Reaction of 1-(2',3'-Epoxy-β-D-lyxofuranosyl)uracil with Hydrogen Fluoride. The Unexpected Formation of 1-(3'-Fluoro-3'-deoxy-β-D-ribofuranosyl)uracil

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Reaction of 1-(2',3'-epoxy-β-D-lyxofuranosyl)uracil (**1**) with hydrogen fluoride afforded 3'-fluoro-3'-deoxyarabinouridine (**2**, 13%) and 3'-fluoro-3'-deoxyuridine (**3**, 11%). The structure of **3** was assigned from spectrometric data and confirmed by an unambiguous synthesis from 2',5'-di-*O*-trityl-2,3'-anhydrouridine (**5**) and hydrogen fluoride.

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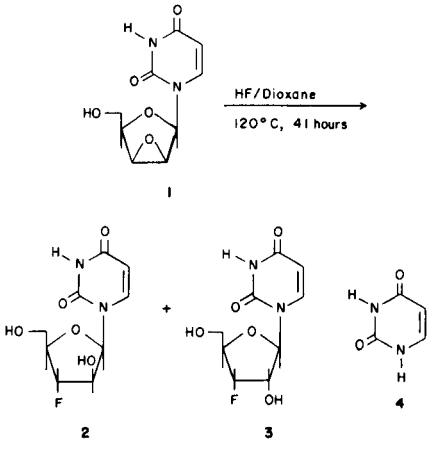
Pyrimidine nucleosides having a halogen atom at the 2'-position of 2'-deoxyuridine [1-4] and the 3'-position of 3'-deoxyarabinouridine [5-7] have been investigated as potential diagnostic agents for tumor localization and as antitumor and antiviral agents [8]. The transport of 2'-halogeno-2'-deoxyuridines by the human erythrocyte nucleoside transporter has been reported [9].

3'-Fluoro-3'-deoxyarabinouridine (**2**) was required for a study to investigate the interaction of 3'-halo-3'-deoxyarabinouridines with the human erythrocyte nucleoside transporter [10]. Reaction of **1** with hydrogen fluoride in anhydrous dioxane at 120° for 41 hours using the procedure reported by Fox [11] gave a complex mixture of products. An extensive chromatographic separation of the reaction mixture using column and thin layer chromatography afforded **2** (13%), the unexpected **3** (11%) and uracil **4**, (67%).



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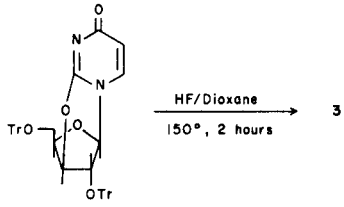
2 3 4

The structure of the unexpected product **3** was initially assigned on the basis of the circular dichroism (cd), ultraviolet (uv), mass (ms) and proton nuclear magnetic resonance (^{1}H nmr) spectra. Ulbricht and coworkers [12] have reported that the cd spectrum can be used to determine the configuration of pyrimidine nucleosides at the anomeric center. This empirical rule is based on the experimental cd curves of pyrimidine β-nucleosides which exhibited positive B_2u ($\pi \rightarrow \pi^*$ transition) Cotton effects. The cd spectrum of **3** in aqueous solution was similar to that of uridine [13] with a positive Cotton effect at 262 nm thereby suggesting that **3** has the β-configuration. The uv spectrum of **3** (water) exhibited a λ max at 262 nm and a λ min at 232 nm (*p*H = 7.2) and a λ max at 262 nm and a λ min at 243 nm (*p*H = 12). These values are consistent with those reported earlier for pyrimidine nucleosides having the ribo configuration suggesting that the sugar moiety is attached to the N-1 position of uracil [14]. The high resolution ms of **3** exhibited a molecular ion at m/z 246 (M^+ Calcd. for $C_9H_{11}N_2O_5F$: 246.0655; Found: 246.0651) as well as a prominent peak (31.8%) at m/z 135 (M^+ Calcd. for $C_5H_8O_3F$: 135.0459; Found: 135.0457) due to the fluorinated sugar moiety.

The ^{1}H nmr spectrum of **3** (acetone-d_6-deuterium oxide) exhibited a *trans*-coupling for $H_{1'}$ ($J_{1',2'}$ = 1.5 Hz) at 5.74 δ which would indicate that **3** must be a β-ribo or α-arabino pyrimidine nucleoside [15]. The cd spectrum for **3** described previously was consistent with that expected for a compound having the β-ribofuranoside configuration [12-13]. The coupling constant $J_{2',3'}$ = 4.5 Hz suggests that the 2'- and 3'-hydrogens must be *cis* to each other ($\phi H_{2'}H_{3'} \sim$ 25-30°). It therefore follows that the 3'-fluoro substituent must be in the ribo (down) configuration. The *trans* $J_{3',4'}$ coupling constant of 2 Hz is compatible with a 3'-ribo fluoro substituent. The assignment of $H_{1'}$ (δ 5.74), $H_{2'}$ (δ 4.09) and $H_{3'}$ (δ 4.82) are consistent with the vicinal ($J_{2',3'-F}$ = 4.5 Hz, $J_{3'-F,4'}$ = 32 Hz) and geminal H-F ($J_{3',3'-F}$ = 50 Hz) coupling constants [11,16]. The large proton-fluorine coupling constants allow ready assignments of the protons by first order analysis. A long range hydrogen-fluorine coupling of 3.0 Hz between the anomeric $H_{1'}$ proton and the C-3' fluorine was also present. The geminal proton-fluorine coupling constant ($J_{3',3'-F}$ = 50 Hz) is within the range normally associated with a sp^3

carbon in a five membered furanose ring [17]. The ^{19}F nmr spectrum in acetone-deuterium oxide relative to hexafluorobenzene appeared as a d, $J_{3',3'\text{-}F}$ = 50 Hz, of d, $J_{3'\text{-}F,4'}$ = 32 Hz of d, $J_{2',3'\text{-}F}$ = 4.5 Hz of d, $J_{1',3'\text{-}F}$ = 3.0 Hz at δ −42.5. A small $J_{3'\text{-}F,5'}$ coupling was also present. The nmr data listed above is consistent with the assigned structure **3**. All assignments were confirmed by double resonance experiments.

The structure assigned to **3** was confirmed by an unambiguous alternate synthesis. Reaction of 2',5'-di-*O*-trityl-2,3'-anhydrouridine (**5**) with anhydrous hydrogen fluoride in dioxane at 150° for 2 hours gave **3** in 66% yield. When the same reaction was carried out at 120° for 21 hours the only product obtained was uracil (**4**). The spectrometric data (uv, ms, ^1H nmr) for **3** prepared in this way was identical to that obtained for **3** prepared from reaction of the epoxide **1** with hydrogen fluoride in dioxane.



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It was also of interest to investigate the reaction pathway by which the unexpected ribo nucleoside **3** was formed. When 3'-fluoro-3'-deoxyarabinouridine (**2**) was heated at 120° in anhydrous hydrogen fluoride-dioxane for 41 hours the ribo nucleoside **3** (25%), uracil **4** (22%) and unreacted **2** (45%) was obtained. On the other hand, a similar treatment of **3** with anhydrous hydrogen fluoride-dioxane afforded uracil (**4**, 95%). These results suggest that **3** arises from further reaction of **2** and uracil (**4**) arises from **3**. The mechanism by which **3** arises from **2** is not known.

EXPERIMENTAL

Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. Ultraviolet spectra were taken on a Pye Unicam SP 1800 spectrometer. The nmr spectra were recorded on Brucker WH 200, AM 300 and WH 400 spectrometers. Mass spectra were determined on an AEI MS-50 spectrometer using direct probe introduction at 200° and 70 eV. The cd spectrum was recorded on a ORD/UV-5 Japan Spectroscopic Co. spectrometer. Thin layer chromatography (tlc) was performed on Whatman MK6F silica gel plates (1 × 3", 200 μ thickness). Preparative tlc was performed on Whatman PLK5F silica gel plates (20 × 20 cm, 1000 μ thickness). The solvent systems used were A, chloroform:methanol (10:1 v/v) and B, the separated upper phase of ethyl acetate:*n*-propanol:-water (4:1:2 v/v/v). Column chromatography was performed using a 1:1 mixture of Merck Kieselgel 60 (70-230 mesh) and Mallinckrodt silicic acid (100 mesh) with chloroform:methanol:acetone (10:1:1 v/v/v) as eluant. Dioxane was dried by distillation from calcium hydride and collection under a nitrogen atmosphere prior to use. 2',5'-Di-*O*-trityl-2',3'-anhydrouridine (**5**) was prepared according to the literature procedure [18]. 3'-Fluoro-3'-deoxyarabinouridine (**2**), 3'-Fluoro-3'-deoxyuridine (**3**) and Uracil (**4**).

A mixture of 1-(2',3'-epoxy-β-D-lyxofuranosyl)uracil (**1**) (1.13 g, 5 mmoles) and liquid hydrogen fluoride (15 g, 15 ml, 0.75 mole) in dry dioxane (150 ml) was heated at 120° for 41 hours in a monel pressure cylinder. Water (60 ml) was added to the cooled reaction mixture which was neutralized with anhydrous sodium carbonate. The solids were removed by filtration and the solvent removed *in vacuo*. The residue was dissolved in absolute ethanol and filtered. The filtrate was concentrated *in vacuo* to give a dark brown residue which was purified on a 2.5 × 40 cm silica gel column. The first eluted product was collected and purified further by preparative tlc using solvent system B. Extraction of the spot having R_f 0.15 with solvent system A and recrystallization from absolute ethanol gave **2** as a white crystalline solid (0.156 g, 13%), mp 179-180° (lit [11] mp 179.5-180.5°); high resolution ms: exact mass calcd. for $C_9H_{11}N_2O_5F$ 246.0655; found: 246.0650. The ^1H nmr spectrum in acetone-d_6-deuterium oxide was identical to the published spectrum and physical data [11]. Further elution gave a second compound which was purified further by preparative tlc using solvent system B. Extraction of the spot having R_f 0.09 with solvent system A and recrystallization from absolute ethanol afforded **3** as a white crystalline solid (0.13 g, 11%), mp 195-196°; uv (water, *p*H = 7.2): λ max 262, λ min 243 nm; cd (water): λ max 262 nm, λ min 232 nm; uv (*p*H = 12): λ max 262 nm, and θ max + 13,300; ^1H nmr: δ 3.75 (d, $J_{5',5''}$ = 11 Hz of d, $J_{4',5'}$ = 11.5 Hz of d, $J_{3'\text{-}F,5'}$ = 1.5 Hz, 1H, H-5'), 3.95 (d, $J_{5',5''}$ = 11 Hz, of d, $J_{4',5''}$ = 5.5 Hz, 1H, H-5''), 4.08 (d, $J_{2',3'}$ = 4.5 Hz, of d, $J_{2',3'\text{-}F}$ = 4.5 Hz of d, $J_{1',2'}$ = 1.5 Hz, 1H, H-2'), 4.12 (d, $J_{3'\text{-}F,4'}$ = 32 Hz of d, $J_{4',5'}$ = 11.5 Hz of d, $J_{4',5''}$ = 5.5 Hz of d, $J_{3',4'}$ = 2.0 Hz, 1H, H-4'), 4.82 (d, $J_{3',3'\text{-}F}$ = 50 Hz of d, $J_{2',3'}$ = 4.5 Hz of d, $J_{3',4'}$ = 2 Hz, 1H, H-3'), 5.6 (d, $J_{5,6}$ = 8 Hz, 1H, H-5), 5.73 (d, $J_{1',3'\text{-}F}$ = 3.0 Hz of d, $J_{1',2'}$ = 1.5 Hz, 1H, H-1'), 7.67 (d, $J_{5,6}$ = 8 Hz, 1H, H-6); high resolution ms: exact mass calcd. for $C_9H_{11}N_2O_5F$: 246.0655; found: 246.0651.

Anal. Calcd. for $C_9H_{11}N_2O_5F$: C, 43.90; H, 4.50; N, 11.38. Found: C, 43.69; H, 4.69; N, 10.94.

Further elution gave uracil (**4**, 0.38 g, 67.8%) identical (tlc, ^1H nmr) with an authentic sample.

Reaction of 3'-Fluoro-3'-deoxyarabinouridine (**2**) With Hydrogen Fluoride.

A mixture of **2** (10 mg) and liquid hydrogen fluoride (0.5 ml) in dry dioxane (2 ml) was heated at 120° for 41 hours. The reaction was completed and the reaction mixture purified as described previously (column and tlc) to yield **3** (2.5 mg, 25%), uracil (1.0 mg, 22%) and unreacted **2** (4.5 mg, 45%).

Reaction of 3'-Fluoro-3'-deoxyuridine (**3**) With Hydrogen Fluoride.

A mixture of **3** (1 mg) and liquid hydrogen fluoride (0.2 ml) in dry dioxane (1 ml) was heated at 120° for 41 hours. The reaction was completed and the reaction mixture purified as described previously to give uracil (95%) as the sole product.

3'-Fluoro-3'-deoxyuridine (**3**).

A mixture of 2',5'-di-*O*-trityl-2,3'-anhydrouridine (**5**) (36 mg, 0.05 mmole) and liquid hydrogen fluoride (0.2 ml) in dry dioxane (2 ml) was heated at 150° for 2 hours in a monel pressure cylinder. Water (1 ml) was added to the cooled solution which was neutralized with solid calcium carbonate. The solids were removed by filtration and the solvent removed *in vacuo*. The residue was dissolved in absolute ethanol and filtered. The filtrate was concentrated *in vacuo* to give a dark brown residue which was separated by column chromatography. An impure fraction was obtained which was purified on preparative tlc using solvent system B:-ethyl acetate (4:1 v/v) to give **3** (8 mg, 66%). 3'-Fluoro-3'-deoxyuridine (**3**) prepared in this way was identical (mp, ^1H nmr, ms) to **3** obtained from reaction of the epoxide **1** with hydrogen fluoride.

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