### Note

# Fluorocarbohydrates Part XXII. 3-Deoxy-3-fluoro-D-gluconic acid

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Metabolic and enzymic studies with 3-deoxy-3-fluoro-D-glucose (1) and Saccharomyces cerevisiae<sup>1,2</sup> and Pseudomonas fluorescens<sup>3</sup> have necessitated the preparation of a number of fluorinated carbohydrates in order to assist with the identification of fluorinated metabolites or to investigate enzyme mechanisms<sup>4</sup>. We now report the synthesis of 3-deoxy-3-fluoro-D-gluconic acid (2) from 3-deoxy-3-fluoro-D-glucose (1).

Compound 1, described initially<sup>5</sup> as a syrup, has now been obtained in a crystalline form (m.p. 108°) which does not mutarotate in water or methyl sulphoxide and which, on the basis of infrared and <sup>19</sup>F n.m.r. data<sup>6</sup>, is considered to be a conglomerate of  $\alpha$ - and  $\beta$ -anomers<sup>\*</sup>. The fluoro sugar 1 gave a crystalline 2,5-dichlorophenylhydrazone.

The electrolytic oxidation of 1 was achived by modification of a procedure<sup>7</sup> applied to D-glucose. 3-Deoxy-3-fluoro-D-gluconic acid (2) was isolated as the calcium salt and displayed electrophoretic and chromatographic properties which were consistent with the structure of an aldonic acid. The acid 2 was identical with a product formed during the biological oxidation of 3-deoxy-3-fluoro-D-glucose by washed cell-suspensions of *Ps. fluorescens*<sup>3</sup>.

A solution of 1 in D<sub>2</sub>O showed <sup>19</sup>F resonances at  $\phi_c$  +194 ( $J_{F,3}$  55,  $J_{F,2}$  13,  $J_{F,4}$  13 Hz) and +200 p.p.m. ( $J_{F,3}$  57,  $J_{F,2}$  13.6,  $J_{F,4}$  13.6 Hz). The resonances at +194 p.p.m. are ascribed to a  $\beta$ -D anomer since a solution of 1,2,4,6-tetra-O-acetyl-3-deoxy-3-fluoro- $\beta$ -D-glucose<sup>5</sup> in CDCl<sub>3</sub> gave one set of peaks with  $\phi_c$  in this region; the resonances at  $\phi_c$  +200 p.p.m. are accordingly ascribed to the  $\alpha$ -D anomer. The resonance centred at  $\phi_c$  +200 p.p.m. is broader than that at +194 p.p.m. and this may well arise from long-range couplings, *e.g.* with the anomeric proton.

The values of  $J_{F,2}$  and  $J_{F,4}$  for 1 are very similar to those observed for methyl 3-deoxy-3-fluoro- $\beta$ -D-xylopyranoside<sup>6</sup>. A solution of crystalline 3-deoxy-3-fluoro-D-

<sup>\*</sup>*Editorial footnote.* A non-mutarotating, crystalline form of 1 (m.p. 114–115°) has also been described [A. B. Foster, R. Hems, and L. D. Hall, *Can. J. Chem.*, 48 (1970) 3937] which, on the basis of <sup>19</sup>F n.m.r. data [A. D. Barford, A. B. Foster, J. H. Westwood, L. D. Hall, and R. N. Johnson, *Carbohyd. Res.*, 19 (1971) 49] contains a near equimolar mixture of  $\alpha$ - and  $\beta$ -anomers.

gluconic acid (2) in  $D_2O$  showed a <sup>19</sup>F resonance at  $\phi_c + 207$  p.p.m., and addition of sodium hydroxide (0.1m final concentration) had no observable effect on the chemical shift.

## EXPERIMENTAL

Melting points were determined using an Electrothermal apparatus and are uncorrected. Thin-layer chromatography (t.l.c.) was performed conventionally on silica gel G (Shandon Scientific Co. Ltd.), using A ethyl acetate-acetic acid-water (3:3:1) and B ethyl acetate-ethanol (4:1); detection was effected by charring with sulphuric acid-ethanol (1:1) at 120° for 5 min, or by treatment with a 1% solution of sodium metaperiodate in acetone followed by benzidine<sup>8</sup>. Electrophoresis was carried out in a Miles high-voltage unit (Miles Hivolt Ltd.), using Whatman No. 1 paper (prewashed with 2M acetic acid and distilled water<sup>9</sup>), at either 6 kV for 2 h in an aceticformic acid buffer<sup>11</sup> (pH 2) or 3 kV for 30 min in a pyridine-acetic acid buffer<sup>11</sup> (pH 5.3); the mobilities ( $M_G$ ) of aldonic acids are relative to D-glucose. Aldonic acids were detected with the benzidine-periodate reagent<sup>8</sup>.

Electrolytic oxidation<sup>7</sup> was carried out with carbon electrodes  $(10 \times 0.5 \text{ cm})$  (Griffin and George) with a voKam power unit (Shandon Scientific Co. Ltd.).<sup>19</sup>F chemical shifts ( $\phi_c$ ) were determined using a Jeol (Model JNM-4H-100) spectrometer at 94 MHz and 33°, with CFCl<sub>3</sub> as internal standard.

Fluorine determination on calcium 3-deoxy-3-fluoro-D-gluconate was made by our hydrolysis procedure<sup>11</sup> and the use of the Fluoride electrode (Orion Research Inc.).

3-Deoxy-3-fluoro- $\alpha\beta$ -D-glucose (1). — 3-Deoxy-3-fluoro-1,2:5,6-di-O-isopropylidene-D-glucose<sup>5</sup> (5 g) was dissolved in acetone (125 ml) and heated under reflux with M sulphuric acid (200 ml) for 3 h. Hydrolysis was then complete (t.1.c., solvent B). The hydrolysate was neutralised with barium carbonate (80 g) and filtered, and the filtrate was concentrated *in vacuo* and dried over phosphorus pentaoxide to yield a thick syrup (3.4 g). This hydrolysis procedure gave better results than those achieved by the use of Amberlite IR-120(H<sup>+</sup>) resin. The dry syrup was crystallised from dry acetone and, after recrystallisation from the same solvent and decolourisation with charcoal, 1 was obtained as colourless needles (2.0 g), m.p. 108°,  $[\alpha]_D^{22} + 64^\circ$  (c 1, water),  $R_F 0.45$  (solvent C) (Found: C, 39.8; H, 6.1; F, 10.8. C<sub>6</sub>H<sub>11</sub>FO<sub>5</sub> calc.: C, 39.6; H, 6.05; F, 10.4%). The i.r. spectra (both KBr disc and Nujol mull) of the crystalline sugar showed type 2a and 2b bands at 852 and 898 cm<sup>-1</sup> which are indicative<sup>12</sup> of  $\alpha$ - and  $\beta$ -D configuration at the anomeric centre.

A mixture of 3-deoxy-3-fluoro- $\alpha\beta$ -D-glucose (100 mg), 2,5-dichlorophenylhydrazine (196 mg), and methanol (15 ml) was heated on a boiling water-bath until all the solvent had evaporated to give a syrup which was treated with ether at 0-5° overnight. The resulting, solid hydrazone, after recrystallisation from ethyl acetatelight petroleum (b.p. 40-60°) or aqueous ethanol, had m.p. 135°,  $R_F$  0.6 (solvent B) (Found: C, 42.5; H, 4.5; N, 8.3; F, 5.8.  $C_{12}H_{15}Cl_2FN_2O_4$  calc.: C, 42.3; H, 4.4; N, 8.2; F, 5.6%). 3-Deoxy-3-fluoro-D-gluconic acid. — The electrolytic oxidation of 1 was conducted in a glass vessel (height 8.7 cm, diameter 8 cm) with a screw-cap lid provided with three holes (6 mm diameter) 3 cm apart. Two of these holes were fitted with carbon electrodes, and the assembly was placed on an efficient magnetic stirrer. To the vessel was added calcium bromide (0.623 g), calcium carbonate (0.9 g), 3-deoxy-3fluoro- $\alpha\beta$ -D-glucose (3 g), and water (67 ml). A steady current of 40 mamp was passed through the stirred mixture for 40 h. During this time, the current was reversed at intervals to prevent deposition of material on one of the electrodes. The electrolysed solution was filtered through Kieselguhr and the filtrate was concentrated *in vacuo* to a thin syrup which crystallised from ethanol. Recrystallisation from water yielded hydrated calcium 3-deoxy-3-fluoro-D-gluconate (3 g), m.p. 198-200° (dec.),  $R_F$  0.38 (solvent A) [Found: C, 29.6; H, 5.2; F, 7.1. Ca(C<sub>6</sub>H<sub>10</sub>FO<sub>6</sub>)<sub>2</sub>·3H<sub>2</sub>O calc.: C, 29.5; H, 5.3; F, 7.8%].

A thick suspension of the calcium salt (3 g) in water (8 ml) was applied to Amberlite IR-120(H<sup>+</sup>) resin (85 g) in a glass column (40 × 2 cm). Elution with water and concentration of the appropriate fractions at 35° (bath) gave a thick syrup. On standing, a crystalline product separated which was washed with ether and recrystallised from *p*-dioxane-ether to give 2, m.p. 119°,  $[\alpha]_D^{21} - 6^\circ$  (c 1, water),  $R_F$  0.38 (solvent A),  $M_G$  1.8×10<sup>2</sup> in pH 2 buffer and 6.0×10<sup>2</sup> in pH 5.3 buffer (Found: C, 36.3; H, 5.6; F, 9.0. C<sub>6</sub>H<sub>11</sub>FO<sub>6</sub> calc.: 36.3; H, 5.6; F, 9.6%).

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