Synthesis of 3-Deoxy-3-fluoro-D-glucose 1- and 6-Phosphates and their Intreaction with Phosphoglucomutase and UDPG-Pyrophosphorylase

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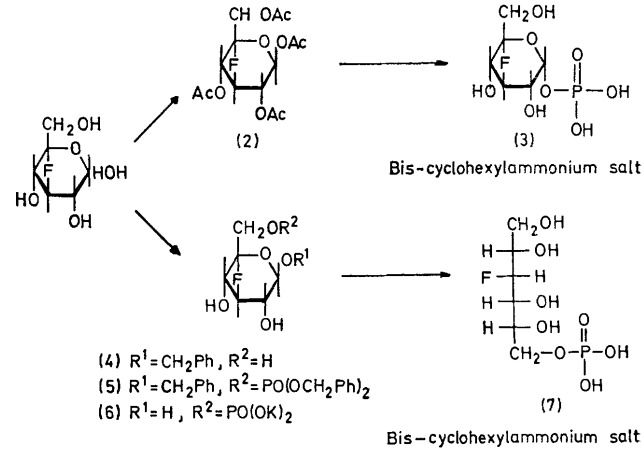
Summary 3-Deoxy-3-fluoro-α-D-glucose 1-phosphate (**3**) and the corresponding 6-phosphate (**6**) have been synthesised and shown to be competitive inhibitors of phosphoglucomutase and UDPG-pyrophosphorylase.

As part of a study of the metabolism and enzymology of fluorinated carbohydrates and related compounds[1] we have examined the biochemical effects of 3-deoxy-3-fluoro-D-glucose (**1**) on resting cells of *Saccharomyces cerevisiae*[2] and have shown that (**1**) is metabolised by whole cells[3] and cell-free extracts[4] of *Ps. fluorescens* to 3-deoxy-3-fluoro-D-gluconic acid and 3-deoxy-3-fluoro-2-oxo-D-gluconic acid. These results indicate that stereospecifically fluorine substituted carbohydrates and related compounds may act as biochemical pseudosubstrates and also give information about enzyme specificity.[5,6] We now report the synthesis and some enzyme inhibitory properties of 3-deoxy-3-fluoro-α-D-glucose 1-phosphate (**3**) and the 6-phosphate (**6**).

The synthesis of (**3**) was based on a method reported for α-D-glucose 1-phosphate.[7] 1,2,4,6-Tetra-*O*-acetyl-3-deoxy-3-fluoro-D-glucose[8] (**2**) (1·013 g) and anhydrous phosphoric acid (2·129 g) were heated at 50—55 °C for 2 h *in vacuo*. The resulting syrup was neutralised with 2N-lithium hydroxide and the filtrate passed through Dowex 50 (H^+). The eluate was collected in a flask containing cyclohexylamine (5·0 ml) and the 3-deoxy-3-fluoro-α-D-glucose 1-phosphate bis-cyclohexylammonium salt (**3**)† isolated as crystalline solid (yield 70%), m.p. 158—162°, $[\alpha]_D^{22}$ + 61·5° (*c* 1·2, H_2O), R_F, 0·4 (CC41 cellulose, solvent A, t-pentyl alcohol–water–toluene-*p*-sulphonic acid 60 ml: 30 ml: 2 g). 3-Deoxy-3-fluoro-D-glucose-6-phosphate (**6**) was synthesised *via* benzyl 3-deoxy-3-fluoro-D-glucopyranoside (**4**) which



was obtained in high yields as a crystalline solid, m.p. 95°, $[\alpha]_D^{21}$ —58°, (*c* 1·0, MeOH) by shaking (**1**) with benzyl alcohol saturated with hydrogen chloride. Treatment of (**4**) with dibenzyl phosphonochloridate[9] at —40° gave crystalline benzyl-3-deoxy-3-fluoro-β-D-glucose 6-dibenzylphosphate (**5**)† m.p. 109—114°, $[\alpha]_D^{22}$ —4·2° (*c* 2·7, $CHCl_3$). Hydrogenolysis of (**5**) with 10% palladium on charcoal yielded (**6**)‡ which was isolated as a hygroscopic potassium salt (90%) R_F 0·35 (CC41 cellulose, solvent A) which consumed 2·0 moles periodate/mole and 0·84 moles formic acid/mole after 72 h. Slow oxidation was probably due to the formation and slow hydrolysis of a formyl ester. No formaldehyde was produced. These results are consistent with the introduction of the phosphate group at C-6 of 3-deoxy-3-fluoro-D-glucose. The structure of (**6**) was further confirmed after reduction with potassium borohydride and isolation of the 3-deoxy-3-fluoro-D-glucitol 1-phosphate as the bis-cyclohexylammonium salt (**7**),† m.p. 162—165°, $[\alpha]_D^{22}$ —30·5° (*c* 1·1, H_2O).

The effect of 3-deoxy-3-fluoro-α-D-glucose 1-phosphate (**3**) on UDPG-pyrophosphorylase and the corresponding 6-phosphate (**6**) on phosphoglucomutase and glucose 6-phosphate dehydrogenase activities was examined using a cell-free extract from *S.cerevisiae*, prepared by the method of Munch-Peterson.[11] UDPG-pyrophosphorylase activity was assayed either by adding excess of UDPG-dehydrogenase, NAD and estimating the production of $NADH_2$ by absorbance changes at 340 nm or by adding excess of pyrophosphatase and measuring the production of inorganic phosphate.[12] The same cell-free extract also contained phosphoglucomutase activity which, since its activity was lower than UDPG-pyrophosphorylase (activity ratio 1 : 4, respectively), could be measured in a similar assay by changing the substrate from glucose 1-phosphate to glucose 6-phosphate, adding the phosphoglucomutase co-enzyme glucose 1,6-diphosphate, and again observing the change in absorbance at 340 nm due to the production of $NADH_2$. The glucose 6-phosphate dehydrogenase activity was also measured in the same cell-free extract by observing the increase in 340 nm absorbance on addition of NADP due to the production of $NADPH_2$. It was shown that neither (**3**) nor (**6**) was a substrate for UDPG-pyrophosphorylase and phosphoglucomutase, respectively, although (**6**) was a poor substrate for glucose 6-phosphate dehydrogenase. Thus at 5 mM (**6**) gave a 1% initial rate of that given by the normal substrate at 0·5 mM.

At concentrations greater than 8 mM and using Lineweaver–Burk plots[13] (**3**) showed competitive inhibition of UDPG-pyrophosphorylase in the presence of the normal substrate with K_i 4 × 10^{-2}M compared with a K_m 6 × 10^{-5}M for α-D-glucose 1-phosphate. A similar analysis showed that (**6**) was essentially a competitive inhibitor of phosphoglucomutase with K_i 4 × 10^{-2}M compared with a K_m 4 × 10^{-4}M for glucose 6-phosphate.

The inhibition of phosphoglucomutase and UDPG-pyrophosphorylase by (**6**) and (**3**), respectively, is consistent with our previous biochemical studies in which it was demonstrated that 3-deoxy-3-fluoro-D-glucose (**1**) can act as a poor substrate for yeast hexokinase[14] as well as inhibit polysaccharide synthesis in resting whole cells of *S. cerevisiae*.[15]

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† Analysed correctly for the monohydrate. Fluorine analyses were carried out with the fluoride electrode[10] which eliminates interference by phosphate.

‡ Gave correct fluorine analysis.

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