Synthesis of the Monodeoxy-monofluoroglycerols and their Interaction with Glycerol Kinase

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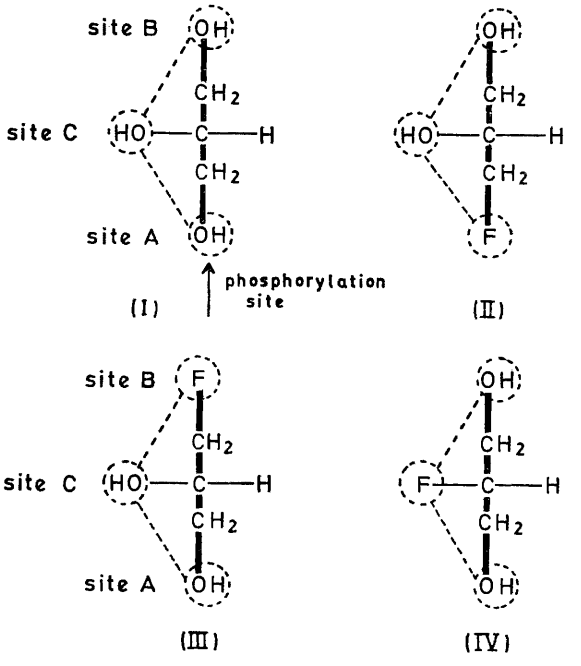
Summary The three monodeoxy-monofluoroglycerols have been synthesised stereospecifically and their behaviour as substrates or inhibitors of glycerol kinase has been found to be dependent on the position of fluorine in the molecule.

MODIFICATION of natural metabolites by substitution of a hydrogen atom by fluorine has resulted in the synthesis of a number of pseudo-substrates or inhibitors which have proved useful as metabolic probes.[1] Less is known of the metabolic effects of compounds in which a hydroxy-group of a natural substrate has been replaced by fluorine. Current interest in such fluorinated carbohydrate analogues,[2] and the recent finding[3] that *rac*-1-deoxy-1-fluoroglycerol-3-phosphoric acid† can act as a substrate of L-α-glycerol

† Stereospecific numbering, as recommended by the I.U.P.A.C.–I.U.B. Commission, 1967, has been used in order to clarify the relationship of the fluoro-analogues to glycerol.

phosphate dehydrogenase prompts us to report the stereospecific synthesis of the three monodeoxy-monofluoroglycerols and their behaviour as substrates of glycerol kinase.



3-Deoxy-3-fluoro-*sn*-glycerol (II), b.p. $55°_{0.2}$, $[\alpha]_D^{22}$ −8·2° (*c* 10, H_2O), was synthesised from D-mannitol (in 12% yield) *via* 1,2-*O*-isopropylidene-3-*O*-tosyl-*sn*-glycerol[4] (V) by treatment of (V) with KF in diethylene glycol, followed by acid hydrolysis. 1-Deoxy-1-fluoro-*sn*-glycerol (III), b.p. $55°_{0.2}$ $[\alpha]_D^{22}$ +8·2° (*c* 10 in water), was prepared by inversion of configuration at C-2 of its enantiomer (II) by treatment of the ditosyl derivative of (II) with sodium benzoate, followed by acid hydrolysis [yield 30% from (II)]. Crystalline 2-deoxy-2-fluoroglycerol (IV), m.p. 39—40°, was synthesised from 2-*O*-tosyl-1,3-di-*O*-trityl glycerol[5] by fluoride exchange using tetra-n-butylammonium fluoride in acetonitrile, followed by acid hydrolysis (yield from glycerol 20%). Tosylation of (IV) gave the di-*O*-tosyl derivative, m.p. 111—112°, (lit.[6] 109°). The monodeoxy-monofluoroglycerols were pure by g.l.c. analysis of their trimethylsilyl ethers, and showed consistent elemental analytical, i.r., and n.m.r. data.

The monodeoxy-monofluoroglycerols, together with their deoxy-, hydroxy-, and oxo-analogues, constitute a simple system for investigating the ability of a fluoro-substituent to act as a hydroxyl analogue in enzyme–substrate interactions, since enzymes are known (liver alcohol dehydrogenase, glycerol dehydrogenase, and glycerol kinase) which catalyse reactions specifically at positions 1-, 2-, and 3- of *sn*-glycerol.

A model has been presented[7] for the substrate requirements for phosphorylation by yeast glycerol kinase which requires the presence of three actual or potential substrate hydroxy-groups, in the spatial arrangement shown (I) for efficient binding to the enzyme. In each of the monodeoxy-monofluoro-glycerols (II, III, and IV) a different hydroxy-group is replaced by fluorine, and the effect of presenting a fluorine atom to each binding site (A, B, and C, respectively) can be assessed.

The behaviour of the monodeoxy-monofluoroglycerols as substrates or inhibitors of yeast glycerol kinase was studied by following the rate of ADP formation.[7] Under the conditions of the assay the K_m values for dihydroxyacetone and glycerol are 7mM and 0·05 mM, respectively. Fluoro-analogues (III) and (IV), in which a fluorine atom approaches binding sites B and C, respectively, both behave as substrates of the enzyme with K_m values of 120 mM and 150 mM. However (II), in which fluorine, rather than oxygen, is presented to the phosphorylation site (site A) of the enzyme, shows no substrate activity at concentrations up to 500 mM, but acts as an effective competitive inhibitor of either dihydroxyacetone or glycerol (K_I = 5 mM).

The model suggested for the substrate specificity of yeast glycerol kinase is based on the stereoselectivity of the enzyme for phosphorylation of the 3-position of *sn*-glycerol, the behaviour of dihydroxyacetone and D- and L-glyceraldehyde as substrates, and the inability of the deoxy-analogues of glycerol (propane- 1,2- and -1,3-diols) to bind effectively to the active site.[7] The observed behaviour of fluoro-analogues (III) and (IV) as substrates, and of fluoro-analogue (II) as a competitive inhibitor can easily be reconciled with the requirements of the model if the fluoro-substituents are able to interact with the "specific" hydroxyl sites on the enzyme. The general applicability of such interactions to other systems could lead to the use of fluoro-substituents as highly specific probes for studying the nature of binding in enzyme–substrate complexes.

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