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ON THE ANALOGY BETWEEN FORSKOLIN AND D-GLUCOSE

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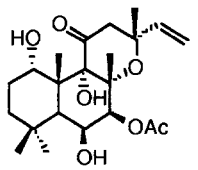
Abstract: 2-*O*-Acetyl-D-glucose was synthesized in order to evaluate the influence of an acyl group on the binding with the glucose carrier protein (GluT); as its affinity neighbours that of glucose itself, the glucose – forskolin analogy appears to be coincidental and several explanations are proposed.

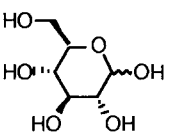
Keywords: carbohydrates, membrane, receptor, terpene.

D-Glucose provides energy to cells through glycolysis. As this process occurs within the cell, a family of transmembranar proteins, coined GluT, facilitate D-glucose transport across the lipid membrane.[1,2] Developing a probe which would selectively bind to these glucose carriers would be an attractive way to evaluate GluT's membrane density and to monitor the abnormal glucose uptake associated with some diseases (Alzheimer, diabetes, cancer).[3]

Diverse classes of compounds (carbohydrates, cytochalasins, flavonoids) are known to interact with GluT proteins[3] but we were particularly attracted by forskolin, **1**.[4] This terpene was first described as an adenylate cyclase activator and was subsequently shown to inhibit glucose transport (EC_{50} = 0.24 μM) in adipocytes plasma membrane vesicles,[5] human erythrocytes,[6] and platelets.[7] The direct interaction of forskolin with the glucose carrier protein has been demonstrated with the observed binding of [^{3}H]-forskolin to human erythrocytes.[8]



forskolin **1**



D-glucose

To explain the affinity of forskolin for the glucose carrier GluT, Seamon *et al.* have proposed a seductive model[9] in which a carbohydrate is recognized within the forskolin functionalities: indeed, α-D-galactopyranose shows spatial superimposition of all its hydroxyl groups with the oxygens linked to the A and B rings of the terpene, as displayed in figure 1. As interactions of carbohydrates with the glucose carrier are believed to occur with the hydroxyl groups located at C-1, C-2 and C-3[10,11] (which would thus correspond to the oxygenated

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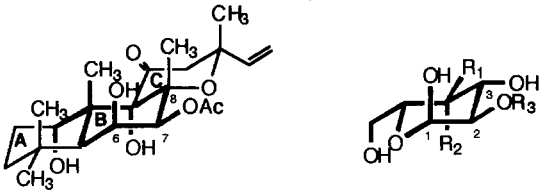
groups located at C-6, C-7 and C-8 in forskolin), this would explain why D-galactose and D-glucose (which differ only in their relative stereochemistries at C-4) can be substituted to each other in this model.



forskolin **1**

α-D-galactopyranose : $R_1 = R_3 = H$, $R_2 = OH$

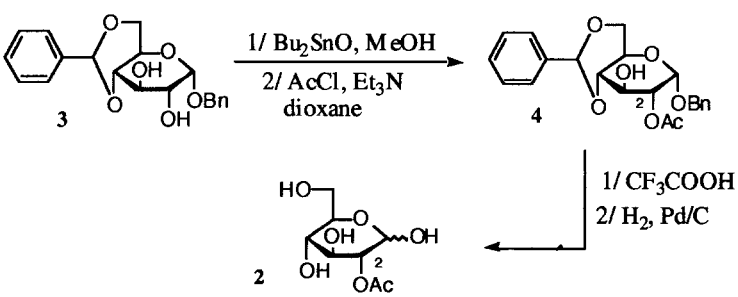
α-D-glucopyranose : $R_1 = OH$, $R_2 = R_3 = H$

2 : $R_1 = H$, $R_2 = OH$, $R_3 = Ac$

Figure 1: structural analogies between forskolin and carbohydrates.[9]

Given this rationalization, the micromolar range affinities of forskolin remains to be explained, when compared with the millimolar-range observed for carbohydrates.[11] *Could the carbonyl group of the C-7 ester of forskolin (or derivatives) be of importance* ? Indeed, good affinities are observed for various esters at C-7 of forskolin (which include radiolabelled / photoaffinity probes) [12] but, conversely, there is a 30 fold loss in affinity for 7-desacetyl-forskolin itself (EC_{50} = 7.1 μM).[9] Thus, we decided to introduce an acetyl group at O-2 on glucose, and to prepare **2**, since this ester derivative of glucose would match more closely the forskolin B-ring sub-structure (see figure 1).

Despite the large number of syntheses of partially acylated glucose derivatives,[13] it is surprising that the preparation of 2-*O*-acetyl-D-glucose, **2**, remains undescribed.[14] The synthetic approach which has been used in the present work aimed at flexibility in the introduction of the O-2 acyl substituent, and started with benzyl glucopyranoside.[15,16] As the anomeric configuration of glucopyranosides has been shown to strongly influence the outcome of acylation regioselectivities,[17] the readily available α anomer **3**,[15,16] was selected. **3** was stannylated[18,19] and acetylation of the intermediate stannyl ether (not isolated), using acetyl chloride/triethylamine, gave a single acetate (80% from **3**) whose structure could be ascertained as **4** (δ H-2 = 4.75 ppm; dd; $J_{2\text{-}1}$ = 4 Hz, $J_{2\text{-}3}$ = 9.5 Hz).[20]



The desired 2-*O*-acetyl-D-glucopyranose, **2**,[21] was then obtained without migration of the acetyl group by acidic deprotection (93%) of the 4,6-benzylidene acetal of **4**, which was followed by reductive removal (73%) of the anomeric protecting group.

2-*O*-Acetyl-D-glucose was tested as an inhibitor of glucose transport activity in rat adipocytes (where the predominant glucose transporter isoform is GluT4) and the Ki found to be 8.5 mM (data courtesy of G.D. Holman, J. Yang - University of Bath, UK). This is in the range of that observed[11] for D-glucose itself and this result, while showing that the presence of an acetyl group at O-2 does not enhance affinity, leads us to question the forskolin / glucose analogy put forward.[9]

It is therefore likely that recognition of forskolin by GluT operates through a different mechanism than that of glucose, especially since its binding is thought to occur at the endofacial binding site; furthermore, allosteric effects may operate, with different sites for D-glucose and forskolin being involved. On structural grounds, it is worth pointing out in comparing D-glucose (or D-galactose) and forskolin, that the pyranose oxygen, which is essential for binding[11], is lacking in forskolin or congeners (position –5 of the B-ring); also, the conformation of the B-ring in forskolin has been predicted to be a rigid distorted chair,[22] thus departing from the carbohydrate pyranose ring structure.

The rationale for the synthesis of 2-*O*-acetyl-D-glucose, **2**, was based on the structural analogy put forward [9] between glucose and forskolin. However, the affinity of **2** for GluT suggests the structural model for a comparison between forskolin and D-glucose[9] (or also for D-galactose)[23] is unlikely to be correct.

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[20] It is of related interest to note that when this acylation procedure was carried out starting with the β anomer, no selectivity was observed as equivalent amounts of O-2 and O-3 acetyl derivatives were formed.

[21] **2:** Oil, $[\alpha]_D^{20}$ = +59° (20 min.) + 64 ° (24 h) (c=0.23; H_2O). ^1H NMR (500 MHz - D_2O): δ 5.40 (1H, d, $J_{1\text{-}2}$ = 3.6 Hz, H-1 α), 4.84 (1H, d, $J_{1\text{-}2}$ = 8.1 Hz, H-1 β), 4.73 (1H, dd, $J_{2\text{-}1}$ = 3.6 Hz, $J_{2\text{-}3}$ = 10 Hz, H-2 α), 4.70 (1H, dd, $J_{2\text{-}1}$ = 8.1 Hz, $J_{2\text{-}3}$ = 10 Hz, H-2 β), 3.95 (1H, dd, $J_{3\text{-}2}$ = 10 Hz, $J_{3\text{-}4}$ = 9.5 Hz, H-3α), 3.94 (1H, d, J = 12.8 Hz, H-6 β), 3.90 (1H, m, H-5 α), 3.88 (1H, dd, $J_{6\text{-}5}$ = 2.5 Hz, $J_{6\text{-}6'}$ = 12.5 Hz, H-6 α), 3.81 (1H, dd, $J_{6'\text{-}5}$ = 5.5 Hz, $J_{6\text{-}6'}$ = 12.5 Hz, H-6' α), 3.77 (1H, dd, $J_{6\text{-}5'}$ = 5.5 Hz, $J_{6\text{-}6'}$ = 12.8 Hz, H-6' β), 3.72 (1H, dd, $J_{3\text{-}2}$ = 10 Hz, $J_{3\text{-}4}$ = 9.5 Hz, H-3 β), 3.56 (1H, dd, $J_{64\text{-}3}$ = 9.5 Hz, $J_{4\text{-}5}$ = 9.85 Hz, H-4 α), 3.55 (2H, M, H-5, H-4 β), 2.21 (3H, s, CH_3), 2.20 (3H, s, CH_3); ^{13}C NMR (125 MHz - D_2O): δ 172.8 (C=O), 93.6 (C-1β), 89.0 (C-1α), 75.6 (C-5β), 74.5 (C-2β), 73.3 (C-3β), 72.9 (C-2α),70.8 (C-5α), 70.0 (C-3α), 69.0 (C-4α), 68.9 (C-4β), 60.1 (C-6β), 59.9 (C-6α), 19.9 (CH_3β), 19.8 (CH_3α); these assignments were secured by correlation spectroscopies.

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