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GLYCOSIDES FOR TESTING GLYCOSIDASES: VINYL, 1-ETHOXYETHYL, AND 1-ETHOXYBUT-3-ENYL D-GLUCOPYRANOSIDES

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ABSTRACT

The reaction of ethyl vinyl ether and 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (**1**) in the presence of $Hg(OAc)_2$ and toluene-*p*-sulphonic acid as catalysts yielded the acetylated vinyl, 1-ethoxyethyl, and 1-ethoxybut-3-enyl glycosides in varying proportions. Crystalline 1-ethoxybut-3-enyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**2**), vinyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (**3**), and 1-ethoxyethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**4**) were isolated by chromatography. Compound **4** was also prepared by the reaction of **1** with cold acetaldehyde diethyl acetal containing a trace of acetic acid, and its α anomer (**5**) by the reaction of **1** with boiling acetaldehyde diethyl acetal containing a trace of acetic acid. Each deacetylated D-glucoside was cleaved by the corresponding D-glucosidase, to yield D-glucose and either acetaldehyde (from deacetylated **3**–**5**) or but-3-enal (from deacetylated **2**).

INTRODUCTION

The enzyme assay described by Warburg and Christian[1], which exploits the difference in absorbance between oxidised and reduced forms of pyridine nucleotides at 340 nm, is widely used in studies of pyridine nucleotide-dependent enzymes and their substrates, and for coupled assays in which such substrates are produced. Vinyl esters of various acids have been used in coupled assays of esterases[2]; the liberated vinyl alcohol, which instantaneously tautomerises to acetaldehyde, can be assayed by using alcohol dehydrogenase (ADH) and NADH. We have found that vinyl glycosides, as well as 1-ethoxyethyl glycosides, can be used as substrates for glycosidases in continuous, kinetic test-systems.

RESULTS AND DISCUSSION

In 1967, Fletcher *et al.*[3] noted that vinyl glycosides had not attracted the attention of organic chemists. Also, mixed acetal glycosides other than tetrahydropyranyl glycosides[4] have not yet been described.

When mercuric acetate was added to a boiling mixture of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose[5] (**1**) and ethyl vinyl ether, little reaction ensued during 24 h.

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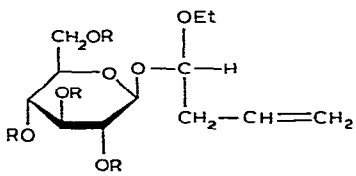
Fletcher *et al.*[3] found that isobutyl vinyl ether reacts readily with 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose in the presence of mercuric acetate, to give the anomeric vinyl D-glucopyranosides. When toluene-*p*-sulphonic acid monohydrate was used as the catalyst, there was significant decomposition of the substrate and polymerisation of the solvent. However, reaction in the presence of both toluene-*p*-sulphonic acid monohydrate and mercuric acetate almost quantitatively converted **1** into a near-equimolar mixture of three products (**2**–**4**), the components of which could be isolated crystalline by chromatography.



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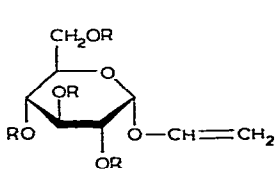

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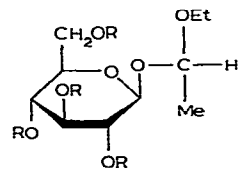

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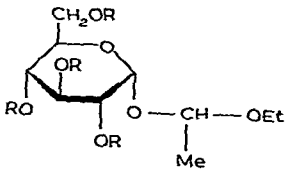
Compound **2** reduced permanganate solution and was shown by ^{1}H-n.m.r. spectroscopy and other criteria to be 1-ethoxybut-3-enyl 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranoside, as was **4** shown to be 1-ethoxyethyl 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranoside. Compound **3** was the known vinyl 2,3,4,6-tetra-*O*-acetyl-*α*-D-glucopyranoside formed[6] by a reversible alkoxy-mercuration. G.l.c. of the original mixture revealed **2**–**4** to be the major components, but minor amounts of other products were present, presumably anomers and, for **2** and **4**, diastereomers.

The formation of **4** (Scheme 1) could involve mercurinium acetate cation–proton interchange with intermediate I (pathway *a*). Pathway *b* explains the presence of **2**, with ethyl vinyl ether being available in excess. Intermediate II, like I, can be converted into its corresponding unsaturated product **2**. It is not clear why, for **3**, preponderantly the *α* anomer is formed, whereas **2** and **4** are mainly *β* anomers.

Deacetylation of **2**–**4** gave the corresponding products **6**–**8**.

An alternative route to mixed acetal glycosides is *via* transacetalation. With toluene-*p*-sulphonic acid monohydrate as catalyst, **1** and acetaldehyde diethyl acetal reacted slowly (~50% after 24 h at 20°), to give an approximately equimolar *α*,*β*-mixture of glycosides. Boiling the reaction mixture caused decomposition. When **1** was heated with acetaldehyde diethyl acetal in the absence of catalyst, no decomposition occurred and 1-ethoxyethyl 2,3,4,6-tetra-*O*-acetyl-*α*-D-glucopyranoside (**5**) was the main product*. When the liberated ethanol was distilled off, the reaction could be driven almost to completion (>90% as shown by g.l.c.).

*It was subsequently found that the presence of a trace of acetic acid, normally present in crude **1**, is necessary.



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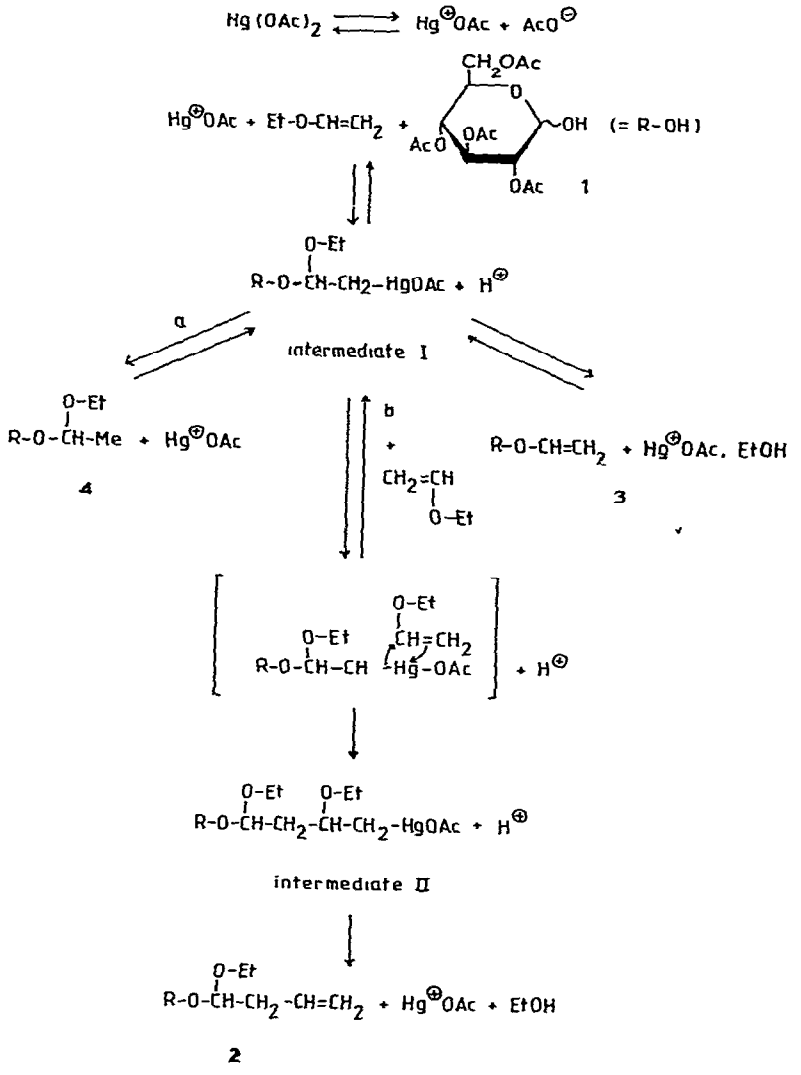

Scheme I. Mechanism of reaction of 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (1) with ethyl vinyl ether.

TABLE I

ACID-CATALYSED REACTION[a] OF 2,3,4,6-TETRA-*O*-ACETYL-β-D-[^{14}C]GLUCOPYRANOSE[b] (**1'**) WITH ACETALDEHYDE DIETHYL ACETAL

Reaction time (*h*)	1	2.5	5	22	28	94
Residual **1'** (%)	89.67	89.53	76.70	58.58	56.46	54.54
4' (%)	10.33	10.48	23.30	41.42	43.53	36.65
5' (%)	—	—	—	—	—	8.79

[a]A 36.5mM solution of **1'** in acetaldehyde diethyl acetal was treated with 0.1 vol % of acetic acid. Aliquots were taken at the indicated intervals and analysed by radiochromatogram-scanning (solvent *A*) [b]Prepared[5] from D-[^{14}C]glucose (0.05 mCi; specific activity, 2.9 mCi/mmol)

Deacetylation of **5** yielded crystalline 1-ethoxyethyl α-D-glucopyranoside (**9**).

When **1** was treated at 20° with acetaldehyde diethyl acetal containing a trace of acetic acid as catalyst, transacetalation occurred slowly without mutarotation and the β-glycoside **4** was formed (Table I).

Compounds **7** and **9** were hydrolysed by α-D-glucosidase and **8** by β-D-glucosidase, thereby confirming the assigned configurations. Acetaldehyde was released in

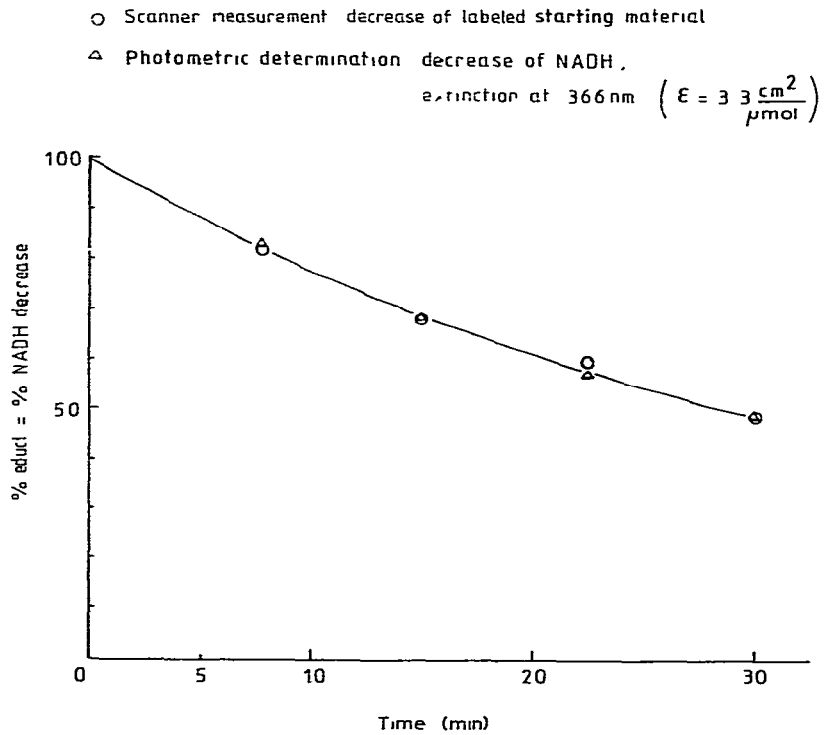

Fig. 1. Cleavage of 1-ethoxyethyl α-D-[^{14}C]glucopyranoside (**9'**) by α-D-glucosidase. Conditions: 0.5 μmol of **9'**, 20 μg of α-D-glucosidase, 0.7 μmol of NADH, and 150 μg of ADH in 1 ml of sodium phosphate buffer (pH 6.8)

TABLE II

KINETIC PARAMETERS[a] FOR 7–9

	K_m (mM)	V_{max} ($\mu mol\ ml^{-1}\ min^{-1}$)	$V_{max}(oNPG)^b/V_{max}$
Vinyl α-D-glucopyranoside (7)	10.04	0.094	55
1-Ethoxyethyl α-D-glucopyranoside (9)	22.9	0 071	73
1-Ethoxyethyl β-D-glucopyranoside (8)	83.3	0.087	1.9

[a]Determined in 0.05M phosphate buffer (pH 6 8) [b]*o*-Nitrophenyl α- or β-D-glucopyranoside as appropriate.

each hydrolysis, and measured by an optical method[7] using aldehyde dehydrogenase (ADH) and NADH as co-substrate. The hydrolysis of **9** is shown in Fig. 1, and the kinetic parameters for **7–9** are given in Table II.

The rate of glycoside cleavage determined by the optical method corresponds with that based on measurement of D-[^{14}C]glucose released from radiolabelled **9'**. 1-Ethoxybut-3-enyl β-D-glucopyranoside (**6**) was also hydrolysed by β-D-glucosidase.

1-Ethoxyethyl glycosides of other monosaccharides and oligosaccharides are being investigated.

EXPERIMENTAL

General. — T.l.c. was performed on silica gel F_{254} (Merck) with *A*, ether–light petroleum (b.p. 60–70°) (4:1) for fully protected compounds; and *B*, ethyl acetate–2-propanol–water (25:14:7) for compounds having free hydroxyl groups. Detection was effected by charring with sulphuric acid. P.c. was performed on Whatman No. 1 paper with 1-butanol–pyridine–water (6:4:3). G.l.c. was performed with a Pye–Unicam GCD chromatograph, with glass columns and 3% of SE-52 on Chromosorb G, AW-DMCS. Radioactive compounds were located and quantified with a Packard 7200 radiochromatogram scanner. N.m.r. spectra (internal Me_4Si) were obtained with Varian A-60 D (60 MHz), EM 390 (90 MHz), or Bruker 90 spectrometers, i.r. data with a Perkin–Elmer 137 Infracord spectrometer, and optical rotations with a Perkin–Elmer 141 polarimeter. Melting points are uncorrected.

Enzyme reactions. — α-D-Glucosidase (maltase, α-D-glucoside glucohydrolase. EC 3.2.1.20) from yeast, β-D-glucosidase (β-D-glucoside glucohydrolase EC 3.2.1.21) from sweet almonds, alcohol dehydrogenase (ADH, alcohol: NAD oxidoreductase, EC 1.1.1.1.) from yeast, and β-nicotinamide adenine dinucleotide (β-NADH) were purchased from Boehringer and used without further purification. All enzyme reactions were performed at 30° in 0.05M sodium phosphate buffer (pH 6 8). Initial rates relate to the decrease in absorbance at 366 nm, which is a measure for the amount of NADH.

Reaction of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranose (**1**) *with ethyl vinyl ether.*— A mixture of **1** (20 g), mercuric acetate (2.15 g), toluene-*p*-sulphonic acid monohydrate (430 mg), and redistilled ethyl vinyl ether (90 ml) was boiled under reflux for 2 h. Dissolution occurred after 30 min. T.l.c. (solvent *A*) then showed, besides very little **1** (R_F 0.12), three main products, **2** (R_F 0.33), **3** (R_F 0.30), and **4** (R_F 0.24). The reaction mixture was diluted with chloroform (500 ml), washed with aqueous 5% potassium bromide (2 × 500 ml), water (2 × 500 ml), and saturated, aqueous sodium hydrogencarbonate (200 ml), dried ($MgSO_4$), and concentrated under diminished pressure.

A portion (0.26 g) of the reaction products (2.6 g) was eluted from a column (5 × 100 cm) of silica gel with solvent *A*, and the fractionation was monitored by t.l.c.. Four major fractions were obtained: *1* (600 mg) contained **2** and a small proportion of **3**, *2* (700 mg) contained equal amounts of **3** and **2**, *3* (500 mg) contained mainly **3** but also **2** and **4**, and *4* (600 mg) contained mainly **4** but some **3**. Fractions *1*, *3*, and *4* yielded solid residues.

Two crystallisations of fraction *1* from ether–light petroleum (b.p. 30–50°) gave vinyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside[3] (**3**; 350 mg, 16.4%), m.p. 105°, $[\alpha]_D^{22}$ +135° (*c* 1, chloroform); lit.[3] m.p. 106–107°, $[\alpha]_D^{20}$ +135.4° (chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 2.0–2.15 (m, 12 H, 4 OAc), 4.63 [dd, 1 H, $J_{7,8'}$ 14 Hz, H-8′ (*trans*)], 4.3 (dd, 1 H, $J_{8,8'}$ 2 Hz, H-8 (*cis*)],6.33 (dd, 1 H, $J_{7,8}$ 6 Hz, H-7), 3.9–4.2 (m, 3 H, H-5,6,6′), 5.08, 5.52 (2 dd, 2 H, H-3,4), 4.9 (dd, 1 H, $J_{2,3} = J_{3,4} = J_{4,5}$ = 9.8 Hz, H-2), and 5.28 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1).

Zemplén deacetylation of **3** (200 mg) gave vinyl α-D-glucopyranoside[3] (**7**; 102 mg, 93%), m p. 118–121° (from ethanol–ethyl acetate–ether); lit.[3] m.p. 118–120°.

Fraction *3* was crystallised from pyridine and water at low temperature, to give 1-ethoxybut-3-enyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**2**) as long, silky needles. Recrystallisation from ether–light petroleum (b.p. 30–50°) yielded material (280 mg, 8.6%) having m.p. 64°, $[\alpha]_{578}^{20}$ −29.5°, $[\alpha]_D^{20}$ −28.5° (*c* 1, chloroform).

Anal. Calc. for $C_{20}H_{30}O_7$: C, 53.80; H, 6.77. Found: C, 53.87; H, 6.82.

Recrystallisation of fraction *4* (600 mg) from ether–light petroleum (b.p. 30–50°) yielded 1-ethoxyethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**4**; 420 mg, 17.4%), m.p. 82°, $[\alpha]_{578}^{20}$ −47°, $[\alpha]_D^{20}$ −45° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 1.7 (t, 3 H, CH_3CH_2), 1.3 (d, 3 H, CH_3CH), 2.0–2.1 (m, 12 H, 4 OAc), 3.3–3.9 (m, 2 H, CH_3CH_2), 4.1–4.2 (m, 3 H, H-5,6,6′), and 4.7–5.4 (m, 5 H, H-1,2,3,4 and CH_3CH).

Anal. Calc. for $C_{18}H_{28}O_{11}$: C, 51.42; H, 6.71. Found. C, 51.65, H, 6.87.

1-Ethoxybut-3-enyl β-D-glucopyranoside (**6**). — Zemplén deacetylation of **2** (223 mg) and crystallisation of the product from ethanol–ether yielded **6** (130 mg, 94%), m.p. 179°, $[\alpha]_{578}^{20}$ −53°, $[\alpha]_D^{20}$ −51° (*c* 1, pyridine). ^{1}H-N.m.r. data (pyridine-d_5): δ 1.19 (t, 3 H, H-12), 2.73, 2.84 (2 m, 2 H, $J_{8,9}$ 7 Hz, H-8), 3.74, 4.32 (2 m, 2 H, $J_{11,12}$ 7 Hz, H-11), 3.97 (ddd, 1 H, $J_{5,6}$ 2 Hz, H-5), 4.02 (dd, 1 H, $J_{3,4}$ 9 Hz, H-3), 4.06 (dd, 1 H, $J_{2,3}$ 9 Hz, H-2), 4.30 (dd, 1 H, $J_{4,5}$ 9 Hz, H-4), 4.32 (dd, 1 H, $J_{5,6'}$ 6.5 Hz, H-6′), 4.52 (dd, 1 H, $J_{6,6'}$ 11.5 Hz, H-6), 5.04 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 5.09

(m, 1 H, $J_{7,8}$ 6 Hz and typical other couplings, H-7), 5.10 (m, 1 H, $J_{10,10'}$ 2 Hz, H-10), 5.20 [dd, 1 H, $J_{9,10'}$ 17 Hz (*trans*), H-10'], and 6.15 [m, 1 H, $J_{9,10}$ 10 Hz (*cis*), H-9].

Anal. Calc. for $C_{12}H_{22}O_7$: C, 51.78; H, 7.97. Found: C, 52.09, H, 8.33.

1-Ethoxyethyl β-D-glucopyranoside (**8**). — Zemplén deacetylation of **4** (210 mg) and recrystallisation of the product from ethanol gave **8** (120 mg, 95%), m.p 180°, $[\alpha]_{578}^{20}$ −45°, $[\alpha]_D^{20}$ −44° (*c* 0.5, water). ^{1}H-N.m.r. data (pyridine-d_5). δ 1.18 (t, 3 H, H-10), 1.59 (d, 3 H, H-8), 3.69, 4.21 (2 m, 2 H, $J_{9,10}$ 7 Hz, H-9), 3.96 (ddd, 1 H, $J_{5,6}$ 2 Hz, H-5), 4.03 (dd, 1 H, $J_{2,3}$ 9 Hz, H-2), 4.18 (dd, 1 H, H-3), 4.29 (dd, 1 H, $J_{4,5}$ 9 Hz, H-4), 4.31 (dd, 1 H, $J_{5,6}$ 6.5 Hz, H-6'), 4.51 (dd, 1 H, $J_{6,6'}$ 11.5 Hz, H-6), 5.00 (d, 1 H, $J_{1,2}$ 5 Hz, H-1), and 5.21 (q, 1 H, $J_{7,8}$ 5.5 Hz, H-7).

Anal. Calc. for $C_{10}H_{20}O_7$: C, 47.61; H, 7.99. Found: C, 47.43; H, 7.85

1-Ethoxyethyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (**5**) — A mixture of **1** (22.5 g) and acetaldehyde diethyl acetal (100 ml) containing acetic acid (0.1 ml) was boiled under reflux for 16 h; then, during 7 h, ~50 ml of the solvent together with the liberated ethanol were slowly distilled off and continuously replaced by fresh acetaldehyde diethyl acetal*†. Finally, the reaction mixture was concentrated to dryness under diminished pressure, and the residue was crystallised from methanol and water, to give **5**. Recrystallisation from methanol–water yielded material (16 g, 59%) having m.p. 80–89°, $[\alpha]_{578}^{20}$ +127°, $[\alpha]_D^{20}$ +122° (*c* 1, chloroform). ^{1}H-N.m.r data ($CDCl_3$): δ 1.15 (t, 3 H, H-10), 1.33 (d, 3 H, H-8), 2.0–2.1 (m, 12 H, 4 OAc), 3.2–3.9 (m, 2 H, $J_{9,10}$ 7.5 Hz, H-9), 4.0–4.3 (m, 3 H, H-5,6,6'), 4.8 (q, 1 H, $J_{7,8}$ 5.5 Hz, H-7), 4.85 (dd, 1 H, $J_{2,3}$ 10.8 Hz, H-2), 5.0 (dd, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 5.3 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), and 5.5 (dd, 1 H, $J_{3,4}$ 10 Hz, H-3).

Anal. Calc. for $C_{18}H_{28}O_{11}$: C, 51.42, H, 6.71. Found: C, 51.57; H, 6.91.

1-Ethoxyethyl α-D-glucopyranoside (**9**). — Zemplén deacetylation of **5** (110 mg) and crystallisation of the product from methanol–ether gave **9** (63 mg, 95%), m.p. 126°, $[\alpha]_{578}^{20}$ +128.5°, $[\alpha]_D^{20}$ +123° (*c* 1, water).

Anal. Calc. for $C_{10}H_{20}O_7$: C, 47.61; H, 7.99. Found: C, 47.48; H, 7.91.

1-Ethoxyethyl β-D-glucopyranoside (**8**) *by transacetalation*. — A mixture of **1** (3.5 g) and acetaldehyde diethyl acetal (60 ml) containing acetic acid (50 μl) was kept at 25°. The reaction was monitored by t.l.c. (solvent *A*). After 30 h, the reaction mixture consisted of nearly equal amounts of **1** and **4** with a trace of **5** (Table I). Amberlite IRA-400 (HO^-) resin (200 ml), prewashed with ethanol, was then added together with ethanol (100 ml), and the mixture was stirred for 1 h. Most of the organic solvent was evaporated *in vacuo* (bath 30–40°), and the residue plus resin was stirred with water (200 ml) and ethanol (100 ml), applied to a column (50 × 3 cm) of Amberlite IRA-400 (HO^-) resin (500 ml), and eluted with water until no more **8**

*If t.l.c. (solvent *A*) did not show predominant formation of **5** (~70% of total) at this stage, the boiling under reflux and evaporation with replacement of solvent were repeated

†With small quantities of substrate (< 1 g), the reaction mixture was evaporated almost to dryness and the refluxing repeated with fresh ethyl acetal and acetic acid (1000:1)

could be detected by t.l.c. (solvent *B*). Lyophilisation then yielded **8** (930 mg) contaminated with a trace of **9**. Recrystallisation from ethanol yielded **8** (789 mg, 32%).

ACKNOWLEDGMENTS

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