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o-NITROPHENYL 6-DEOXY-3-*O*-(6-DEOXY-β-D-*xylo*-HEX-5-ENOPYRANOSYL)-β-D-*xylo*-HEX-5-ENOPYRANOSIDE, THE MAJOR PRODUCT OF β-D-GLUCOSIDASE ACTION ON *o*-NITROPHENYL 6-DEOXY-β-D-*xylo*-HEX-5-ENOPYRANOSIDE*

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ABSTRACT

Incubation of *o*-nitrophenyl 6-deoxy-β-D-*xylo*-hex-5-enopyranoside (**1**) with emulsin β-D-glucosidase gave, instead of the expected 6-deoxy-D-*xylo*-hexos-5-ulose (**3**), *o*-nitrophenyl 6-deoxy-3-*O*-(6-deoxy-β-D-*xylo*-hex-5-enopyranosyl)-β-D-*xylo*-hex-5-enopyranoside (**2**) in high yield (~90% under optimal conditions) The structure of **2** was established from spectroscopic data and by correlation with compounds synthesised definitively The specificity of the transfer reaction is discussed as an argument for an acceptor or aglycon binding-site

INTRODUCTION

The enzymic cleavage of glycosides by glycosidases yields the free sugar if the glycosyl moiety is transferred to water, or a new glycoside if it is transferred to a suitable acceptor Good acceptors are polyhydric alcohols, especially glycerol, which can compete with water even at low concentrations Such acceptors as water, glycerol, or other monosaccharides play a passive role However, in a transfer reaction catalysed by β-D-glucosidase[1], D-glucal played an active role as acceptor, since there was selective formation of a glycosidic link to HO-3 (allylic position) The transfer reaction described herein is similarly specific

RESULTS AND DISCUSSION

o-Nitrophenyl 6-deoxy-β-D-*xylo*-hex-5-enopyranoside (**1**) was prepared by reaction of 2,3,4-tri-*O*-acetyl-6-bromo-6-deoxy-α-D-glucopyranosyl bromide with *o*-nitrophenol and treatment of the product with silver fluoride When **1** was incubated with emulsin β-D-glucosidase in phosphate buffer at pH 6 8, *o*-nitrophenol was

*Uncommon Results of Glycosidase Action Part V For Part IV, see J Lehmann and B Zieger, *Carbohydr Res*, 58 (1977) 73–78

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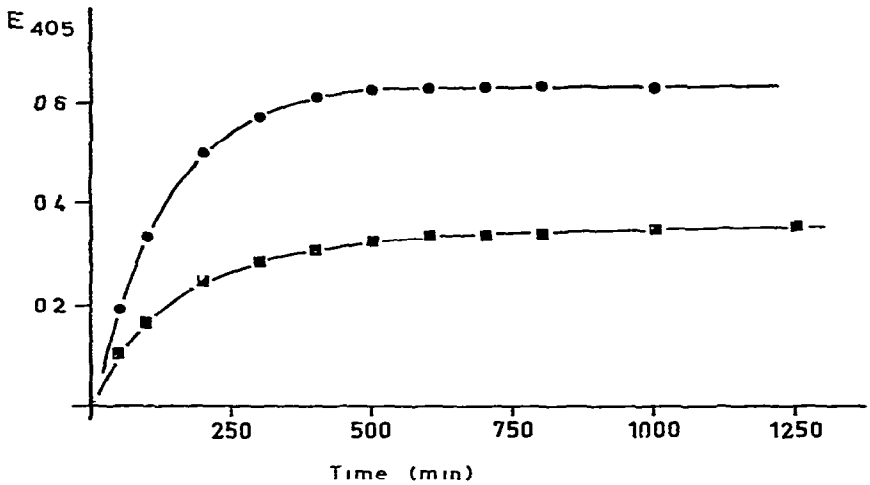
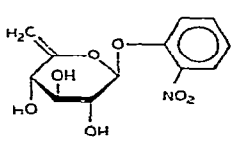


Fig 1. Liberation of *o*-nitrophenol from equimolar (0 05mM) solutions of *o*-nitrophenyl β-D-glucopyranoside (—●—) and *o*-nitrophenyl 6-deoxy-β-D-*xylo*-hex-5-enopyranoside (**1**, —■—) by β-D-glucosidase in 0 1M sodium phosphate buffer (pH 6 8) at 27° Enzyme concentration was 5 μg/ml for the saturated, and 50 μg/ml for the unsaturated substrate

liberated at an initial rate of ~10% of that for *o*-nitrophenyl β-D-glucoside, but only 50–60% of the expected *o*-nitrophenol was formed (Fig 1) T l c showed that a new compound (**2**) was formed which was still unsaturated and contained the aromatic residue Compound **2**, for which yields of ~90% were obtained when the substrate concentration was 0 2M, was isolated crystalline after column chromatography Hydrolysis of **2** with aqueous mineral acid gave *o*-nitrophenol and 6-deoxy-D-*xylo*-hexos-5-ulose (**3**) The complex n m r spectrum of **2**, which showed one



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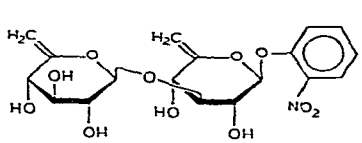

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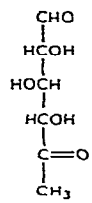

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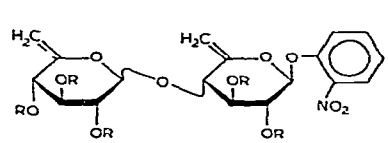
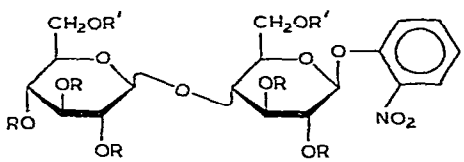
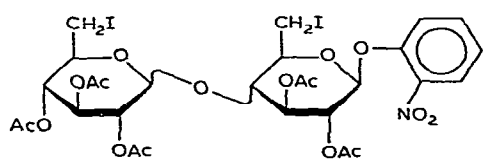


4 R = H
11 R = Ac



5 R = R′ = H
6 R = R′ = Me_3Si
7 R = Me_3Si, R′ = Ts
8 R = H R′ = Ts
9 R = Ac, R = Ts



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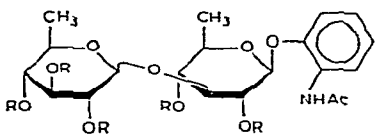
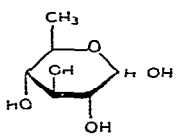
aromatic residue per two unsaturated glycosyl moieties, was closely similar to that of the starting material The respective i r spectra also were very similar Although the position of the glycosidic link could not be determined, the foregoing data are consistent with the structure of an *o*-nitrophenyl 6-deoxy-*O*-(6-deoxy-*β*-D-*xylo*-hex-5-enopyranosyl)-*β*-D-*xylo*-hex-5-enopyranoside

o-Nitrophenyl 6-deoxy-4-*O*-(6-deoxy-*β*-D-*xylo*-hex-5-enopyranosyl)-*β*-D-*xylo*-hex-5-enopyranoside (**4**) was prepared from *o*-nitrophenyl *β*-cellobioside (**5**) by the sequence **5**→**6**→**7**→**8**→**9**→**10**→**11**→**4** (see Experimental) The mobilities of **4** and **2** in t l c were similar but not identical, hence **2** is not (**1**→**4**)-linked

Catalytic hydrogenation with tritium gas of a solution of **2** in acetic anhydride followed by the addition of pyridine gave a labelled compound **12** contaminated with minor products due to non-stereospecific hydrogenation Deacetylation of **12** and hydrolysis of the product (**13**) yielded mainly labelled 6-deoxy-D-glucose (D-quinovose, **15**), which was identified by co-crystallisation with an authentic sample Methylation of **13** and methanolysis of the product yielded four major labelled compounds (**16**–**19**) that were separable by t l c The mobilities of **16** and **17** could not be changed by benzylation Treatment of either **16** or **17** with methanolic hydrogen chloride gave a mixture of **16** and **17** Compound **16** had chromatographic properties identical with those of methyl 6-deoxy-2,3,4-tri-*O*-methyl-*α*-D-glucopyranoside[3] Equilibration of the latter compound in methanolic hydrogen chloride gave a mixture of products with chromatographic properties identical to those of the mixture of **16** and **17**, thus proving the latter compounds to be methyl 6-deoxy-2,3,4-tri-*O*-methyl-*α*- and *β*-D-glucopyranoside, and indicating that the non-glycosidic terminus of **2** is a 6-deoxy-D-*xylo*-hex-5-enopyranosyl residue



12 R = Ac
13 R = H
14 R = Me



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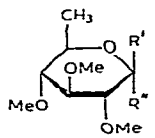
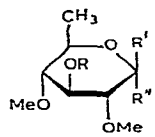


16 R′ = H R″ = OMe
17 R = OMe R = H

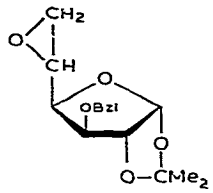


18 R = R = H R″ = OMe
19 R = R = H R′ = OMe
20 R = Bzl R = H R″ = OMe
21 R = Bzl R = OMe R″ = H

Compounds **18** and **19** were also anomeric methyl glycosides which could be benzylated to give **20** and **21**, respectively, that were identical (by t l c) with methyl 3-*O*-benzyl-6-deoxy-2,4-di-*O*-methyl-*α*- and *β*-D-glucopyranoside The identity of **21** was proved by co-crystallisation with an authentic sample

The foregoing data, coupled with the fact that **13** could be hydrolysed with *β*-D-glucosidase under conditions used for the synthesis of **2** from **1**, indicate **2** to be *o*-nitrophenyl 6-deoxy-3-*O*-(6-deoxy-*β*-D-*xylo*-hex-5-enopyranosyl)-*β*-D-*xylo*-hex-5-enopyranoside

Authentic **20** and **21** were synthesised from 5,6-anhydro-3-*O*-benzyl-1,2-*O*-isopropylidene-*α*-D-glucofuranose[4] (**22**) *via* **23**, **24**, and **25**



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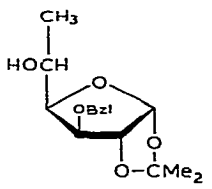

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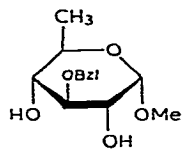

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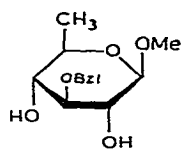

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The formation of **2** is another example (*cf* Ref 1) of unique acceptor specificity in the action of a β-D-glycosidase As in the enzymic dimerisation of D-glucal, it is likely that the *threo* relationship of two hydroxyl groups vicinal to a C=C bond in the acceptor molecule is responsible for its exceptionally high affinity for the aglycon binding-site in a mode which finally leads to a (1→3)-link exclusively

EXPERIMENTAL

General — T l c was performed on silica gel F_{254} (Merck) with *A*, 4 1 benzene–methanol, *B*, 4 1 ether–light petroleum (b p 60–70°), or *C*, 63 7 2 ethyl acetate–methanol–water Detection of unlabelled compounds was effected by charring with conc sulphuric acid at 120° Radioactive materials were detected autoradiographically (Kodak Medical Film, blue sensitive, single coated) or with a Packard 7200 Radiochromatogram scanner. Melting points are uncorrected I r and n m r data were obtained with Perkin–Elmer Infracord 137 and Varian A-60, HA-100, or Bruker 90 spectrometers Optical rotations were measured with a Perkin–Elmer 141 Polarimeter β-D-Glucosidase (crystal suspension, 5 mg/ml in 3 2M ammonium sulphate) with a specific activity of 40 U/mg (salicin) was purchased from Boehringer (Mannheim) and used without further purification

o-Nitrophenyl 2,3,4-tri-O-acetyl-6-bromo-6-deoxy-β-D-glucopyranoside — A solution of 2,3,4-tri-*O*-acetyl-6-bromo-6-deoxy-α-D-glucopyranosyl bromide[2] (acetodibromoglucose, 43 g) in acetone (300 ml) was added to a solution of *o*-nitrophenol (20 g) and sodium hydroxide (85 g) in water (185 ml) The mixture was stored at 4° for 14 h to give the title product (34 8 g, 71%), m p. 192° (from methanol), $[\alpha]_{578}^{23}$ +31 5° (*c* 1, chloroform), ν_{max}^{KBr} 1530 (NO_2) and 1760 cm^{-1} (C=O)

Anal Calc for $C_{18}H_{20}BrNO_{10}$ C, 44.10, H, 4 11, Br, 16 30, N, 2 86 Found C, 43 98. H, 4 08, Br, 16 20, N, 2 92

o-Nitrophenyl 2,3,4-tri-O-acetyl-6-deoxy-β-D-xylo-hex-5-enopyranoside. — A solution of the foregoing compound (38 g) in pyridine (490 ml) was shaken with silver fluoride (technical grade, 35 g) for 20 min The reaction was monitored by t l c (solvent *B*) Dry acetone (1 litre), dry, powdered calcium chloride (20 g), and charcoal (~10 g) were added, the mixture was shaken for 30 min and then filtered, and the solution was concentrated under diminished pressure A solution of the residue in chloroform (1 litre) was washed with water (3 × 100 ml), dried ($CaCl_2$), and concentrated *in vacuo* to yield the title compound (23 g, 72 5%), m p 78–79°

(from methanol), $[\alpha]_{578}^{22}$ +10° (*c* 1, chloroform), ν_{max}^{KBr} 1610 (NO_2), 1670 (C=C), and 1750 cm^{-1} (C=O)

Anal Calc for $C_{18}H_{19}NO_{10}$ C, 52 82, H, 4 68, N, 3 42 Found C, 52 59, H, 4 75, N, 3 45

*o-Nitrophenyl 6-deoxy-β-*D-xylo-*hex-5-enopyranoside* (**1**) — Conventional deacetylation of the foregoing compound (4 1 g) with methanolic sodium methoxide yielded **1** (2 7 g, 94%), m p 106° (from water), $[\alpha]_{578}^{22}$ −123° (*c* 0 5, water), ν_{max}^{KBr} 1520 (NO_2), 1670 (C=C), and 3340 cm^{-1} (OH) N m r data (Me_2SO-d_6) δ 3 29 (q, 1 H, $J_{2,3}$ 7, $J_{3,4}$ 8 5 Hz, H-3), 3 53 (q, 1 H, $J_{1,2}$ 5 5 Hz, H-2), 3 92 (sex, 1 H, $J_{4,6} = J_{4,6'}$ = 2 Hz, H-4), 4 58 (d, 1 H, H-6), 4 63 (d, 1 H, H-6'), 5 31 (d, 1 H, H-1), and 7 1–8 0 (m, 4 H, aromatic)

Anal Calc for $C_{12}H_{13}NO_7$ C, 50 89, H, 4 63, N, 4 95 Found C, 50 90, H, 4 68, N, 4 75

*o-Nitrophenyl 6-deoxy-3-O-(6-deoxy-β-*D-xylo-*hex-5-enopyranosyl)-β-*D-xylo-*hex-5-enopyranoside* (**2**) — A solution of **1** (1 5 g) in 0 2M sodium phosphate buffer (pH 6 8, 100 ml) was incubated with *β*-D-glucosidase (3 ml) at 35° for 12 h The reaction was monitored by t l c (solvent *C*) The mixture was concentrated to 50 ml under reduced pressure (bath, 35°), diluted with water (100 ml), and freeze-dried The residue was extracted with acetone (2 × 100, 5 × 50 ml), and the extract was concentrated *in vacuo* The crude product was decolorised with charcoal and eluted from a column (70 × 4 cm) of silica gel with solvent *C* to yield **2** (0 6 g), m p 148° (from water), $[\alpha]_{578}^{22}$ −65° (*c* 1, chloroform), ν_{max}^{KBr} 1525 (NO_2), 1675 (C=C), and 3450 cm^{-1} (OH) N m r data (Me_2SO-d_6) δ 3 05–3 95 (m, 5 H) 4 18 (d, 1 H, *J* 8 Hz), 4 52 (d, 2 H, *J* 2 Hz, H-6), 4 60 (d, 2 H, *J* 2 Hz, H-6'), 4 72 (d, 1 H, $J_{1,2}$ 5 5 Hz, H-1'), 5 48 (d, 1 H, $J_{1,2}$ 5 5 Hz, H-1), and 7 1–8 0 (m, 4 H, aromatic)

Anal Calc for $C_{18}H_{21}NO_{11}$ C, 50 59. H, 4 95, N, 3 28 Found C, 50 40, H, 4 92, N, 3 34

*o-Nitrophenyl 2,3,6,2',3',4',6'-hepta-*O*-acetyl-β-cellobioside* — A solution of sodium hydroxide (4 5 g) and *o*-nitrophenol (11 25 g) in water (110 ml) was added to a solution of acetobromocellobiose[5] (33 g) in acetone (150 ml) The mixture was stirred at room temperature for 4 h and then poured into ice–water followed by extraction with chloroform (300 and 100 ml) The combined extracts were washed with water (2 × 100 ml), dried ($CaCl_2$), and concentrated under reduced pressure to yield the title compound (21 5 g, 60%), m p 204° (from methanol), $[\alpha]_{578}^{22}$ −5 5° (*c* 1, chloroform), ν_{max}^{KBr} 1750 (C=O) and 1550 cm^{-1} (NO_2)

Anal Calc for $C_{32}H_{39}NO_{20}$ C, 50 73, H, 5 19, N, 1 85 Found C, 50 50, H, 5 33, N, 2 07.

o-Nitrophenyl β-cellobioside (**5**) — The foregoing compound (5 9 g) was stirred with conc methanolic ammonia for 20 h The reaction was monitored by t l c (solvent *A*) After concentration under reduced pressure, methanol (10 ml) was added to the residue. The resulting crystalline material was triturated with methanol (10 ml) to yield **5** (2 2 g, 61 %), m p. 220°, $[\alpha]_{578}^{22}$ −87 5° (c 1, H_2O), ν_{max}^{KBr} 1530 (NO_2) and 3450 cm^{-1} (OH)

Anal. Calc for $C_{18}H_{25}NO_{13}$ C, 46 64, H, 5 44, N, 3 02. Found C, 46 47, H, 5 59, N, 3 29

o-*Nitrophenyl hepta*-O-*trimethylsilyl-β-cellobioside* (**6**) — To a solution of **5** (10 g) in pyridine (160 ml) were added chlorotrimethylsilane (16 5 g) and hexamethyldisilazane (27 g) with cooling After 2 h, the mixture was concentrated to dryness under reduced pressure, and a solution of the residue in tetrachloromethane (275 ml) was filtered through Celite, washed with water (2 × 200 ml), dried (Na_2SO_4), and concentrated *in vacuo* to give **6** (21 g, ~100%), m p 128° [from light petroleum (b.p 30–50°)], $[\alpha]_{578}^{22}$ −60 5° (*c* 1, chloroform), ν_{max}^{KBr} 1250 (SiMe) and 1530 cm^{-1} (NO_2).

Anal Calc for $C_{39}H_{81}NO_{13}Si_7$ C, 48 36, H, 8 43, N, 1.45 Found C, 48 55, H, 8 28; N, 1 68

o-*Nitrophenyl 6,6'-di*-O-*tosyl-β-cellobioside* (**8**) — To crude **6** were added, in sequence, pyridine (200 ml), water (3 6 ml), and glacial acetic acid (2 4 g) When the reaction was complete (t l c , solvent *B*), the mixture was poured into ice–water (1 5 litres), and extracted with chloroform (400 and 200 ml) The combined extracts were washed with water (200 ml), dried ($CaCl_2$), and concentrated *in vacuo*, yielding a pale-yellow syrup, to a solution of which (20 g) in dry pyridine (200 ml) was added tosyl chloride (23 g) in two portions The reaction was carried out at 4° and monitored by t l c (solvent *B*) When the reaction was complete, the mixture was poured into ice–water (2 litres) and stirred, the product **7** was collected and dissolved in methanol (400 ml), and water was added to slight turbidity The mixture was boiled under reflux for 4 h and then concentrated *in vacuo*, and the residue was crystallised from ethanol to yield **8** (7 g), m p 138°, $[\alpha]_{578}^{22}$ −68° (*c* 1, acetone), ν_{max}^{KBr} 1530 (NO_2) and 3450 cm^{-1} (OH)

Anal Calc for $C_{32}H_{37}NO_{17}S_2$ C, 49 79, H, 4 83, N, 1 81, S, 8 31. Found C, 49.51, H, 4 85, N, 1 86, S, 8 06

o-*Nitrophenyl 2,3 2',3',4'-penta*-O-*acetyl-6,6'-di*-O-*tosyl-β-cellobioside* (**9**) — Conventional treatment of **8** (9 g) with pyridine (22 ml) and acetic anhydride (20 ml) gave **9** (8 7 g, 76%), m p 122° (from methanol), $[\alpha]_{578}^{22}$ +2° (*c* 1, chloroform), ν_{max}^{KBr} 1520 (NO_2) and 1760 cm^{-1} (C=O)

Anal. Calc. for $C_{42}H_{47}NO_{22}S_2$ C, 51 38, H, 4 82, N, 1 43, S, 6 53. Found C, 50 65, H, 5 08, N, 1 75, S, 6 78

o-*Nitrophenyl 2,3,2',3',4'-penta*-O-*acetyl-6,6'-dideoxy-6,6'-di-iodo-β-cellobioside* (**10**) — A solution of **9** (2 2 g) and sodium iodide (6 g) in acetic anhydride (50 ml) was boiled under reflux for 25 min, the reaction was monitored by t l c (solvent *B*) The cooled mixture was filtered, and concentrated *in vacuo*, and a solution of the resulting syrup in chloroform (300 ml) was washed with 0 1% aqueous sodium bisulphite and water (2 × 200 ml), dried ($CaSO_4$), and concentrated under reduced pressure Crystallisation of the residue from methanol yielded **10** (6 g), m p 210°, $[\alpha]_{578}^{22}$ +1° (*c* 1, chloroform), ν_{max}^{KBr} 1530 (NO_2) and 1770 cm^{-1} (C=O)

Anal Calc for $C_{28}H_{33}I_2NO_{16}$· C, 37 64, H, 3 72, N, 1 57 Found C, 37 83, H, 3 89, N, 1 81

o-Nitrophenyl 2,3-di-O-acetyl-6-deoxy-4-O-(2,3,4-tri-O-acetyl-6-deoxy-β-D-xylo-hex-5-enopyranosyl)-β-D-xylo-hex-5-enopyranoside (**11**) — A solution of **10** (7 g) in pyridine (40 ml) was shaken with silver fluoride (7 g, technical grade) for 20 min; the reaction was monitored by t l c (solvent *B*) The liquid phase was then decanted dropwise into vigorously stirred, dry ether (1 5 litres) Charcoal (~5 g) was added, and stirring was continued for 10 min The mixture was filtered, and concentrated *in vacuo*, and toluene was repeatedly evaporated from the residue to remove residual pyridine Crystallisation of the resulting syrup from methanol yielded **11** (3 g, 60%), m p 125°, $[\alpha]_{578}^{22}$ −61.5° (*c* 1, chloroform), ν_{max}^{KBr} 1520 (NO_2), 1680 (C=C), and 1770 cm^{-1} (C=O) N m r data ($CDCl_3$) δ 2 06 (s, 3 H, Ac), 2 08 (s, 3 H, Ac), 2 13 (s, 6 H, 2 Ac), 2 16 (s, 3 H, Ac), 4 5–5 3 (m, 10 H), 5 65 (d, 2 H, 2 H-1), and 7 1–8 0 (m, 4 H, aromatic)

Anal Calc for $C_{28}H_{31}NO_{16}$ C, 52 75, H, 4 90, N, 2 20 Found C, 52 67, H, 5 04, N, 2 42

Deacetylation of **11** (20 mg) with either 0 01M methanolic sodium methoxide (1 ml) or methanolic ammonia (5%, 5 ml) yielded **4** as a highly unstable syrup which was homogeneous (t l c , solvent *C*), decolorised sodium permanganate, but was not identical with **2**

Methylation analysis of **2** — A solution of **2** (500 mg) in acetic anhydride (5 ml) was hydrogenated over 5% palladium-on-charcoal (500 mg) which previously had been equilibrated with tritium gas (25 mCi, specific activity, 500 mCi/mmol) After 5 h, when hydrogen uptake was complete, the catalyst was removed, pyridine (10 ml) was added, and the mixture was stored at room temperature for 10 h and then concentrated under reduced pressure The resulting syrup **12** (0 7 g) which was nearly homogeneous (t l c , solvent *B*), was deacetylated with 0 01M methanolic sodium methoxide (10 ml, t l c , solvent *A*) After the addition of water (1 drop), carbon dioxide was bubbled through the solution for 30 min which was then filtered through a column (12 × 2 cm) of silica gel and eluted with methanol The radioactive eluate was concentrated *in vacuo*, yielding syrupy **13** (460 mg) A small amount of **13** (2 × 10^6 d p m) gave D-quinovose (**15**) as the only sugar component on hydrolysis with β-D-glucosidase [10 μl, 5 mg/ml, in 200 μl of phosphate buffer (pH 6 8) at 37°] and with 0 05M hydrochloric acid (room temperature) The radioactively labelled D-quinovose was identified by co-crystallisation with an authentic sample

A mixture of **13** (400 mg), *N,N*-dimethylformamide (10 ml), methyl iodide (5 ml), and silver oxide (3 g) was stirred vigorously for 16 h Ether (50 ml) was added, and the inorganic material was collected and washed with ether The combined ether solutions were concentrated under reduced pressure, yielding **14** as a pale-yellowish oil (265 mg) which was homogeneous in t l c (solvent *B*)

A mixture of **14** (0 1 g) and 2% methanolic hydrogen chloride (10 ml) was boiled under reflux for 6 h The products (**16–19**) were isolated by p l c (solvent *B*. R_F 0 35, 0 42, 0 14, and 0 23) When either **16** or **17** was equilibrated with methanolic hydrogen chloride, a mixture of **16** and **17** resulted (t l c , solvents *A* and *B*) Radiolabelled **16** co-chromatographed [solvents *A* and *B* and ethyl acetate–propan-2-ol–

water (25·14 7)] with methyl 6-deoxy-2,3,4-tri-*O*-methyl-α-D-glucopyranoside[3]. Similar equilibration of either **18** or **19** yielded a mixture of **18** and **19** (t l.c., solvents *A* and *B*).

A mixture of **18** or **19** (5 mg), *N*,*N*-dimethylformamide (2 ml), benzyl bromide (1 ml), and silver oxide (1 g) was stirred vigorously for 8 h at room temperature. Methanol (20 ml) was added, and the inorganic material was collected and washed with methanol (10 ml) and then with chloroform (20 ml) The combined filtrates and washings were concentrated to dryness under reduced pressure. A solution of the residue in chloroform (50 ml) was washed with water (2 × 20 ml), dried ($CaSO_4$), and concentrated *in vacuo*, to yield radioactive **20** and **21**, which were identified by cc chromatography with authentic compounds and in one case (**21**) by co-crystallisation with the corresponding, unlabelled, authentic sample

Methyl 3-O-benzyl-6-deoxy-2,4-di-O-methyl-α- and -β-D-glucopyranoside (**20** and **21**) — A solution of 5,6-anhydro-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose[4] (**22**, 5 g) in dry ether (60 ml) was added dropwise to a suspension of lithium aluminium hydride (1 g) in dry ether (100 ml) The vigorously stirred mixture was boiled under reflux for 10 min More reductant (1 g) was added and boiling was continued for 1 h After treatment with ethyl acetate (5 ml) and water (100 ml), the mixture was extracted with chloroform (4 × 50 ml) The combined and dried ($CaSO_4$) extracts were concentrated *in vacuo* to yield **23** as a colourless syrup (4 3 g), a solution of which in 10% methanolic hydrogen chloride was boiled under reflux for 5 h The mixture was neutralised with 20% ammonia in methanol and concentrated under reduced pressure, and the residue was extracted with acetone (2 × 100 ml) The extract was concentrated and a solution of the residue in chloroform (100 ml) was washed with water (2 × 50 ml), dried ($CaSO_4$), and concentrated to give an oil (3 g) that consisted of two components **24** and **25** (t l c. solvent *B*)

A solution of the mixture of **24** and **25** (1 6 g) in *N*,*N*-dimethylformamide (160 ml) was treated with sodium hydride (2 8 g) and methyl iodide (12 ml) Work-up[6] gave a syrupy mixture (0 95 g) of two components (t l c , solvent *B*) Elution of the mixture from a column (80 × 4 cm) of silica gel with ether–light petroleum (b p 60–70°) gave, firstly, **21** (95 mg), m p 57° [from light petroleum (b p 30–50°)], $[\alpha]_{578}^{27}$ −6° (*c* 0 5, chloroform) N.m r data ($CDCl_3$) δ 4.17 (d, 1 H, $J_{1,2}$ 7 5 Hz, H-1), 4 83 (s, 2 H, PhCH_2), and 7 36 (s, 5 H, Ph)

Anal Calc for $C_{16}H_{24}O_5$· C, 64 85, H, 8 16 Found C, 64 86, H, 8 30

Eluted second was **20**, which was distilled to give a colourless oil (220 mg), b p 130° (bath)/0 1 mmHg, $[\alpha]_{578}^{27}$ +95° (*c* 1 2, chloroform). N m r data ($CDCl_3$). δ 4 78 (d, 1 H, $J_{1,2}$ 3 5 Hz, H-1), 4 85 (s, 2 H, PhCH_2), and 7 36 (s, 5 H, Ph)

Anal Found C, 64 96, H, 7 97

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