Synthesis of β-D-Mannopyranosides

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β-D-Mannopyranosides have been synthesized *via* the corresponding 3,4,6-tri-*O*-benzyl-β-D-glucosides by oxidation to the β-D-*arabino*-hexopyranosiduloses, stereoselective hydrogenation and deblocking.

The Koenigs-Knorr reaction, starting from 2,3,4,6-tetra-*O*-acetyl-α-D-mannosyl bromide yields α-D-mannopyranosides. β-D-Mannopyranosides are less readily available but Gorin and Perlin [1] prepared them in a Koenigs-Knorr reaction, using 4,6-di-*O*-acetyl-2,3-di-*O*-carbonate-α-D-mannosyl bromide. Theander [2] demonstrated that hydrogenation of methyl β-D-*arabino*-hexopyranosidulose over a platinum catalyst yielded a mixture of β-D-glucoside and β-D-mannoside in the proportion 6:94. Synthesis of a β-D-glucopyranoside, suitably protected in all positions but C-2 in the D-glucose moiety, oxidation and hydrogenation should thus offer a route to β-D-mannopyranosides. The synthesis of β-D-mannopyranosides by this method is described in the present communication.

2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-α-D-glucosyl bromide (V), which has a participating group at C-2 and consequently should yield β-D-glucopyranosides in a Koenigs-Knorr reaction, was prepared as follows. Methyl 2-*O*-tosyl-α-D-glucopyranoside [3,4] was benzylated [5] to form I and the tosyl group was removed by reduction yielding II. Acid hydrolysis to III followed by benzoylation to IV and treatment with hydrogen bromide in chloroform gave V.

Treatment of V with methanol and silver oxide yielded VI, which was debenzoylated to methyl 3,4,6-tri-*O*-benzyl-β-D-glucoside (VII). Oxidation of VII with dimethyl sulphoxide and acetic anhydride [6] yielded the methyl β-D-*arabino*-hexopyranosidulose (VIII), which on catalytic hydrogenation first over platinum, to reduce the carbonyl group, and then over palladium, to remove the *O*-benzyl groups, yielded methyl β-D-mannopyranoside (IX) characterised as the crystalline tetraacetate. The overall yield from IV, which is a stable substance that can be kept for long time, was 29 %.

The disaccharide 6-*O*-β-D-mannopyranosyl-D-galactose (XIV) was next prepared, starting from V and 1,2:3,4-di-*O*-isopropylidene-α-D-galactose. The sequence of reactions (V — XIII) was analogous to that described above, and

as a last step XIII was subjected to a mild acid hydrolysis, which removed the isopropylidene groups. Crude XIV, on acid hydrolysis, yielded D-mannose, D-glucose, and D-galactose in the proportions 95:5:100 so that the desired epimer constituted 95 % of the products. The overall yield of crystalline XIV, starting from IV, was 17 %.

A hydrolysate of the disaccharide was found to contain equimolar amounts of D-mannose and D-galactose. Methylation analysis [7] of the disaccharide alditol yielded 2,3,4,6-tetra-O-methyl-D-mannose and 1,2,3,4,5-penta-O-methyl D-galactitol in agreement with the assumed structure. The permethylated disaccharide alditol gave the expected mass spectrum [8] and the disaccharide alditol acetate was readily oxidized with chromium trioxide in acetic acid,[9] confirming the β-linkage. NMR spectra, determined for most of the compounds, were consistent with the assigned structures.

Even if the present route to β-D-mannopyranosides is laborious, it compares favourably with the other route [1] leading to these glycosides.

VI-IX: R = CH$_3$

X-XIII: R = 1,2:3,4-di-O-isopropylidene-α-D-galactose
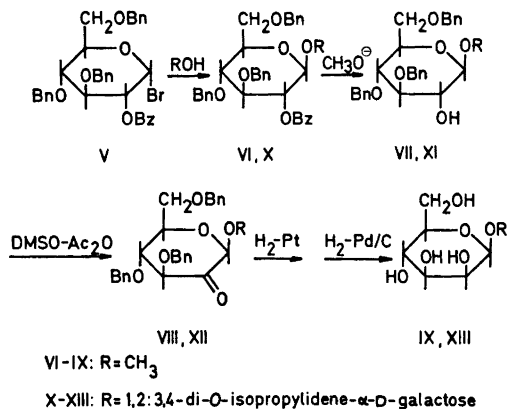
EXPERIMENTAL

General methods. Melting points are corrected. Microanalyses were performed by Dr. A. Bernhardts laboratory, Elbach über Engelskirchen, Germany. Concentrations were performed under reduced pressure, at bath temperatures not exceeding 40°. NMR spectra were recorded on a Varian A 60A instrument in chloroform-d, using TMS as internal standard unless otherwise stated. IR spectra were recorded on a Perkin-Elmer 257 instrument. Optical rotations were determined using a Perkin-Elmer 141 polarimeter and 1 dm microtubes. For GLC a Perkin-Elmer 990 instrument and glass columns (180 × 0.15 cm) containing 3 % ECNSS-M on Gas chrom Q (100 – 120 mesh) at 190° (alditol acetates) or 170° (partially methylated alditol acetates) and 3 % XE-60 on the same support at 190° (permethylated disaccharide alditol) were used. For GLC-MS a Perkin-Elmer 270 instrument and the same columns as above were used.

Analytical and preparative TLC was performed on plates (20 × 20 cm) with a 0.25 mm and 2 mm layer, respectively, of Silica Gel F$_{254}$ (E. Merck, Darmstadt, Germany). Solvent systems A (ethyl acetate:light petroleum, 1:2), B (chloroform-methanol, 95:5), C (ethyl acetate:light petroleum, 1:3), and D (ethyl acetate:light petroleum, 2:3) were used. Compounds were detected by UV-illumination or by spraying with 8 % aqueous sulphuric acid followed by heating to 120°. For column chromatography Silica Gel (< 0.08 mm,

E. Merck, Darmstadt, Germany) was used. Fractionations were monitored polarimetrically and by TLC. For paper chromatography, on Whatman No. 1 paper, the solvent systems E (ethyl acetate:acetic acid:water, 3:1:1) and F (ethyl acetate:pyridine:water, 2:1:2, upper phase) were used. The spots were detected with 3 % p-anisidine hydrochloride in ethanol at 120°.

Methyl 3,4,6-tri-O-benzyl-2-O-tosyl-α-D-glucoside (I). Benzyl bromide (80 ml) was added to a solution of methyl 2-O-tosyl-α-D-glucopyranoside (21.5 g) in dimethyl formamide (150 ml). The reaction mixture was cooled to 0° and silver oxide (80 g) was added portionwise with stirring during 1 h. Stirring was continued for 72 h in the dark at room temperature. After addition of methanol (60 ml) the reaction mixture was stirred for another 24 h. The solids were filtered off and washed with dimethyl formamide (2 × 75 ml) and chloroform (3 × 75 ml). The combined filtrate and washings were extracted with a 1 % aqueous solution of potassium cyanide (1000 ml) which was then extracted with chloroform (3 × 200 ml). The combined organic phases were then washed with water (6 × 500 ml), dried (magnesium sulphate) and evaporated to a syrup. The crude syrup was fractionated on a Silica Gel column (8 × 68 cm, solvent system A) yielding a chromatographically pure syrup (28.8 g, 80 %), $[α]_{578}^{22} = +47°$ (c 1.5, chloroform), $R_F = 0.50$, solvent system A. NMR showed, *inter alia*, $τ$ 2.0 – 3.0, 19 H (aromatic protons), $τ$ 5.13, $J_{1,2}$ 3.5 Hz, 1 H, doublet (anomeric proton), $τ$ 6.68, 3 H, singlet (methoxyl group), $τ$ 7.69, 3 H, singlet (aryl methyl group).

Methyl 3,4,6-tri-O-benzyl-α-D-glucoside (II). A solution of I (28.8 g) and lithium aluminium hydride (17.5 g) in diethyl ether (750 ml) was refluxed overnight. Excess hydride was destroyed by adding ethyl acetate (200 ml), ethanol (40 ml) and 5 % hydrochloric acid (500 ml). The organic layer was separated and the aqueous layer was extracted with ether (200 ml). The combined organic phases were washed with saturated aqueous sodium hydrogen carbonate solution (6 × 300 ml) and water (3 × 300 ml), dried (magnesium sulphate) and concentrated to a syrup, which crystallized spontaneously (20.0 g, 93 %). Crystallization from hexane yielded the pure substance, m.p. 87 – 89°, $[α]_{578}^{23} + 100°$ (c 1.0, chloroform). NMR showed, *inter alia*, $τ$ 2.42 – 2.95, 15 H (aromatic protons), $τ$ 6.58, 3 H, singlet (methoxyl group). (Found: C 72.5; H 7.09. $C_{28}H_{32}O_6$ requires: C 72.4; H 6.96.)

3,4,6-Tri-O-benzyl-α-D-glucose (III). A solution of II (20 g) in 60 % formic acid (1300 ml) was kept at 100° for 12 h and then concentrated to a small volume. The remaining formic acid was codistilled with water (3 × 100 ml) and the residue was dissolved in chloroform (300 ml). The resulting solution was washed with saturated aqueous sodium hydrogen carbonate (3 × 200 ml) and water (150 ml), dried (magnesium sulphate), and concentrated to a syrup. Crystallization from ethanol yielded the pure substance (6.5 g, 34 %), m.p. 85 – 87°, $[α]_{578}^{22} + 71°$ (2 min) → + 65° (24 h) (c 1.1, 0.1 % 2-pyridone in $CHCl_3$). The decrease in optical rotation demonstrates that the substance crystallized as the α-form. This was confirmed by NMR, which showed, *inter alia*, $τ$ 2.50 – 3.00, 15 H (aromatic protons), $τ$ 4.78, $J_{1,2}$ 3 Hz, 1 H, doublet (anomeric proton). (Found: C 71.9; H 6.61. $C_{27}H_{30}O_6$ requires: C 72.0; H 6.71.) A second crop (4.0 g) was obtained by chromatography of the mother liquors on a Silica Gel column (70 × 8 cm), using solvent system B. The total yield of IV was therefore 54 %.

1,2-Di-O-benzoyl-3,4,6-tri-O-benzyl-α-D-glucose (IV). Benzoyl chloride (10.5 ml) was added to a solution of III (8.0 g) in pyridine (60 ml), kept at 0° by external cooling. The mixture was kept at 4° overnight and then at room temperature for 1 h. Water (2.0 ml) was added, and the solution was stirred for 24 h before dilution with chloroform (500 ml). It was then washed with 1.5 M sulphuric acid (3 × 200 ml), saturated aqueous sodium hydrogen carbonate (3 × 200 ml), and water (3 × 200 ml) and dried over magnesium sulphate. On concentration a syrup (11.0 g, 93 %), which crystallized on standing, was obtained. Crystallization from hexane yielded the pure substance, m.p. 83.5 – 85°, $[α]_{578}^{22} + 174°$ (c 1.0, chloroform). (Found: C 74.7; H 5.64. $C_{41}H_{38}O_8$ requires: C 74.8; H 5.81.)

That the α-form was obtained is evident from the high optical rotation and further corroborated by NMR which showed, *inter alia*, $τ$ 1.83 – 3.00, 25 H (aromatic protons), $τ$ 3.28, $J_{1,2}$ 3.5 Hz, 1 H, doublet (anomeric proton).

2-O-Benzoyl-3,4,6-tri-O-benzyl-α-D-glucosyl bromide (V). In a typical experiment IV (1.6 g) was dissolved in hydrogen bromide-saturated chloroform (110 ml). The solution was kept at room temperature for 35 min, concentrated, codistilled with toluene (100 ml) and dissolved in toluene (100 ml). The toluene solution was washed at 0° with saturated aqueous sodium hydrogen carbonate (3 × 100 ml) and water (100 ml), dried (magnesium

sulphate), and concentrated to a syrup. TLC (solvent system C) indicated the presence of a main component ($R_F = 0.69$), assumed to be the glucosyl bromide. That this had the α-configuration was demonstrated by NMR (CCl_4) which showed, *inter alia*, τ 3.25, $J_{1,2}$ 4 Hz, 1 H, doublet (anomeric proton) and also by the optical rotation $[α]_{578}{}^{20} + 128°$ (*c* 1.6, chloroform) of a crude sample. The glucosyl bromide was not very stable and was used in the following step immediately after its preparation without purification.

Methyl 2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-glucoside (VI). A solution of V (from 1.5 g IV) in dry chloroform (20 ml) was added dropwise under stirring to a suspension of silver oxide (3.2 g) in methanol (75 ml). Stirring was continued overnight, after which the mixture was filtered and the resulting solution was concentrated. Fractionation by preparative TLC (3 plates, solvent system A) yielded pure VI, which crystallized from hexane (0.93 g, 72 % calc. on IV), m.p. 93 − 96°, $[α]_{578}{}^{22} + 41°$ (*c* 1.0, chloroform). (Found: C 73.9; H 6.59. $C_{35}H_{36}O_7$ requires: C 73.9; H 6.38.) NMR showed, *inter alia*, τ 1.75 − 3.00, 20 H (aromatic protons), τ 6.55, 3 H, singlet (methoxyl group).

Methyl 3,4,6-tri-O-benzyl-β-D-glucoside (VII). To a solution of VI (0.93 g) in methanol (30 ml) was added M methanolic sodium methoxide (9 ml) and the solution was refluxed for 1 h. The solution was cooled, neutralized (Dowex 50, H^+) and concentrated. The product was purified by preparative TLC (2 plates, solvent system D) and crystallized from hexane, yielding the pure substance (0.51 g, 67 %), m.p. 72 − 75°, $[α]_{578}{}^{22} − 5°$ (*c* 1.1, chloroform). NMR showed, *inter alia*, τ 2.53 − 3.00, 15 H (aromatic protons), τ 6.44, 3 H, singlet (methoxyl group). (Found: C 72.2; H 6.93. $C_{28}H_{32}O_6$ requires: C 72.4; H 6.96.)

Methyl 3,4,6-tri-O-benzyl-β-D-arabino-hexosidulose (VIII). A solution of VII (0.5 g) and acetic anhydride (2.5 ml) in dimethyl sulphoxide (50 ml) was kept under nitrogen for 4 days, after which the reagents were removed by lyophilization. TLC of the product (solvent system D) revealed the presence of a major component ($R_F = 0.28$) accounting for about 80 % of the material and two minor components ($R_F = 0.59$ and 0.75) presumably the 2-O-methylthiomethyl ether and 2-acetate. The major component was isolated by preparative TLC (2 plates) as a chromatographically pure syrup (0.35 g, 70 %), $[α]_{578}{}^{22} − 29°$ (*c* 3.3, chloroform), that did not crystallize.

In the IR spectrum (CCl_4) a strong absorption at 1763 cm^{-1} (C=O) was observed. NMR (CCl_4) showed, *inter alia*, τ 2.39 − 2.89, 15 H (aromatic protons), τ 6.49, 3 H, singlet (methoxyl group).

Methyl β-D-mannopyranoside (IX). A solution of VIII (0.35 g) in ethanol (25 ml) was hydrogenated at room temperature and atmospheric pressure over Adams' catalyst (50 mg). When hydrogen consumption had ceased the catalyst was filtered off, and the solution was concentrated, dissolved in ethanol (25 ml) and further hydrogenated, using 10 % palladium on charcoal (400 mg). Processing yielded a syrup. A hydrolysate of the syrup contained D-glucose and D-mannose in the ratio 5:95, as revealed by analysis of the derived alditol acetates by GLC.[10] Crystallization from isopropanol yielded the glycoside (0.16 g, 84 %) with solvent of crystallization, m.p. 64 − 71°, $[α]_D{}^{22} − 49°$ (*c* 1.0 water). The tetraacetate was prepared and showed $[α]_D{}^{22} − 47°$ (*c* 0.8, chloroform) and melted at 161 − 163°, undepressed on admixture with an authentic sample.

6-O-(2-O-Benzoyl-3,4,6-tri-O-benzyl-β-D-glucosyl)-1,2:3,4-di-O-isopropylidene-α-D-galactose (X). A mixture of 1,2:3,4-di-O-isopropylidene-α-D-galactose[11] (2.0 g), silver oxide (2.0 g) and Drierite (10.0 g) in dry chloroform (50 ml) was stirred in the dark for 1 h before addition of iodine (0.5 g). A solution of V (from 5.0 g IV) in chloroform (30 ml) was added over a period of 30 min and stirring was continued for 48 h at room temperature. The mixture was filtered through a layer of Celite and the filtrate was concentrated to a syrup (6.8 g). Fractionation of this on a Silica Gel column (8 × 68 cm, solvent system D) yielded X as a chromatographically pure syrup (3.25 g, 54 %, calc. on IV) which slowly crystallized. Crystallization from hexane yielded the pure substance, m.p. 93 − 95°, $[α]_{578}{}^{22} − 17°$ (*c* 1.1, chloroform). NMR showed, *inter alia*, τ 1.83 − 3.00, 20 H (aromatic protons), τ 4.63, $J_{1,2}$ 5 Hz, 1 H, doublet (anomeric proton of D-galactose residue), τ 8.55 − 8.92, 12 H (isopropylidene groups). (Found: C 69.1; H 6.75. $C_{46}H_{52}O_{12}$ requires: C 69.3; H 6.58.)

6-O-(3,4,6-Tri-O-benzyl-β-D-glucosyl)-1,2:3,4-di-O-isopropylidene-α-D-galactose (XI). Treatment of X (2.8 g) with methanolic sodium methoxide as described above and fractionation of the product on a Silica Gel column (58 × 4 cm, solvent system D) yielded XI ($R_F = 0.60$) as a colourless, chromatographically pure syrup (2.16 g, 89 %), $[α]_{578}{}^{20} − 42°$ (*c* 1.0, chloroform), NMR showed, *inter alia*, τ 2.53 − 3.00, 15 H (aromatic protons), τ 4.47,

$J_{1,2}$ 5 Hz, 1 H, doublet (anomeric proton of D-galactose residue), τ 8.38 − 8.83, 12 H (isopropylidene groups).

6-O-(3,4,6-Tri-O-benzyl-β-D-arabino-hexosidulose)-1,2:3,4-di-O-isopropylidene-α-D-galactose (XII). Oxidation of XI (2.16 g) in dimethyl sulphoxide (200 ml) and acetic anhydride (10 ml) was performed as described above. The main component ($R_F = 0.27$, solvent system D) was obtained as a chromatographically pure syrup (1.7 g, 79 %), $[\alpha]_{578}{}^{20} - 55°$ (c 1.0, chloroform) by preparative TLC (6 plates). NMR (CCl$_4$) showed, *inter alia*, τ 2.33 − 3.00, 15 H (aromatic protons), τ 4.53, $J_{1,2}$ 5 Hz, 1 H, doublet (anomeric proton of D-galactose residue), τ 8.33 − 8.82, 12 H (isopropylidene groups). A strong absorption was observed in the IR spectrum (CCl$_4$) at 1763 cm^{-1} (C = O).

6-O-(β-D-Mannopyranosyl)-1,2:3,4-di-O-isopropylidene-α-D-galactose (XIII) was prepared by hydrogenation of XII (1.7 g) first over Adams' catalyst (200 mg) and then over palladium on charcoal (1.7 g) as described above. The resulting syrup (0.92 g, 90 %) was not characterized.

6-O-β-D-Mannopyranosyl-D-galactose (XIV). A solution of XIII (0.40 g) in 60 % aqueous acetic acid (25 ml) was kept at 100° for 1 h and concentrated to a syrup. The remaining acetic acid was removed by codistillation with water (3 × 10 ml). A hydrolysate of the syrup, analysed as described above, contained D-mannose, D-glucose, and D-galactose in the proportions 95:5:100. The syrup crystallized spontaneously and was recrystallized from ethanol − water yielding the pure substance (0.17 g, 50 %), m.p. 178 − 180°, $[\alpha]_{578}{}^{23} + 23°$ (5 min)→ + 0.7° (24 h) (c 0.75, water). (Found: C 42.0; H 6.49. C$_{12}$H$_{22}$O$_{11}$ requires: C 42.1; H 6.49.)

Characterisation of the disaccharide. The disaccharide showed $R_{Glc} = 0.35$ and 0.75 on paper chromatography in solvent systems E and F, respectively. A hydrolysate of the disaccharide contained equimolecular amounts of D-mannose and D-galactose, analysed as their alditol acetates by GLC, using an ECNSS-M column.[10] A sample of the disaccharide was reduced with sodium borodeuteride and methylated by Hakomori's method.[12] GLC-MS of the permethylated disaccharide alditol, using an XE-60 column, gave a peak with $T_{Mel} = 1.91$ (retention time relative to permethylated melibiitol) and with the expected mass spectrum.[8] Part of the permethylated disaccharide alditol was hydrolysed, the product reduced with sodium borohydride, acetylated and investigated by GLC-MS,[7] using an ECNSS-M column. Two peaks were obtained, which from their T-values (0.51 and 1.00, respectively) and mass spectra were identified as deriving from 1,2,3,4,5-penta-O-methyl-D-galactitol and 2,3,4,6-tetra-O-methyl-D-mannose, respectively. The deuterium labelling was present at C-1 in the D-galactitol derivative.

The optical rotation of the disaccharide, in water, decreased from $[\alpha]_{578}{}^{23} + 23°$ to + 0.7°, demonstrating that it had crystallised in the α-form. The final low value for the optical rotation is in agreement with a β-D-mannosidic linkage. This was further demonstrated by treating the fully acetylated disaccharide alditol with chromium trioxide in acetic acid, in the presence of *myo*-inositol hexaacetate as an internal standard as described for other disaccharide derivatives.[9] α-D-Mannopyranosides are stable under these conditions but β-D-mannopyranosides are oxidised and a sugar analysis revealed that, as a result of the oxidation, the D-mannose content was considerably reduced, relative to the contents of D-galactitol and *myo*-inositol.

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