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SYNTHESIS OF SOME *O*-D-XYLOSYL-D-MANNOSES AND THEIR DERIVATIVES*

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ABSTRACT

Methyl and benzyl 3-*O*-β-D-xylopyranosyl-α-D-mannopyranoside were prepared by way of D-xylosylation (Koenigs–Knorr) of methyl and benzyl 4,6-*O*-benzylidene-α-D-mannopyranoside (**1** and **17**). Analogous 2-*O*-β-D-xylopyranosyl-α-D-mannopyranosides could not be prepared efficiently by this procedure. However, methyl and benzyl 3-*O*-acetyl-4,6-*O*-benzylidene-α-D-mannopyranoside, prepared by limited acetylation of **1** and **17**, respectively, could be D-xylosylated by the same method, and afforded, after removal of protective groups, methyl and benzyl 2-*O*-β-D-xylopyranosyl-α-D-mannopyranoside. Hydrogenolysis of benzyl 2-*O*- and 3-*O*-β-D-xylopyranosyl-α-D-mannopyranoside yielded the corresponding, reducing disaccharides. In addition to these disaccharides, disaccharides containing an α-D-xylopyranosyl group, and trisaccharides having D-xylopyranosyl groups at both O-2 and O-3 were obtained as minor products.

INTRODUCTION

D-Xylose occupies a unique place among the constituent sugars of glycoconjugates, as it is the linking point between the core protein and several glycosaminoglycans[1]. In the glycoproteins of the *N*-glycosyl type, pineapple-stem bromelain[2,3] constitutes one of the four well-documented cases of the presence of D-xylosyl residues as an integral part of the oligosaccharide chain. Although, in a published report[4], a β-D-xylopyranosyl residue was located on the penultimate 2-acetamido-2-deoxy-D-glucose residue of the common core-structure[2], more-recent work[5,6] does not support this contention. The most plausible experimental data at present[5,6] indicate a β-D-Xyl-(1→2)-D-Man linkage. Therefore, we have synthesized

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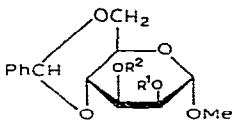
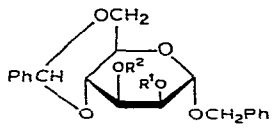
β-D-Xyl*p*-(1→2)-D-Man*p* (**32**), as well as β-D-Xyl*p*-(1→3)-D-Man*p* (**27**), as an aid to clarifying this ambiguity.

The synthetic scheme used involved 4,6-*O*-benzylidenation of methyl or benzyl α-D-mannopyranoside, followed by partial acetylation and D-xylopyranosylation [for the (1→2)-disaccharide] or direct D-xylopyranosylation without acetylation [for the (1→3)-disaccharide].



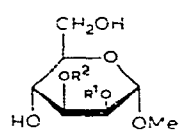
	R^1	R^2
1	H	H
2	H	Ac
3	Ac	H
4	Ac	Ac
5	H	βXAc3
6	H	αXAc3
7	βXAc3	βXAc3
12	βXAc3	Ac
13	αXAc3	Ac



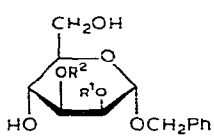
	R^1	R^2
17	H	H
18	H	Ac
19	H	βXAc3
20	H	αXAc3
21	βXAc3	βXAc3
22	βXAc3	αXAc3
	(or αXAc3)	(or βXAc3)
28	βXAc3	Ac
29	αXAc3	Ac

βXAc3 = 2,3,4-Tri-*O*-acetyl-β-D-xylopyranosyl

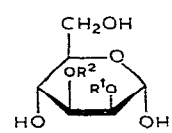
αXAc3 = 2,3,4-Tri-*O*-acetyl-α-D-xylopyranosyl



	R^1	R^2
9	H	βX
10	H	αX
11	βX	βX
14	βX	H
15	αX	H



	R^1	R^2
16	H	H
23	H	βX
24	H	αX
25	βX	βX
26	βX	αX
	(or αX)	(or βX)
30	βX	H
31	αX	H



	R^1	R^2
27	H	βX
32	βX	H

βX = β-D-Xylopyranosyl

αX = α-D-Xylopyranosyl

EXPERIMENTAL

Materials. — D-Mannose and methyl α-D-mannopyranoside were obtained from Sigma Chem. Co (St. Louis, MO), and D-xylose from Pfanstiehl Labs., Inc. (Waukegan, IL). 2,4,6-Tri-2-pyridyl-*s*-triazine (TPTZ) is a product of G. Frederick Smith Chem. Co. (Columbus, OH), and silica gels (DC-2 and LP-1) for column chromatography were obtained from Quantum Industries (Fairfield, NJ).

General methods. — Melting points (uncorrected) were measured with a Fisher–Johns apparatus. Proton magnetic resonance (p.m.r.) spectra were recorded with a JEOL NMH-100 spectrometer and optical rotations were measured with a Cary 60 spectropolarimeter. A Perkin–Elmer gas chromatograph 990 and a DuPont mass spectrometer 491 were used for analyses by gas–liquid chromatography–mass spectrometry (g.l.c.–m.s.). Elemental analyses were performed by Galbraith Labs. Inc. (Knoxville, TN).

All evaporations were conducted under diminished pressure below 40°. Thin-layer chromatography (t.l.c.) was performed on layers of silica gel F-254 precoated on aluminum (Merck). Components on t.l.c. plates were detected by fluorescent quenching of u.v. absorption, and also by spraying with 10% sulfuric acid in 50% ethanol, followed by charring at 140°.

For routine separations, a column (2.5 × 35 cm, or 5 × 40 cm) of silica gel, type DC-2 (60–200 μm) was used. For cleaner separations, rechromatography on a column (3.5 × 38 cm) of silica gel type LP-1 (10–20 μm) at a flow rate of 15–20 $ml.h^{-1}$ was employed. Solvent systems used for both t.l.c. and column chromatography were: (*A*) 1:1 (v/v) benzene–ethyl acetate, (*B*) 1:4 (v/v) benzene–ethyl acetate, (*C*) 9:4:2 (v/v) ethyl acetate–isopropyl alcohol–water, (*D*) 8:2:1 (v/v) ethyl acetate–acetic acid–water, and (*E*) 3:2:1 (v/v) ethyl acetate–isopropyl alcohol–water.

For gel filtration of acetylated compounds, a column (4 × 190 cm) of Sephadex LH-20 was used, with 95% ethanol as the eluant, and collection of 18 ml per fraction. For purification of deacetylated compounds, a column (2.5 × 142 cm) of Sephadex G-15 was routinely used with 0.1M acetic acid as the eluant and collection of 4 ml per fraction.

The phenol–sulfuric acid method[7] was used for routine, carbohydrate analysis of column effluents, as well as for determination of the concentrations of non-crystalline products. For these analyses, crystalline di- and tri-saccharides were used as the standards. It was found that the millimolar absorbance of an *O*-Xyl→Man disaccharide (33) is approximately the sum of the millimolar absorbances of a mannose (12.5) and a xylose (19.2). For trisaccharides consisting of one mannose and two xylose residues, the millimolar absorbance is 52. For analyses of the composition of neutral sugar, samples were hydrolyzed in 2M trifluoroacetic acid for 2 h at 100°, evaporated, and then analyzed by an automated method[8]. Deacetylated and deacetalized di- and tri-saccharides were methylated by the Hakomori method[9] with methylsulfinyl sodium. Permethylated material was hydrolyzed with 2M trifluoroacetic acid in a Teflon-lined, screw-capped tube for 3 h at 100°. After the hydrolysis,

the acid was evaporated off, and the tube was kept overnight over pellets of sodium hydroxide in a vacuum desiccator. The hydrolyzate was reduced by adding a solution of sodium borohydride (10 mg) in water (0.4 ml). After a few hours of reduction, 60% acetic acid (0.3 ml) was added, and the solution was evaporated to dryness. Borate was removed as methyl borate by repeated addition of methanol and evaporation. Acetylation was accomplished by heating with acetic anhydride (0.5 ml) for 20 h at 100°, cooling, and pouring into water (10 ml). Partially methylated alditol acetates were extracted into chloroform (2 × 5 ml), which was then successively washed with saturated sodium hydrogencarbonate (2 × 5 ml) and M sodium chloride (1 × 5 ml), dried (sodium sulfate), filtered, and evaporated. The dried residue was dissolved in acetone for use in g.l.c.–m.s. analysis. Methylation analyses were performed essentially as described by Lindberg[10] with a column of 3% of ECNSS-M coated on Gas Chrom Q. Identification of g.l.c. peaks was made by comparison of the retention times with those of reference compounds, and by further confirmation by m.s. analyses.

Preparation of methyl 3-O-acetyl-4,6-O-benzylidene-α-D-mannopyranoside (**2**). — Methyl 4,6-*O*-benzylidene-α-D-mannopyranoside (**1**) (2.82 g, 10 mmol), prepared according to the method of Bebault and Dutton[11], was suspended in a mixture of dry pyridine (70 ml) and toluene (20 ml), and cooled to 4°. A solution of acetic anhydride (1.0 ml, 10.6 mmol) in dry pyridine (10 ml) was added dropwise to the cooled suspension with gentle stirring. After a few hours, the same amount of acetic anhydride solution was added, and the suspension was stirred overnight in the cold. T.l.c. (solvent *A*) of the mixture then showed three products, in addition to a mall amount of unchanged starting-material **1**. The mixture was poured into a stirred mixture of cold water (400 ml) and toluene (200 ml); the aqueous layer was separated and extracted once with toluene (50 ml), and the toluene solutions were combined, washed thrice with cold 0.5M sulfuric acid, once with cold saturated sodium hydrogencarbonate, and once with M sodium chloride. The toluene solution contained acetylation products; compound **1** was contained in the water layer. The toluene layer was dried (sodium sulfate), filtered, and evaporated. The product with the highest R_F value (0.75) coincided with fully acetylated **1**, prepared by peracetylation of **1**. The p.m.r. spectrum of this product indicated the presence of acetyl and methyl groups in the ratio of 2:1, as expected: it is, therefore, methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-α-D-mannopyranoside (**4**). The two remaining products were identified by p.m.r. spectroscopy and periodate oxidation as, in the order of decreasing R_F value, methyl 2-*O*-acetyl-4,6-*O*-benzylidene-α-D-mannopyranoside (R_F 0.59) (**3**) and **2** (R_F 0.41), **2** being the major product. These compounds were separated by chromatography on a column of LP-1 silica gel. Elution with solvent *A* (6-ml fractions collected) separated the diacetyl derivative almost completely, but there was a considerable overlap between the 2- and 3-*O*-acetyl derivatives; these were separated by rechromatography on the same column, using eluant *B*. All three products were obtained as amorphous solids by evaporation from ethanolic solution. The yield of **2** was 0.98 g (30%).

Characterization of **2** *and* **3**. — P.m.r. signals for 2- and 3-*O*-acetyl groups were well separated, with the axial acetyl group at O-2 farther downfield than that on O-3

(2-*O*-acetyl signal at 2.17 and 3-*O*-acetyl at 2.05 p.p.m.), in accordance with the known generality. Diacetyl derivatives gave signals at 2.05 and 2.20 p.p.m. In further confirmation of the structural assignment, periodate oxidation was conducted on all three derivatives, as follows: ~50 μmol of each of **2**, **3**, and **4** was accurately weighed out and deacetalized with 60% acetic acid (0.5 ml) for 3 h at 55° in a Teflon-lined, screw-capped tube. The mixture was evaporated, and dried overnight in a vacuum desiccator. The residue was dissolved in water, and an aliquot (2.5 μmol) was treated with sodium periodate (0.05M in the reaction mixture). At various times, an aliquot was taken for analysis of the remaining periodate by the TPTZ method[12]. As expected, the 2-acetate **3** consumed 0.95 mol of periodate per mol in 4 h, without further significant change, whereas the 3-acetate **2** and the 2,3-diacetate **4** consumed less than 0.1 mol of periodate per mol.

Preparation of methyl 4,6-O-benzylidene-3-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-α-D-mannopyranoside (**5**) *and byproducts* **6** *and* **7**. — 2,3,4-Tri-*O*-acetyl-α-D-xylopyranosyl bromide (**8**) was prepared according to the method of Weygand[13]. Compound **1** (1.41 g, 5 mmol), **8** (3.39 g, 10 mmol), and mercuric cyanide (2.53 g, 10 mmol) were stirred in 1:1 (v/v) dry benzene–dry nitromethane (40 ml), and the progress of the reaction was monitored by t.l.c. (solvent *A*). After 5 h, an additional 5 mmol each of **8** and mercuric cyanide were added, and the process was repeated until **1** was almost used up: a total of 20 mmol of **8** was used. The mixture was filtered, the filtrate was evaporated, the residue was dissolved in chloroform (50 ml), and the undissolved solid was filtered off. The filtrate was washed with M sodium chloride (2 × 50 ml), to remove residual mercuric salt, dried (sodium sulfate), filtered, and evaporated. The residue was dissolved in 95% ethanol (25 ml) and fractionated on a column of Sephadex LH-20. Fractions were analyzed by the phenol–sulfuric acid method, and by t.l.c. (solvent *A*). The elution profile was similar to that shown in Fig. 1; there were three u.v.-absorbing bands (*i.e.*, benzylidene derivatives). The fast-moving band (**7**, R_F 0.49), which was located in fractions 92 to 97, was later found to be a trisaccharide. Two overlapping, slower-moving bands (**6**, R_F 0.46, and **5**, R_F 0.43) were present in fractions 107 to 118, and were later found to be disaccharides. The trisaccharide crystallized out on standing overnight, and, on concentration of the mother liquor, more crystals were obtained. Fractions containing disaccharides were combined, and evaporated, and the resulting syrupy residue was then separated on a column of LP-1 silica gel with solvent *B*. Purified **5**, **6**, and **7** were analyzed for neutral sugars after acid hydrolysis: 1 mol of **5** (and **6**) contained 1 mol each of mannose and xylose, whereas 1 mol of **7** contained 2 mol of xylose and 1 mol of mannose. The p.m.r. spectra confirmed these results: the ratios of aromatic (7.3–7.7 p.p.m.), methyl (3.41 p.p.m.), and acetyl (2.0–2.1 p.p.m.) protons were 5:3:9–10 for **5** and **6**, and 5:3:18 for **7**. Anomeric assignment was made later, after deacetylation and deacetalization. Combination of all of the data indicated that **6** was methyl 4,6-*O*-benzylidene-3-*O*-(2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl)-α-D-mannopyranoside, and **7** was methyl 4,6-*O*-benzylidene-2,3-di-*O*-(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)-α-D-mannopyranoside. Compound **5** was obtained as an amorphous solid in 41%

yield; compound **6** had m.p. 198–199° (crystallized from 95% ethanol), 5% yield; and compound **7** had m.p. 108° (crystallized from 95% ethanol), 6% yield.

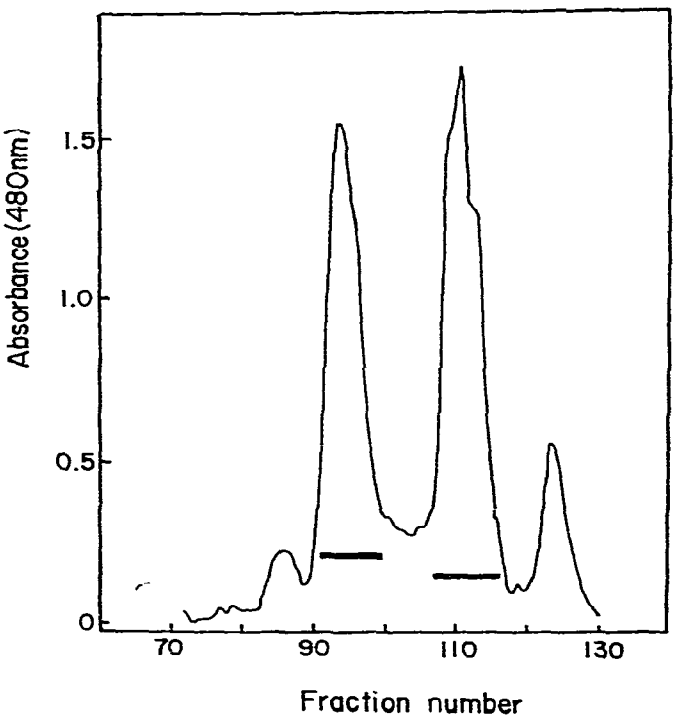


Fig. 1. Elution profile of the mixture obtained from the reaction of **8** with **17** on a column (4 × 190 cm) of Sephadex LH-20; 95% ethanol as eluant; 18 ml per fraction. [An aliquot (5 μl) from each fraction was analyzed by the phenol–sulfuric acid method.]

Preparation of methyl 3-O-β-D-xylopyranosyl-α-D-mannopyranoside (**9**) *and byproducts* **10** *and* **11**. — Compound **5** (0.4 g, 0.74 mmol) was deacetylated with 25mM sodium methoxide in dry methanol (2 ml) overnight at room temperature. The solution was then evaporated, the residue dissolved in 60% acetic acid (2 ml), and heated for 3 h at 55°. The mixture was evaporated, and the residue was dissolved in water (2 ml), and purified on a column of Sephadex G-15. Fractions containing **9** only (t.l.c., solvent *C*) were combined, and evaporated, and the residue was crystallized from 95% ethanol; yield 76%, m.p. 218–219°, $[\alpha]_D^{24}$ +60.2° (*c* 1.14, water).

Anal. Calc. for $C_{16}H_{22}O_{10}$ (326.30): C, 44.18; H, 6.80. Found: C, 44.59; H, 7.04.

Compounds **10** and **11** were obtained similarly. Compound **10** was obtained as an amorphous solid, $[\alpha]_D^{25}$ +115.4° (*c* 2.28, water); **11** was crystallized from isopropyl alcohol, m.p. 122–124° (hygroscopic), $[\alpha]_D^{25}$ −21.7° (*c* 4.86, water). The anomeric-proton signals of these compounds were observed clearly, after D_2O

exchange. In Me_2SO-d_6, **9** had one α-linkage (δ 4.62, d, 1, *J* 1–2 Hz) and one β-linkage (δ 4.27, d, 1, *J* 6 Hz); **10** had two α-linkages: α_1 (δ 4.50, s, 1) and α_2 (δ 4.89, d, 1, *J* 3 Hz); **11** had one α-linkage (δ 4.72, d, 1, *J* 2 Hz) and two β-linkages (δ 4.29, d, 2, *J* 5 Hz).

From these data, the title structure was assigned to **9**, methyl 3-*O*-α-D-xylopyranosyl-α-D-mannopyranoside to **10**, and methyl 2,3-di-*O*-β-D-xylopyranosyl-α-D-mannopyranoside to **11**.

Preparation of methyl 3-O-acetyl-4,6-O-benzylidene-2-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-α-D-mannopyranoside (**12**) *and its isomer* (**13**). — Compound **8** (1.43 g, 4.2 mmol), **2** (1.2 g, 3.7 mmol), and mercuric cyanide (1.08 g, 4.2 mmol) were stirred in 1:1 (v/v) benzene–nitromethane (20 ml) at room temperature. The progress of the reaction was monitored by t.l.c. (solvent *A*). Mercuric cyanide and **8** (4.2 mmol each) were added twice more during the 48-h reaction. The mixture was treated exactly as for the preparation of **5**. The elution profile from the column of Sephadex LH-20 is shown in Fig. 2. T.l.c. showed two overlapping, u.v.-absorbing bands in the di-

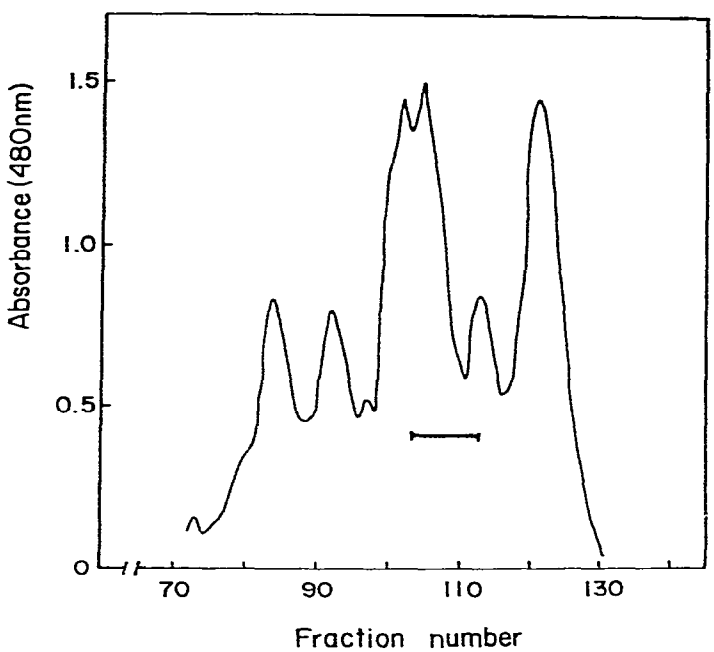


Fig. 2. Elution profile of the mixture obtained from the reaction of **8** with **2** on a column (4 × 190 cm) of Sephadex LH-20; 95% ethanol as eluant; 18 ml per fraction. [An aliquot (5 μl) from each fraction was analyzed by the phenol–sulfuric acid method.]

saccharide area (fractions 102–115) (**12**, R_F 0.64; **13**, R_F 0.72), and none in the trisaccharide area. The two disaccharides were separated as before, by chromatography on LP-1 silica gel with solvent *A*. Pure fractions from the column effluent were combined, and evaporated, and **12** was crystallized from absolute ethanol–benzene–

petroleum ether (b.p. 35–60°); yield, 0.61 g (36%), m.p. 100–101°. Similarly, evaporation of the column effluent containing **13** yielded ~0.1 g (6%) of solid **13**. Both **12** and **13** contained 1 mol each of mannose and xylose per mol. The p.m.r. spectrum of **12** showed signals from aromatic, methyl, and acetyl protons in the correct ratios (5:3:12). Together with p.m.r. and other data obtained (see the next section), the title structure was assigned to **12**, and methyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-*O*-(2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl)-α-D-mannopyranoside to **13**.

Preparation of methyl 2-O-β-D-xylopyranosyl-α-D-mannopyranoside (**14**) *and its isomer* **15**. — Compounds **12** and **13** were deacetylated and deacetalized as described for **5**. Pure fractions from the column of Sephadex G-15 were combined, and evaporated. Neither **14** nor **15** could be obtained crystalline, but they were pure by t.l.c. (solvent *C*). For **14**, $[\alpha]_D^{25}$ −14.3° (*c* 5.19, water); for **15**, $[\alpha]_D^{25}$ +102.4° (*c* 3.34, water). The p.m.r. spectra were recorded for solutions in Me_2SO-d_6 after D_2O exchange. Compound **14** had one α- (δ 4.59, d, 1, *J* 1 Hz) and one β-linkage (δ 4.25, d, 1, *J* 6.5 Hz); **15** had two α-linkages (δ 4.64, s, 1; and δ 4.80, d, 1, *J* 3 Hz). From these data, **14** was assigned to be the title compound, and **15**, methyl 2-*O*-α-D-xylopyranosyl-α-D-mannopyranoside.

Benzyl α-D-mannopyranoside (**16**). — To a suspension of D-mannose (20 g, 111 mmol) in dry benzyl alcohol (200 ml) were added toluene (50 ml) and a catalytic amount of recrystallized *p*-toluenesulfonic acid (2.3 g, 12 mmol). The mixture was boiled under a reflux condenser fitted with a Dean–Stark apparatus for 30 min, 2.8 ml (155 mmol) of water being collected. The mixture was cooled, diluted with ether (400 ml) and petroleum ether (b.p. 35–60°, 600 ml), and kept in a freezer. The solid that separated out was filtered off, and recrystallized from ethanol–ether, yielding 10.2 g (37.5%) of **16**, m.p. 131° (lit.[14] 132–133°). The filtrate and the mother liquor of crystallization were combined and evaporated, the residue was dissolved in water, and the solution was extracted with benzene. The aqueous phase was concentrated, and then fractionated on a column (5 × 162 cm) of Bio Gel P-2 by elution with 0.1M acetic acid (20-ml fractions). Fractions containing mainly **16** (t.l.c., solvent *D*) were combined and evaporated, and the residue was crystallized from absolute ethanol, to yield additional crystals of **16**. The mother liquor contained, in addition to **16** (R_F 0.50), a large amount of a contaminant (R_F 0.41, solvent *D*) that could not be removed by repeated recrystallization. However, chromatography of the mother liquor on Dowex-1 X-2 (OH^-) resin (200–400 mesh) with carbonate-free water yielded pure **16**. A combination of these two procedures yielded a further 6 g (15%) of crystalline **16** (total yield, 52.5%).

Benzyl 3-O-acetyl-4,6-O-benzylidene-α-D-mannopyranoside (**18**). — Benzyl 4,6-*O*-benzylidene-α-D-mannopyranoside (**17**) was prepared from **16** according to the method of Shaban *et al.*[14]. Monoacetylation of **17** was conducted as described for the preparation of **2**, with slight modifications. To a cold, stirred solution of **17** (2 g, 5.6 mmol) in dry pyridine (30 ml) and toluene (30 ml) was added dropwise a cold solution of acetic anhydride (0.58 ml, 6.1 mmol) in dry pyridine (10 ml). The solution was kept overnight in the cold, and then acetic anhydride (0.58 ml in 10 ml

of pyridine) was added. After 40 h, t.l.c. (solvent *A*) clearly showed the 2,3-diacetate (R_F 0.72). The reaction was stopped by pouring the mixture into a beaker containing cold water (500 ml) and toluene (200 ml). The layers were separated, and the water phase was extracted once with toluene (100 ml). The extract and toluene layer were combined, and successively washed with cold 0.5M sulfuric acid (3 × 75 ml), saturated sodium hydrogencarbonate (50 ml), and M sodium chloride (50 ml). A precipitate (shown by t.l.c. to be unreacted **17**) that began to form during the acid washing was removed by filtration, and the filtrate was dried (sodium sulfate), filtered, and evaporated.

Separation of **18** from 2-*O*- and 2,3-di-*O*-acetyl derivatives, as well as **17**, was accomplished as described for **2**, with a column of LP-1 silica gel, with solvent *A* as the eluant. Fractions containing only **18** were evaporated; the residue was dissolved in 95% ethanol, and evaporated to give **18** as a white solid, 0.9 g (40%).

The p.m.r. spectra of **18** and its 2-*O*-acetyl isomer showed the correct ratios of aromatic protons to acetyl protons. Signals of 2-*O*-acetyl protons were at 2.08–2.10 p.p.m., and those of 3-*O*-acetyl protons, at 2.00–2.02 p.p.m.

Preparation of benzyl 4,6-O-benzylidene-3-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-α-D-mannopyranoside (**19**) *and byproducts* **20**, **21**, *and* **22**. — Compound **17** (0.9 g, 2.5 mmol), **8** (1.7 g, 5 mmol), and mercuric cyanide (1.26 g, 5 mmol) in 1:1 (v/v) benzene–nitromethane (25 ml) were stirred at room temperature. The reaction was complete within 2 h, as judged by t.l.c. (solvent *A*). The mixture was treated exactly as described for **5**: the elution profile from a column of Sephadex LH-20 is shown in Fig. 1. T.l.c. analysis (solvent *A*) showed that there were two trisaccharides and only one detectable disaccharide. In another experiment, only a 50% excess of **8** was used, in order to decrease the formation of trisaccharide. Under these conditions, a very small amount of a second disaccharide was observed. The major disaccharide product was later identified as **19**; a minor disaccharide and a major and a minor trisaccharide were assigned the respective structures **20**, **21**, and **22**. Separation of **19** from **20**, and of **21** from **22** was accomplished as before, using chromatography on a column of LP-1 silica gel (solvent *A*). The p.m.r. spectra of **19** and **21** showed the expected ratios of aromatic protons to acetyl protons for the disaccharide and the trisaccharide, respectively. Neutral-sugar analyses of acid hydrolyzates gave the expected ratios of xylose to mannose in all four of these compounds: **19** and **20**, one mole each of xylose and mannose per mole; **21** and **22**, two moles of xylose and one mole of mannose per mole. From these results, and anomeric assignments as described later, **20** was identified as benzyl 4,6-*O*-benzylidene-3-*O*-(2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl)-α-D-mannopyranoside, the anomeric assignment of the D-xylosyl group being tentative, owing to an insufficiency of this compound. Compound **21**, the major trisaccharide, was probably produced by attachmentof the second β-D-xylosyl group at O-2 of **19** (one α-anomeric and two β-anomeric signals). Compound **22** had one β- and two α-anomeric linkages, but its complete structure was not elucidated.

Compound **19** was obtained in a yield of 0.72 g (48%), m.p. 100–101° (crystal-

lized from 95% ethanol–ether); **21**, yield 0.69 g (37%), m.p. 92–94° (crystallized from 95% ethanol); **22**, yield 0.03 g (2%), m.p. 89–90° (crystallized from 95% ethanol).

Anal. (for **21**). Calc. for $C_{42}H_{50}O_{20}$ (874.82): C, 57.66; H, 5.76. Found: C, 57.80; H, 5.90.

Preparation of benzyl 3-O-β-D-xylopyranosyl-α-D-mannopyranoside (**23**) *and byproducts* **24**, **25**, *and* **26**. — Compound **19** was deacetylated and deacetalized (as described for **9**) to yield **23**. Similarly, **24**, **25**, and **26** were respectively obtained from **20**, **21**, and **22**. The p.m.r. spectrum of D_2O-exchanged samples in Me_2SO-d_6 yielded the following information. Signals at 4.52–4.54 and 4.66–4.69 p.p.m. were assigned to the two protons of the benzyl methylene groups, by comparison with the spectra of **9**, **10**, and **16**. Compound **23** had one α-linkage (δ 4.80, d, 1, *J* 1 Hz) and one β-linkage (δ 4.36, d, 1, *J* 5 Hz). A satisfactory spectrum of **24** was not obtained, due to an insufficiency of the material. Compound **25** had one α-linkage (δ 4.93, d, 1, *J* 2 Hz) and two β-linkages (δ 4.30, d, 2, *J* 6 Hz); **26** gave two, overlapping, α-linkage signals (δ 4.92 and 4.89) and one β-linkage (δ 4.34, d, 1, *J* 6 Hz). Compound **23** had $[\alpha]_D^{25}$ +20.2° (*c* 4.47, water); **24**, $[\alpha]_D^{25}$ +26.2° (*c*, 0.14, water); **25**, crystallized from 2-propanol, m.p. 123–125°, $[\alpha]_D^{25}$ −8.3° (*c*, 5.98, water); and **26**, $[\alpha]_D^{25}$ +57.4° (*c* 2.56, water). Thus, compound **24** was tentatively assigned the structure of benzyl 3-*O*-α-D-xylopyranosyl-α-D-mannopyranoside (see **20**), and **25**, the structure benzyl 2,3-di-*O*-β-D-xylopyranosyl-α-D-mannopyranoside. A complete structure was not established for **26**.

3-O-β-D-Xylopyranosyl-D-mannose (**27**). — In a microflask of a Brown hydrogenator (Micro-hydro-analyzer, Delmar Scientific Lab.) was placed a solution of **23** (0.25 g, 0.62 mmol) in 60% acetic acid (4 ml) in which 10% palladium-on-carbon (30 mg) was suspended. Hydrogen was generated externally by introduction of sodium borohydride from a microburet into 60% acetic acid. The uptake of hydrogen was monitored by the volume of the sodium borohydride solution consumed; the progress of the hydrogenolysis was also checked by t.l.c. in solvent *C* (R_F of **23**, 0.46; R_F of **27**, 0.18). After 24 h, more catalyst (30 mg) was added, and, after a total of 48 h, the mixture was filtered through Celite, and the solid washed with 50% ethanol. The filtrate was evaporated, and the residue was dissolved in a small volume of water, and purified on a column of Sephadex G-15. Fractions containing **27** were combined and evaporated, and the resulting syrup was dissolved in warm 95% ethanol. On standing, crystals were obtained; 0.17 g (0.55 mmol); m.p. 194–196°.

Preparation of benzyl 3-O-acetyl-4,6-O-benzylidene-2-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-α-D-mannopyranoside (**28**) *and its isomer* **29**. — Compound **8** (2.0 g, 5.9 mmol), mercuric cyanide (1.5 g, 5.9 mmol), and **18** (0.75 g, 1.9 mmol) were suspended in 1:1 (v/v) dry benzene–nitromethane (15 ml), and the suspension was stirred at room temperature. After 4 h, the same weights of **8** and mercuric cyanide were added, stirring was continued overnight, and the mixture was treated as for **5**. The elution profile from a column of Sephadex LH-20 was similar to that of the reaction mixture for **12** (see Fig. 2). As with **12**, two overlapping disaccharides were observed. The major product, **28**, and the minor product, **29**, were separated by

chromatography on a column of LP-1 silica gel (solvent *A*). Compound **28** crystallized from isopropyl ether–petroleum ether (b.p. 35–60°); m.p. 75–76° (37% yield); **29** crystallized from 95% ethanol, m.p. 82° (15% yield). Neutral-sugar analyses of purified **28** and **29** showed one mole each of mannose and xylose per mole for both compounds. On the basis of these results, together with the anomeric assignment of the deacetylated, deacetalized materials by p.m.r. spectroscopy, **28** was assigned the title structure, and **29** that of benzyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-*O*-(2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl)-α-D-mannopyranoside.

Preparation of benzyl 2-O-β-D-xylopyranosyl-α-D-mannopyranoside (**30**) *and its isomer* **31**. — Deacetylation and deacetalization of **28** and **29** were conducted as before, to yield **30** and **31**, respectively. Compound **30** crystallized from 95% ethanol–ether, m.p. 94–95°, $[\alpha]_D^{25}$ −13.2° (*c* 3.1, water); **31** had $[\alpha]_D^{25}$ +118.0° (*c* 1.81, water). The p.m.r. spectra of purified **30** and **31** in Me_2SO-d_6 (after D_2O exchange) had anomeric-proton signals as follows: **30**, one α-linkage (δ 4.90, s, 1) and one β-linkage (δ 4.33, d, 1, *J* 6 Hz); **31**, two α-linkages (δ 4.91, s, 1; and δ 4.86, d, 1, *J* 3–4 Hz). From these results, the structure of benzyl 2-*O*-α-D-xylopyranosyl-α-D-mannopyranoside was assigned to **31**.

2-O-β-D-Xylopyranosyl-α-D-mannose (**32**). — Compound **32** was obtained by hydrogenolysis of either **30** or deacetylated (but not deacetalized)* **28**. The conditions of hydrogenolysis were similar to those used for **27**. The progress of the reaction was monitored by t.l.c. in solvent *C* (R_F of **30**, 0.42; R_F of **32**, 0.12). The mixture was treated as before, and purified on a column of Sephadex G-15. Fractions containing pure **32** were combined and evaporated, and the residue was dried overnight in a vacuum desiccator over sodium hydroxide pellets, and crystallized from absolute ethanol. However, the crystals were hygroscopic.

Characterization of the oligosaccharides. — Sugar-composition analyses, periodate oxidation, and methylation were applied to the synthesized oligosaccharides. The analyses for sugar composition are given in Table I. Methylation analyses were made for six of the products, as shown in Table I, and all yielded the results expected. Periodate oxidation was performed on all of the methyl and benzyl glycosides of disaccharides, in order to differentiate between (1→3)- and (1→2)-linkages. Sample solutions (0.05 ml, containing 1–2 μmol of disaccharide) were mixed with 0.1M sodium periodate (0.05 ml). At appropriate time-intervals, aliquots were taken for analysis, by the TPTZ method, of the periodate remaining. Oxidation was complete within 4 h at room temperature; the concentration of periodate then remained constant. The results, given in Table I, support the structures assigned the compounds tested: (1→3)-linked disaccharides consumed 2 mol of periodate per mol, and (1→2)-linked consumed 3 mol per mol.

*Hydrogenolysis of this compound was considerably slower than that of **30**, requiring 72 h and three additions of Pd/C catalyst during the reaction. For this reason, hydrogenolysis was routinely conducted after mild, acid treatment for removal of the benzylidene group.

TABLE I

CHARACTERIZATION OF THE *O*-D-XYLOSYL-D-MANNOSIDES SYNTHESIZED

Starting D-*mannose derivative*	*Products*		*Yield from glycosylation* (%)	*Sugar composition (mol/mol) of di- or tri-saccharide*		*Periodate consumed (mol/mol)*[a]	*Linkage*	R_F *of deacetylated products in solvent* E
	Acetylated form	*Deacetylated form*		*Xylose*	*Mannose*			
	5[b]	9[c]	41	1.05	1.0	2.1 (2)	β-(1→3)	0.41
1	6[b]	10	5	1.05	1.0	2.16 (2)	α-(1→3)	0.43
	7[b]	11[c]	6	1.98	1.0		β-(1→3) β-(1→2)	0.19
2	12[b]	14[c]	36	1.04	1.0	3.38 (3)	β-(1→2)	0.30
	13	15	6	0.99	1.08	3.08 (3)	α-(1→2)	0.39
	19	23[c]	48	0.91	1.00	1.92 (2)	β-(1→3)	0.65
	20	24	negligible	0.89	0.95	2.22 (2)	α-(1→3)	0.60
17	21	25	37	1.98	1.0/		β-(1→3) β-(1→2)	0.50
	22	26	2	1.86	0.93		β- or α-(1→3) α- or β-(1→2)	0.51
18	28	30[c]	37	1.18	0.86		β-(1→2)	0.60
	29	31[c]	15	1.03	1.10	2.9 (3)	α-(1→2)	0.62

[a]The numbers in parentheses indicate theoretical values. [b]For these compounds, the sugar composition is given relative to D-mannose as 1.0. [c]Methylation analyses were performed on these compounds, and the expected results were obtained.

Comparative D-*xylosylation of* **1** *and* **17**. — From the previous experiments, it appeared that D-xylosylation of the benzyl D-mannoside **17** proceeds much more readily than that of the corresponding methyl D-mannoside **1**. In order to check this, D-xylosylation of **1** and **17** was performed under exactly the same conditions, using freshly prepared **8**. The reaction mixture contained 1.25 mmol of **1** or **17**, a 50% molar excess of **8**, and mercuric cyanide (1.88 mmol) in 1:1 (v/v) dry benzene–nitromethane (15 ml). T.l.c. (solvent *A*) showed that **17** was consumed in less than 2 h, but **1** was still present in the mixture after reaction overnight. At this point, the reaction was stopped, and the mixture was treated as described before, and then fractionated on a column of Sephadex LH-20. Di- and tri-saccharide regions of the column effluent were located (by analysis by the phenol–sulfuric acid method, and by t.l.c. in solvent *A*), and separately evaporated, to give almost pure di- and tri-saccharides as solids; the yields were: for methyl glycoside of the disaccharide 34%, of the trisaccharide negligible; for benzyl glycosides of the disaccharide 74%, of the trisaccharide, 28%. Thus, D-xylosylation of **17** was much more facile than that of **1** under the conditions described.

DISCUSSION

In the 4,6-*O*-benzylidene derivatives of methyl and benzyl α-D-mannopyranosides, there is an axial OH on C-2, and an equatorial OH on C-3. Although OH-2 groups are generally the more reactive, the axial OH-2 in α-D-mannopyranosides would be expected to be much less reactive than normal. Indeed, 3-*O*-β-D-xylopyranosyl-D-mannopyranose derivatives (**5** and **19**) could be prepared with ease by a direct Koenigs–Knorr reaction using D-mannopyranosides (**1** and **17**) having both OH-2 and OH-3 free. However, for effective preparation of 2-*O*-β-D-xylopyranosyl-D-mannopyranose derivatives, it was necessary first to protect OH-3; this was readily accomplished by selective acetylation of **1** and **17**, utilizing the relative ease of acetylation of the OH-3 group. Garegg[15] prepared **2** and **3** by acetylation of **1** (in pyridine at room temperature) with 1.1 mol of acetic anhydride per mol of **1**, and found **2** to be the preponderant product. The difference in reactivity of OH-2 and OH-3 was also shown during the Koenigs–Knorr reaction. In order to use up the D-mannopyranoside derivatives (**17** or **18**), it was necessary to employ an almost 6-fold excess of **8** to react with **18**, whereas less than a 50% excess of **8** was needed for **17**.

The nature of the aglycon seems to influence the rate of glycosylation under the Koenigs–Knorr conditions used in this work. D-Xylosylation occurred more readily with the benzyl glycoside **17** than with the methyl glycoside **1**, as shown by the results of the parallel D-xylosylation experiment. In a similar experiment* on 3-*O*-D-galactosylation of 2-acetamido-4,6-*O*-benzylidene-2-deoxy-D-glucose, no such difference was found between methyl and benzyl glycosides.

*Unpublished results.

Utille and Vottero[16] reported the formation of both α- and β-linked D-xylopyranosyl derivatives in a Koenigs–Knorr reaction that included a catalytic amount of mercuric bromide in addition to mercuric cyanide. The ratio of β- to α-linked products was 3:1. In this work, we have also observed formation of α-D-xylopyranosyl products in all of the Koenigs–Knorr reactions. In our reaction mixtures, mercuric bromide was not added (although this would be formed during the course of the reaction). The ratio of β- to α-linked products varied considerably, depending on the ease of the reaction. Thus, the D-xylosylation of **17** at OH-3 proceeded readily, to yield 85% of β-linked products (**19** and **21**) and 2% of α-linked products (**20** and **22**), whereas the forced D-xylosylation of **18** to afford 2-linked compounds gave β-linked product (**28**) in 37%, and α-linked product (**29**) in 15%, yield.

Modification of OH-2 of a D-mannoside is usually accomplished *via* acyclic derivatives. For example, Kaifu *et al.*[17] prepared 2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-mannose by treating an oxazoline derivative prepared from 2-acetamido-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride with 3,4:5,6-di-*O*-isopropylidene D-mannose dimethyl acetal. Our results demonstrate that there is an alternative approach to 2-substitution of D-mannose that is suitable for use where D-mannose residues are already glycosidically bound. When the ultimate product is a glycoside, rather than a reducing sugar, the present approach may have considerable advantages over the use of acyclic derivatives.

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