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STEREOSELECTIVE SYNTHESIS AND ^{13}C-N.M.R. SPECTRA OF TWO ISOMERIC METHYL β-GLYCOSIDES OF TRISACCHARIDES RELATED TO ARABINOXYLAN

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ABSTRACT

Methyl 2-*O*-acetyl-4-*O*-(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)-β-D-xylopyranoside (**4**) and methyl 2,3-di-*O*-acetyl-4-*O*-(2,4-di-*O*-acetyl-β-D-xylopyranosyl)-β-D-xylopyranoside were separately condensed with 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl bromide under modified Koenigs–Knorr conditions, to give high yields (>90%) of trisaccharide derivatives containing a (1→3)-linked α-L-arabinofuranosyl residue. Removal of the protecting groups gave methyl 3-*O*- and 3′-*O*-α-L-arabinofuranosyl-β-D-xylobioside. ^{13}C-N.m.r. data for these compounds are presented and the homo- and hetero-nuclear 2D-n.m.r. spectra of **4** are discussed.

INTRODUCTION

Arabinoxylans of low molecular weight are found as hemicelluloses of annual plants and softwoods. Their backbone is slightly branched[1], and some of the (1→4)-linked β-D-xylosyl residues bear an α-L-arabinofuranosyl group at O-3. Trisaccharides containing two xylopyranose residues and one arabinofuranosyl group have been isolated in small proportions from the products of partial enzymic hydrolysis of xylans from wheat straw[2,3], rye flour[4], cocksfoot grass[4], and rice straw[5], but the structures of some have not been proved. We now report syntheses of the methyl β-glycosides of 3- (**7**) and 3′-*O*-α-L-arabinofuranosylxylobiose (**9**). These trisaccharides are model compounds in that they contain the basic structural features of arabinoxylans.

RESULTS AND DISCUSSION

We have described[6] the synthesis of 3-*O*-α-L-arabinofuranosyl-D-xylose and its methyl β-glycoside, and have discussed conditions of condensation of 2,3,5-tri-

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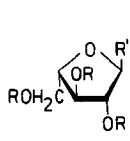
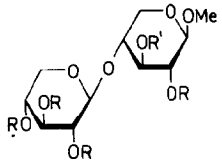
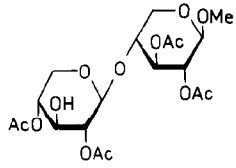
O-benzoyl-α-L-arabinofuranosyl bromide (**1**) with methyl 2,4-di-*O*-acetyl-β-D-xylopyranoside which are highly stereoselective for the formation of a (1→3)-α-glycosidic linkage. The starting points in the present synthesis were the crystalline disaccharide derivatives methyl 2-*O*-acetyl-4-*O*-(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)-β-D-xylopyranoside (**4**) and methyl 2,3-di-*O*-acetyl-4-*O*-(2,4-di-*O*-acetyl-β-D-xylopyranosyl)-β-D-xylopyranoside[7] (**5**). Compound **4** was obtained from methyl 2,3-anhydro-4-*O*-β-D-xylopyranosyl-β-D-ribopyranoside[8] by reaction with the benzyloxy anion followed by acetylation and catalytic hydrogenolysis. The bromide **1** was prepared from methyl 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranoside[9]. The blocking groups (acetyl and benzoyl) in both reaction components were chosen so that they could be removed in one reaction step after the condensation reaction.



1 R = Bz , R' = Br
10 R = H , R' = OMe
11 R = Bz , R' = OMe



2 R = H , R' = Bzl
3 R = Ac , R' = Bzl
4 R = Ac , R' = H



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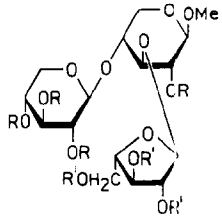
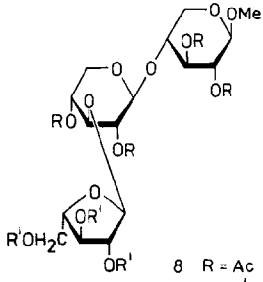
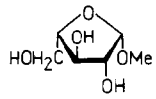


6 R = Ac , R' = Bz
7 R = R' = H



8 R = Ac , R' = Bz
9 R = R' = H



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Treatment of **4** with an excess of **1** in dry benzene in the presence of mercuric cyanide yielded 96% of the expected (1→3)-α-linked trisaccharide derivative **6**; formation of the (1→3)-β-linked isomer was not detected. Likewise, condensation of **5** and **1** gave the (1→3)-α-linked trisaccharide derivative **8** (91%) and no trace of the β isomer.

Deacylation of the trisaccharide derivatives **6** and **8** by methanolic sodium methoxide afforded crystalline methyl 3-*O*-α-L-arabinofuranosyl-4-*O*-β-D-xylopyranosyl-β-D-xylopyranoside (**7**) and methyl 4-*O*-(3-*O*-α-L-arabinofuranosyl-β-D-xylopyranosyl)-β-D-xylopyranoside (**9**), respectively. The structures of **2**–**4** and **6**–**9** were confirmed by ^{13}C-n.m.r. spectroscopy, and the relevant data are given in Table I. The resonances of **4**, **7**, and **9** were assigned by the combined use of

TABLE I

^{13}C-N.M.R. CHEMICAL SHIFTS FOR DI- (**2–4**), TRI- (**6–9**), AND MONO-SACCHARIDES (**10–12**)

Compound	*Ring*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*Me*	
2	C	104.93	73.35	82.32	76.73[a]	63.74	58.34	[b]
	C′	103.30	74.39	76.99[a]	70.62	66.47		
3	C	102.01	71.54	78.94	76.67	62.18	56.53	[c]
	C′	99.99	71.15	71.54	68.61	62.18		
4	C	101.49	71.86	71.91	78.97	62.28	56.01	[d]
	C′	100.51	70.50	71.35	68.09	61.94		
6	C	102.27	72.32	74.07[a]	75.11[a]	62.50	56.59	[c]
	C′	99.28	71.21	71.99	68.61	62.50		
	C″	105.58	82.45	77.97	81.09	63.61		
7	C	106.24	75.89	79.93	76.20	65.25	59.80	[e]
	C′	104.03	75.54	78.19	71.78	67.70		
	C″	110.20	83.24	79.82	87.34	63.90		
8	C	102.01	71.21	72.64	75.89	63.02	56.92	[c]
	C′	100.97	71.21	81.87	69.78	62.24		
	C″	106.69	82.26	77.06	81.87	63.67		
9	C	106.51	75.53	76.46	79.16	65.59	59.89	[e]
	C′	104.43	75.53	84.21	70.48	67.71		
	C″	110.84	83.90	79.16	86.69	63.92		
10	C	110.99	83.37	79.02	86.57	63.87	57.59	[f]
11	C	106.87	82.19	77.92	80.85	63.71	54.99	[g]
12	C	104.81	78.98	77.17	84.63	65.75	57.78	[f]

[a]These assignments may be interchanged. [b]Measured at 25 MHz, using a Jeol JNM FX-100 spectrometer and solutions in D_2O at 20° (internal methanol, δ 50.15). [c]As in [b], but for solutions in $CDCl_3$ (internal Me_4Si). [d]Measured at 50.3 MHz, using a Varian XL-200 spectrometer and solutions in $CDCl_3$ (internal Me_4Si). [e]As in [d], but for solutions in D_2O (internal sodium 2,2-dimethyl-2-silapropylsulfonate, δ_{Me} 0.00; see ref. 11). [f]Measured as in [e]; the assignment taken from ref. 18. [g]Measured as in [d]; assigned by selective heteronuclear decoupling.

homonuclear (^{1}H) and heteronuclear (^{1}H, ^{13}C) two-dimensional n.m.r. spectroscopy[10], the details of which will be described elsewhere[11]. The same technique was also applied to the model compound methyl 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranoside (**11**). The results[11] clearly demonstrate that the chemical shifts of the signals for trisaccharides can be predicted on the basis of those for suitable model mono- or di-saccharides obtained under similar conditions. This is now a routine practice when a direct assignment is not feasible. Thus, the resonances in the spectra of the trisaccharide derivatives **6** and **8** were assigned on the basis of a comparison with the spectra of disaccharide derivatives **3** and **4**, methyl β-D-xylobioside[12], and the model compounds **10** and **11**.

For the disaccharide derivative **2**, the chemical shifts of the carbon signals in the non-reducing unit are similar to those of the corresponding carbon atoms in methyl β-D-xylobioside[13]. The remaining signals can also be assigned according to this model by taking into account a large positive α-effect of the benzyl group[12]. The occurrence of two signals at δ 76.8, one of which must be due to C-4 (and the other to C′-3), proves the presence of a β-glycosidic bond in **2**. This conclusion is

further supported by the values of chemical shifts of carbon signals for the non-reducing unit. Similarly, the chemical shifts of the carbon resonances of **3** and **4** could be assigned by comparison with the assigned signals of xylobiose hexa-acetate[14,15] and methyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside[16], taking into account the effect of the substituents[12,16].

The values of the chemical shifts of the carbon signals in the non-reducing units of the disaccharide derivatives **3** and **4** are close to those for xylobiose hexa-acetate[14,15] and, hence, it can be concluded that these derivatives also contain a β linkage (the signals would occur at lower δ values if an α linkage were present).

The chemical shifts of the C′-1 and the C-4 resonances in the disaccharide derivative **4** are 2 p.p.m. to higher field than those of the corresponding signals in similar oligosaccharides. Therefore, two-dimensional spectroscopy was applied,

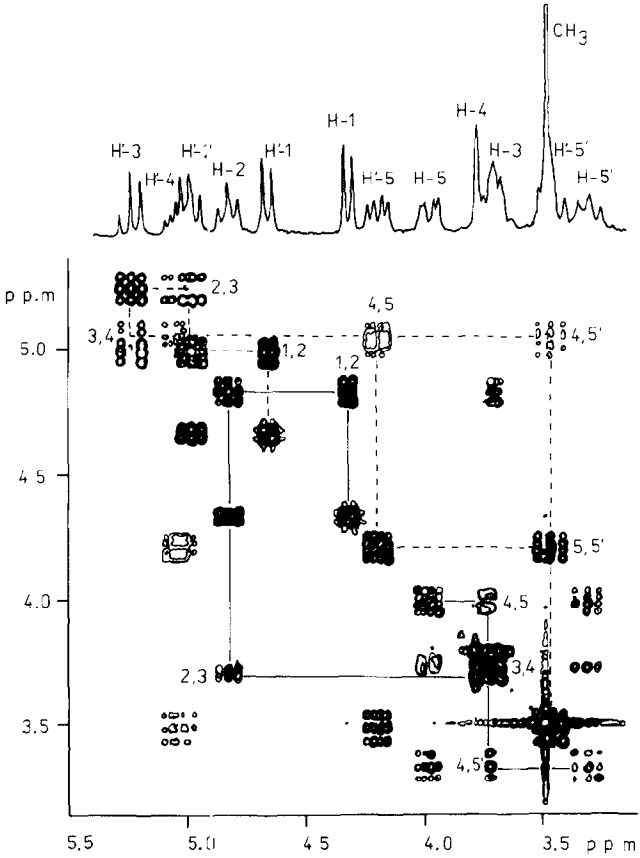

Fig. 1. ^{1}H-N.m.r. spectrum ($CDCl_3$) and a contour plot of the homonuclear, chemical-shift correlated, 2D-n.m.r. spectrum of **4**. The 2D map is composed of 512–512 data points which were acquired with quadrature phase detection and pseudo-echo shaping in both dimensions on a Varian XL-200 spectrometer. The data were symmetrised. The broken lines indicate connections between the dia and cross peaks used for the line assignments.

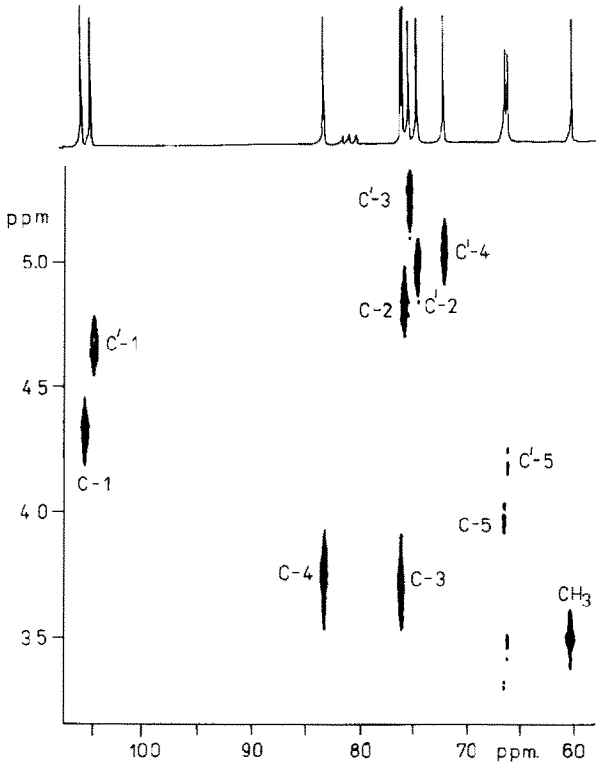

Fig. 2. ^{13}C-N.m.r. spectrum ($CDCl_3$) and a contour plot of the heteronuclear (^{13}C, ^{1}H), chemical-shift correlated, 2D-n.m.r. spectrum of **4**. Exponential weighting was used for sensitivity enhancement, a 1024 × 1024 data matrix was used to store the FIDs, and the spectrum was recorded with a Varian XL-200 spectrometer.

and the spectra shown in Figs. 1 and 2 prove the correctness of the assignments given in Table I. In seeking to elucidate the origin of these anomalous chemical shifts, apparently not observed hitherto, the concentration dependence (in $CDCl_3$) and the effect of added methyl sulfoxide were investigated. As shown in Fig. 3, the addition of methyl sulfoxide, a recognised proton acceptor, had a dramatic effect on the C'-1 and C-4 resonances (broadening and shifting to "normal" lower δ values) and a small (opposite) effect on the C-3 resonance line. Since the anomalous chemical shifts are not markedly concentration-dependent, it is concluded that the anomaly is due to an intramolecular interaction of HO-3 with either AcO'-2 or O'-5.

Comparison of the chemical shifts of the C-4 signals in **6–9** with earlier results[13,17] permits the conclusion that the xylose residues are β-linked. Comparison of the chemical shifts of the C''-1 signals of the arabinofuranosyl rings in **6–9** and in the model compounds **10–12** clearly indicates that the L-arabinofuranosyl groups are α.

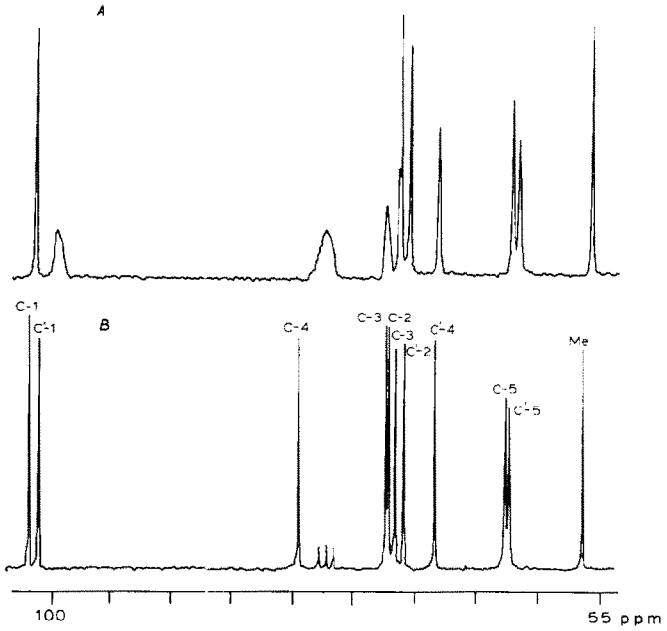

Fig. 3. ^{13}C-N.m.r. spectra of **4**: *A*, in $CDCl_3$ containing a few drops of methyl sulfoxide; *B*, in $CDCl_3$ (Varian XL-200).

EXPERIMENTAL

General methods. — Melting points were determined on a Kofler hot-stage. Optical rotations (22°, *c* 1) were measured with a Perkin–Elmer Model 141 automatic polarimeter. Microanalyses were performed with a Perkin–Elmer Model 240 automatic analyser. Preparative chromatography was performed by gradient elution from columns of dry-packed Silica Gel 60 (Merck) which, prior to packing, had been equilibrated with 40% of the mobile phase. All reactions were monitored by t.l.c. on Silica Gel G with detection by charring with sulfuric acid. Benzene and toluene were dried with sodium and calcium hydride, respectively, and freshly distilled. Solution were dried with anhydrous sodium sulfate and concentrated at 40°/2 kPa.

Methyl 3-O-benzyl-4-O-β-D-xylopyranosyl-β-D-xylopyranoside (**2**). — Methyl 2,3-anhydro-4-*O*-β-D-xylopyranosyl-β-D-ribopyranoside[8] (5.4 g, 19.4 mmol) was added to a solution of benzyl-alcoholic sodium benzoxide (4.6 g of sodium hydride in 90 mL of benzyl alcohol), and the mixture was stirred at 100° for 90 min with the exclusion of atmospheric moisture and carbon dioxide. The mixture was then cooled to 20°, diluted with ethanol (100 mL), deionised with Dowex 50W (H^+) resin, filtered, and concentrated, and benzyl alcohol was removed at 100° (bath)/20 Pa. The dark-yellow residue was eluted from a column of silica gel (600 g) with chloroform–acetone (1:1) and crystallised from acetone, to give **2** (6

g, 80%), m.p. 159–160°, $[\alpha]_D$ −57° (chloroform), R_F 0.25 (chloroform–acetone, 1:1) (Found: C, 55.86; H, 6.86. $C_{18}H_{26}O_9$ calc.: C, 55.95; H, 6.78%).

Methyl 2-O-acetyl-3-O-benzyl-4-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-β-D-xylopyranoside (**3**). — Acetic anhydride (12 mL) was added to a solution of **2** (5.9 g, 15.3 mmol) in pyridine (20 mL), and the mixture was left at room temperature for 6 h. Conventional work-up gave material (R_F 0.4: benzene–acetone, 8:1) which, after crystallisation from methanol, afforded **3** (7.6 g, 89.7%), m.p. 141.5–142.5°, $[\alpha]_D$ −67° (chloroform) (Found: C, 56.27; H, 6.27. $C_{26}H_{34}O_{13}$ calc.: C, 56.31; H, 6.18%).

Methyl 2-O-acetyl-4-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-β-D-xylopyranoside (**4**). — A solution of **3** (7.5 g) in acetone–methanol (1:4, 300 mL) was hydrogenolysed at room temperature over 5% Pd/C (0.5 g) for 5 h. T.l.c. (benzene–acetone, 8:1) then showed complete conversion of **3** into **4** (R_F 0.25). Conventional isolation of the product and crystallisation from ethanol gave **4** (5.8 g, 92.4%), m.p. 157.5–158.5°, $[\alpha]_D$ −66° (chloroform) (Found: C, 49.08; H, 6.21. $C_{19}H_{28}O_{13}$ calc.: C, 49.13; H, 6.07%).

Methyl 2-O-acetyl-4-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-3-O-(2,3,5-tri-O-benzoyl-α-L-arabinofuranosyl)-β-D-xylopyranoside (**6**). — A solution of **1** (6.4 g, 13.4 mmol, freshly prepared from methyl 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranoside[9]) in the minimum amount of benzene was added to a stirred mixture of **4** (2.5 g, 5.4 mmol), mercuric cyanide (1.7 g, 6.7 mmol), and benzene (100 mL), and the mixture was stirred with the exclusion of atmospheric moisture for 3 h. T.l.c. (benzene–acetone, 20:1) then showed the absence of **4** (R_F 0.05), and the presence of the condensation product **6** (R_F 0.2) and the hydrolysis product (R_F 0.4) of **1**. Small amounts of by-products (R_F >0.5) were also present. The mixture was worked-up[19], and the product was subjected to chromatography, using linear-gradient elution with benzene–acetone (20:1 →15:1). Crystallisation from acetone then gave **6** (4.7 g, 96.1%), m.p. 89–92°, $[\alpha]_D$ −44° (chloroform) (Found: C, 59.44; H, 5.31. $C_{45}H_{48}O_{20}$ calc.: C, 59.47; H, 5.32%).

Methyl 3-O-α-L-arabinofuranosyl-4-O-β-D-xylopyranosyl-β-D-xylopyranoside (**7**). — Methanolic M sodium methoxide (1.5 mL) was added to a solution of **6** (3.6 g) in methanol (150 mL), and the solution was kept for 1 h at room temperature. T.l.c. then showed deacylation to be complete and the presence of a product (R_F 0.35; chloroform–methanol, 4:1). The solution was neutralised with Dowex 50W (H^+) resin, filtered, and concentrated, and the residue was freed from methyl benzoate by chromatography and then crystallised from acetone to give **7** (1.5 g, 88.2%). Recrystallisation from methanol gave material having m.p. 206–209°, $[\alpha]_D$ −110° (water) (Found: C, 44.70; H, 6.73. $C_{16}H_{28}O_{13}$ calc. C, 44.86; H, 6.59%).

Methyl 2,3-di-O-acetyl-4-O-[2,4-di-O-acetyl-3-O-(2,3,5-tri-O-benzoyl-α-L-arabinofuranosyl)-β-D-xylopyranosyl]-β-D-xylopyranoside (**8**). — Compound **5**[7] was condensed with **1** as described for the preparation of **6**. Conventional isolation gave amorphous **8** (91.1%), $[\alpha]_D$ −48° (chloroform) (Found: C, 59.38; H, 5.34. $C_{45}H_{48}O_{20}$ calc. C, 59.47; H, 5.32%).

Methyl 4-O-(3-O-α-L-arabinofuranosyl-β-D-xylopyranosyl)-β-D-xylopyranoside (**9**). — Deacylation of **8**, as described for the preparation of **7**, afforded **9** (89.4%), m.p. 146–147° (from methanol–acetone, 2:1), $[\alpha]_D$ −121° (water) (Found: C, 44.83, H, 6.65. $C_{16}H_{28}O_{13}$ calc. C, 44.86; H, 6.59%).

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