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LOCATION OF *O*-ACETYL GROUPS IN THE ACIDIC D-XYLAN OF *Mimosa scabrella (bracatinga)*. A STUDY OF *O*-ACETYL GROUP MIGRATION

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ABSTRACT

Extraction with dimethyl sulfoxide of wood-meal of the stem of bracatinga (*Mimosa scabrella*), a south Brazilian hardwood, that was defatted and delignified by treatment with aqueous chlorine at 0–5° followed by extraction with cold ethanol, gave a soluble *O*-acetylated 4-*O*-methyl-D-glucurono-D-xylan having (1→4)-linked β-D-xylopyranosyl residues that were unsubstituted (65%) and 2-*O*- (14%), 3-*O*- (16%), and 2,3-di-*O*-acetylated (5%), as determined by methylation analysis. Another preparation obtained by use of refluxing ethanol in the delignification process showed neither removal nor migration of acetyl groups. By comparison with synthetic, partly *O*-acetylated D-xylans of known composition, ^{13}C-n.m.r. spectroscopy indicated that *O*-acetyl group migration does not occur during treatment with cold aqueous chlorine, refluxing ethanol, or water at 70°. Methyl 2-*O*-acetyl-4-*O*-methyl-β-D-xylopyranoside (**6**) was also unaffected by aqueous chlorine. *O*-Acetyl group migration took place more readily in aqueous and dimethyl sulfoxide solutions of **6** than of *O*-acetyl-D-xylans. The lowest temperatures at which migration was observed in monosaccharides was at 50 and 70° for solutions in D_2O and $(CD_3)_2SO$, respectively.

INTRODUCTION

Hägglund *et al.*[1] showed that delignification of birch wood (a technical mixture of *Betula verrucosa* and *Betula pubescens*) using the sodium chlorite method[2], followed by dimethyl sulfoxide extraction (ambient temperature) of the resulting holocellulose, gave a glucuronoxylan having 11% of acetyl groups. The distribution of the *O*-acetyl groups in this birch glucuronoxylan (13% of *O*-acetyl), prepared by

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TABLE I

DISTRIBUTION OF ACETYL GROUPS IN *O*-ACETYL-D-XYLANS

Polysaccharide fraction	*O-Acetyl group substitution in β-D-xylopyranosyl residues (%)*[a]			
	None	*2-O-*	*3-O-*	*2,3-di-O-*
Birch wood[b]	58	12	24	6
Fraction A	60(65)	15(14)	19(15)	6(5)
Fraction B	62(67)	15(14)	18(15)	5(4)
Synthetic C[c]	74(78)	18(16)	6(5)	1(1)
Synthetic D[d]	57(63)	25(23)	11(9)	6(5)
Fraction E	43(48)	15(14)	26(22)	17(16)

[a]Percentages corrected for different rates of degradation of D-xylose and its 2-, 3-, and 2,3-di-*O*-methyl derivatives during hydrolysis of the per-*O*-methylated polysaccharide are in parentheses. [b]Ref. 4. The positions of substitution were determined by g.l.c. examination of methyl *O*-methylxylosides. [c]Prepared with 0.5 equiv. of acetic anhydride. [d]Prepared with 1.0 equiv. of acetic anhydride.

delignification of acetone-extracted wood-meal by the chlorite method[3] at 60° and pH 4.7, was determined by Bouveng[4]. This author used phenylcarbamyl groups as protective substituents, prior to removal of acetate groups and subsequent methylation and removal of the protecting groups. Of the (1→4)-linked β-D-xylopyranosyl residues, 58% were unacetylated, 12% and 24% 2- and 3-*O*-acetylated, respectively, and 6% di-*O*-acetylated (Table I).

Lindberg *et al.*[5] determined the distribution of the *O*-acetyl groups in natural acetylated birch xylan by use of an analagous method in which the free hydroxyl groups were protected as acetals by reaction with methyl vinyl ether[6]. The proportions of nonacetylated and 2-, 3-, and 2,3-*O*-acetylated xylosyl residues were 22:12:11:5. It was observed that these values did not correspond to those determined by acetyl group migration. In studies on migration of *O*-acetyl groups, Garegg[7] found that under the conditions for preparing holocellulose, *i.e.*, with 3% ethanolic 2-aminoethanol, the monoacetates of benzyl 4-*O*-methyl-β-D-xylopyranoside were converted into a mixture in which the 3-acetate preponderated somewhat over the 2-acetate; some deacetylation also occurred. No acetyl group migration was observed on treatment of the 3-acetate with dimethyl sulfoxide under the conditions used for preparation of *O*-acetylated 4-*O*-methyl-D-glucurono-D-xylans. Surprisingly, a slight migration occurred with the 2-acetate isomer.

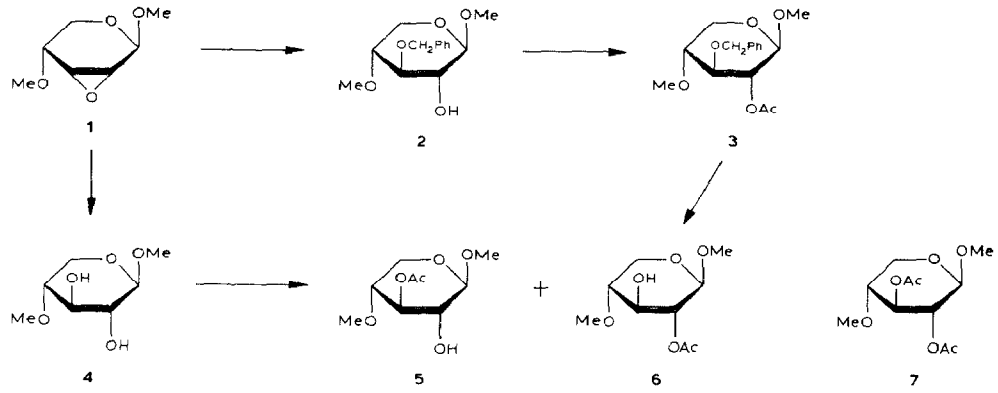
RESULTS AND DISCUSSION

In the present study, we have investigated the location of the acetyl groups in the D-xylan portion of the 4-*O*-methyl-D-glucurono-D-xylan obtained from the branches of the hardwood, bracatinga (*Mimosa scabrella*), previously designated *Mimosa bracatinga*. It provided a hemicellulose A that had a main chain of (1→4)-linked β-D-xylopyranosyl residues substituted at O-2 by single 4-*O*-methyl-α-D-

glucopyranosyluronic acid groups[8]. An *O*-acetyl-D-xylan (Fraction A) was obtained by a procedure that avoided high temperatures and treatment with basic 2-aminoethanol, which might result in *O*-acetyl group migration or removal. A number of branches were obtained at different heights from five different trees and converted into a powder that was defatted with 2:1 benzene–ethanol at 28°. The residue was treated[9] with aqueous chlorine at 0–5°, and then extracted with cold ethanol to remove degraded lignin, followed by cold dimethyl sulfoxide to give a soluble Fraction A containing 4-*O*-methyl-D-glucuronic acid (15%; carbazole method[10]), D-xylose (76%; phenol–sulfuric acid method[11]), and *O*-acetyl groups (9.0%; hydroxylamine method[12]). The acetyl groups were located and estimated by the phenylcarbamate–methylation procedure of Bouveng[4], as simplified by Corrêa *et al.*[13]. The resulting, partially *O*-methylated product was converted into a mixture of partially *O*-methylated xylitol acetates, the positions of the methyl groups of which corresponded to those of the *O*-acetyl groups of the original polysaccharide [the presence of 1,3,5-tri-*O*-acetyl-2,4-di-*O*-methylxylitol indicated 2-*O*-acetyl substitution because of the (1→4)-linked D-xylopyranosyl units in the polymer]. The mixture was analyzed by capillary gas-liquid-chromatography–mass spectrometry (g.l.c.–m.s.). Resolution of the acetates of 2-*O*- and 3-*O*-methylxylitol was achieved with coatings of OV-17 and DB-210 following unsuccessful trial experiments with Durowax-4 and OV-225. SP-1000, which is capable of separating the isomers[14], was not available. Since more symmetrical peaks were obtained with DB-210 than with OV-17, the former coating was used in the present study.

Under the conditions of hydrolysis of the per-*O*-methylated polysaccharide to give a mixture of D-xylose and *O*-methyl derivatives, the decomposition of each fragment took place at a different rate. After subjecting a standard mixture of the four resulting compounds to the complete procedure, g.l.c. analysis indicated that the 3-*O*-methyl derivative was the most stable. By comparison with it, the recovery of the derivatives was 92% for the 2- and 94% for the 2,3-di-*O*-methyl derivative, and 77% for D-xylose. On the basis of the corrected peak areas, Fraction A contains 65% of unacetylated, (1→4)-linked β-D-xylopyranosyl residues; 14 and 15% of 2-*O*- and 3-*O*-acetylated residues, respectively; and 5% of di-*O*-acetylated residues (Table I). These values are similar to those obtained by Bouveng[4] for birch-wood hemicellulose (without correction factors). In the present experiments, no attempt was made to determine the positions of *O*-acetyl groups in the 4-*O*-methyl-D-glucuronic acid units.

Because more vigorous extraction procedures may cause *O*-acetyl group migration, the same batch of bracatinga meal was subjected to a similar extraction process, except that boiling ethanol was used in the delignification step. Methylation analysis of the product (Fraction B) showed that the distribution of *O*-acetyl groups was virtually the same as that in Fraction A (Table I). Also the contents of 4-*O*-methyl-D-glucuronic acid (15%), D-xylose (77%), and acetyl groups (8.2%) were similar.



These results appear to indicate that *O*-acetyl group migration does not take place during treatment with aqueous chlorine, or hot ethanol. It is possible, however, that the ratio of 2-*O*- and 3-*O*-acetylated units in the *O*-acetyl-D-xylan is close to that of an equilibrated mixture, so that any *O*-acetyl group migration would not be detected. Therefore, further studies on the migration of *O*-acetyl groups were carried out on two synthetic, partially acetylated D-xylans (C and D) having different compositions which contained[13] more substituents at OH-2 than -3, and were obtained by treatment with acetic anhydride of commercial D-xylan in formamide containing a little pyridine. On analysis by the methylation method[13] (Table I) they showed more 2-*O*- than 3-*O*-acetyl groups, which agrees with the results of Garegg[7], who showed that acetylation of benzyl 4-*O*-methyl-β-D-xylopyranoside with 1.1 molar equivalent of acetic anhydride in pyridine gave the 2- and 3-acetates in a 1.7:1 ratio.

For the study of migration of acetyl groups of natural and synthetic *O*-acetyl-

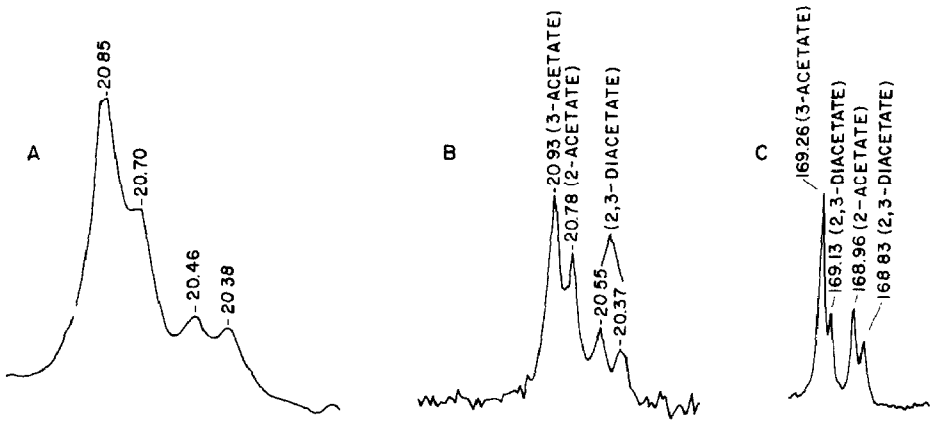


Fig. 1. ^{13}C-N.m.r. spectra of natural *O*-acetyl D-xylan in solution in di($^2H_3$)methyl sulfoxide: (A) CH_3 signals, 100-MHz spectrometer; (B) CH_3 signals, 360-MHz spectrometer; and (C) C=O signals, 360-MHz spectrometer.

TABLE II

^{13}C-N.M.R. DATA (δ) FOR C-1, C=O, AND CH_3CO OF *O*-ACETYL DERIVATIVES OF D-XYLAN AND MODEL COMPOUNDS **4**, **5**, **6**, AND **7**[a]

Compound	*C-1*	*C=O*	*CH_3CO*
Partly acetylated natural D-xylan	101.67	169.25	20.85
	103.06	168.94	20.70
	99.37		20.46
			20.38
D-Xylan diacetate	99.64	169.10	20.35
		168.81	20.22
D-Xylan	101.69		
4	104.40		
5	104.10	169.36	20.79
6	101.50	169.04	20.70
7	100.96	169.34	20.40
		168.95	20.28

[a]As determined at 100 MHz for solutions in $(CD_3)_2SO$ at 50°. The signals are listed in order of decreasing intensity from top to bottom (see Fig. 1A).

D-xylans, ^{13}C-n.m.r. spectroscopy was found to be more rapid and convenient than the methylation procedure, as 2-*O*-, 3-*O*-, and 2,3-di-*O*-acetyl groups show typical, resolved signals for methyl and carbonyl groups, the CH_3 signals of $COCH_3$ being the most suitable. At low field, these signals (Fig. 1B) are better resolved (at 360 MHz) than those of the carbonyl group (Fig. 1C). The CH_3 signals of the natural D-xylan at δ 20.37 and 20.55 were assigned to di-*O*-acetyl groups as they were present in the spectrum of the fully acetylated D-xylan. The methylation results (Table I) showed that natural *O*-acetyl-D-xylan contains more 3-*O*- than 2-*O*-acetyl groups, and these gave a predominant signal at δ 20.93 and another at δ 20.78, respectively. In the cases of synthetic xylans C and D, the signal at δ 20.67 (Fig. 2) is larger, which corresponds to the higher proportion of 2-*O*-acetyl groups. (Some contributions to the signals could arise from acetylated 4-*O*-methyl-D-glucopyranosyluronic acid residues that were not detected in the methylation procedure). Similarly, the signals contributed by the carbonyl groups of the natural *O*-acetyl-D-xylan were assigned. In terms of quantitative determination, the ^{13}C-n.m.r. and methylation methods gave different results, but in our opinion the latter method is more reliable. The synthetic, monomeric analogs of the *O*-acetylated units of the β-D-(1→4)-xylans, the 2-*O*- (**6**), 3-*O*- (**5**), and 2,3-di-*O*-acetyl (**7**) derivatives of methyl 4-*O*-methyl-β-D-xylopyranoside, gave methyl and carbonyl signals having related absolute values and the same, relative chemical-shift values (see Table II).

The partly acetylated D-xylans proved resistant to acetyl group migration. No spectral changes occurred when natural and synthetic *O*-acetyl-D-xylans were subjected to (a) boiling at reflux for 18 h in ethanol as a suspension, (b) being kept in aqueous solution of chlorine for 1 h at 4°, (c) heating in a deuterium oxide solution

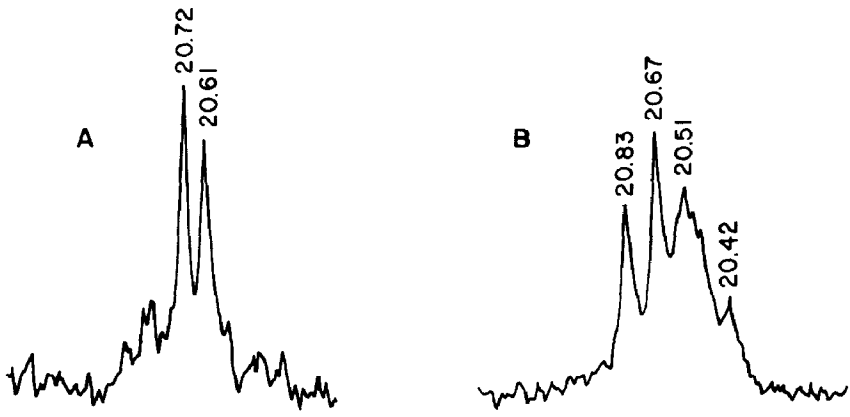


Fig. 2. ^{13}C-N.m.r. spectra of CH_3 region of synthetic acetylated D-xylan in solution in di($^2H_3$)methyl sulfoxide: (A) Prepared with 0.5 equiv., and (B) with 1.0 equiv. of acetylating reagent per sugar residue (360-MHz spectrometer).

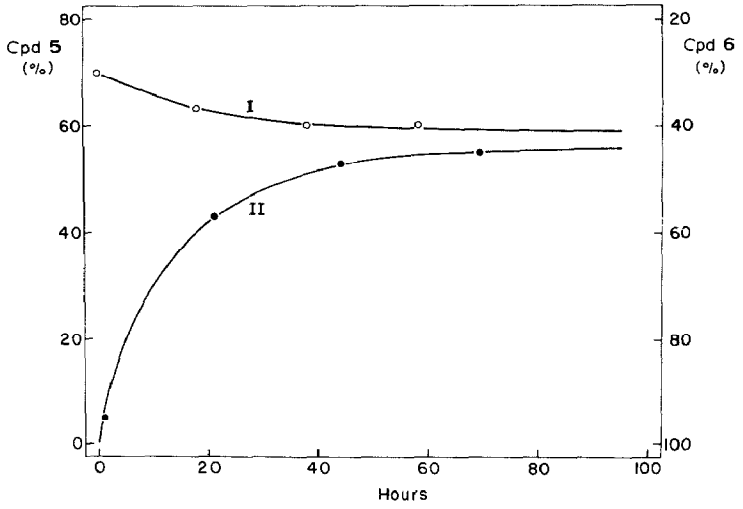


Fig. 3. Equilibrium values of **6** (II) and a mixture containing mainly **5** (I) in solution in deuterium oxide at 70°.

for 64 h at 70°, and (d) heating a di($^2H_3$)methyl sulfoxide solution for 64 h at 70°. In contrast to the D-xylan derivatives, the *O*-acetyl group of the model compound **6** migrated much more readily. By use of the C-1 signals of the ^{13}C-n.m.r. spectrum, which differed in chemical shift from that of **5**, it was possible to rapidly determine, for a solution in deuterium oxide, the proportion of each component. This approach is valid as the ratios corresponded exactly to those obtained under conditions which guaranteed quantitative determination[15]. For a solution at room temperature for 3 days no migration was observed. However, at 50°, 8 and 29% of **5** were detected after 3 and 21 h, respectively. At 70°, the reaction proceeded virtually to equili-

brium after 64 h, 45 and 55%, respectively, of **6** and **5** being present. This was close to the values of 40 and 60%, respectively, observed for a mixture containing mainly **5** (70%) after a treatment of 58 h (see Fig. 3). Under these conditions no free acid was formed. Thus, it appears that the ratios of **6** to **5** in the equilibrated mixture of monosaccharides is close to those observed in the natural *O*-acetyl-D-xylans.

As a solvent of **6**, di($^2H_3$)methyl sulfoxide was found not to promote acetyl group migrations as readily as deuterium oxide. Kept for 21 h at room temperature and at 50°, **6** remained unchanged. However, at 70°, 6 and 20% of **5** were formed after 3 h and 19 h, respectively. No acetyl group migration took place when chlorine was bubbled through a solution of **6** maintained at 0–5°.

For the preparation of **5** and **6**, the following procedures were used. Methyl 2,3-anhydro-4-*O*-methyl-β-D-ribopyranoside[16] (**1**) was converted into the 3-*O*-benzyl derivative **2** by the action of benzyl alcohol and potassium. Acetylation gave **3** and *O*-debenzylation by hydrogenolysis **6**. The 3-*O*-acetyl derivative **5** could not be conveniently prepared in a pure form. Methyl 2,3-anhydro-4-*O*-methyl-β-D-ribopyranoside (**1**) was treated with hot aqueous barium hydroxide (Hough and Jones[16] used hot aqueous sodium hydroxide) to give methyl 4-*O*-methyl-β-D-xylopyranoside (**4**). This was partly benzylated in silver oxide and benzyl bromide in *N,N*-dimethylformamide. The mono-*O*-benzylated fraction was isolated by silicic acid column chromatography to give a fraction that contained, according to ^{13}C-n.m.r. spectroscopy, the 2-*O*- and 3-*O*-benzyl derivatives in a 2.5:1 ratio. Successive acetylation and hydrogenolysis gave a mixture of **5** and **6** in a ratio of 2.3:1 according to ^{13}C-n.m.r. spectroscopy. Although the product was not pure, it was suitable for the study of acetyl group migration in which the equilibrium composition was determined (Fig. 2). The structures of **2** and **3** were confirmed by examination of the ^{13}C-n.m.r. spectra. The shifts of the C-1 and -4 signals of **6**, when compared with those of the unacetylated compound **4**, were displaced by −3.0 and −0.2 p.p.m. respectively. These results would be expected on the basis of shifts of β-carbon atoms occurring on mono-*O*-acetylation[17]. In the case of **5**, the signals for C-1 and -4 underwent displacements of −0.3 and −2.3 (or −3.2) p.p.m., respectively, corresponding to acetylation at O-3.

Preliminary results obtained with another preparation of *O*-acetyl-D-xylan, obtained from bracatinga, showed a possible variation of content and distribution of acetyl groups from sample to sample of wood. A different batch of wood was extracted with 2:1 benzene–ethanol, and the dried residue extracted successively with ammonium oxalate and ethylenediaminetetraacetate (EDTA) prior to treatment with chlorine and dimethyl sulfoxide. The resulting Fraction E contained 4-*O*-methyl-D-glucuronic acid (13%), D-xylose (76%), and acetyl content (11.4%) much higher than those of Fractions A and B. This higher content was also reflected in the content of 2-*O*-, 3-*O*-, and 2,3-di-*O*-acetyl groups (Table I). Although the extraction conditions just mentioned are not expected to cause acetyl group migration, they may be responsible for the selective isolation of a more highly *O*-

acetylated product. The distribution of *O*-acetyl groups in the polysaccharide may not necessarily result from the biosynthesis, as slow migration could occur after polysaccharide formation in the plant.

EXPERIMENTAL

Preparation of O-acetylated D-xylan (Fractions A and B) from Mimosa scabrella. — Powdered stems from bracatinga were delignified according to a combination of methods[9]. Powdered wood was extracted with cold 2:1 benzene–ethanol three times (12 h each) with shaking[18,19]. The residue was suspended in water at 0–5° and Cl_2 passed through for 5 min[20]. The filtered off and dried meal was extracted 3 times with ethanol by shaking for 12 h, and the insoluble material extracted with dimethyl sulfoxide[1]. The extract (Fraction A) was obtained in 0.4% yield. In a similar experiment, except that boiling ethanol was used in place of cold ethanol, Fraction B was obtained in 0.5% yield.

Preparation of O-acetylated D-xylan (Fraction E). — Wood meal, defatted with 2:1 benzene–ethanol in a Soxhlet apparatus (55–60°), was extracted three times with 0.5% aqueous ammonium oxalate for 5 h at 70°, followed by extraction with 0.2% EDTA[21]. (Ammonium oxalate was used originally without EDTA by Bishop[22]). Following aqueous Cl_2 treatment at 0–5°, the dried meal was extracted in a Soxhlet apparatus (55–60°) with ethanol. The product (Fraction E) was obtained in 0.3% yield. This yield is much lower than that obtained on boiling the meal with 3% of 2-aminoethanol in 95% ethanol[4,20], which gave, in 6% yield, a product containing 14 of acetyl groups, 13 of uronic acid, and 72% of D-xylose; and having D-xylopyranosyl units 2-*O*- (19%), 3-*O*- (20%), and 2,3-di-*O*-substituted (13%) with acetyl groups.

Composition of O-acetyl-D-xylans. — The D-xylans were analyzed for content of D-xylose[11], 4-*O*-methyl-D-glucuronic acid[10], and *O*-acetyl groups[12].

Treatment of O-acetylated D-xylans with chlorine. — The solutions of *O*-acetylated D-xylan (100 mg) in water (20 mL) were cooled to 0° and Cl_2 bubbled through for 15 min. The solutions were added to a large amount of ice–water, the mixtures dialyzed, and after 18 h lyophilized.

Treatment of O-acetylated D-xylans with water at 70°. — The *O*-acetylated D-xylans (100 mg) were heated in water (5 mL) for 64 h at 70°.

Synthetic partly acetylated D-xylans. — D-Xylan (0.50 g; Nutritional Biochemicals Corp.) was kept with formamide (20 mL) on a steam bath overnight. Pyridine (0.3 mL) was added with stirring, and acetic anhydride (0.18 mL; 0.5 molar equiv.) in formamide (3 mL) added dropwise with stirring. The mixture was kept overnight at room temperature. It was added to ice–water, and the mixture dialyzed and lyophilized. In another experiment, 1.0 molar equiv. of acetic anhydride (0.36 mL) per unit of D-xylan was added.

G.l.c.–m.s. of O-methylxylitol acetates. — Partially *O*-methylated polysaccharides were prepared from *O*-acetyl-D-xylans by a modification[13] of the

method of Bouveng[4]. Samples (10 mg each) were converted into mixtures of partially *O*-methylated alditol acetates as follows. Each sample was dissolved in 72% aqueous H_2SO_4 (0.5 mL) and, after 1 h at room temperature, water (4 mL) was added, and the solution maintained for 4 h at 100°. The solution was made neutral with $BaCO_3$, the suspension filtered, and the filtrate evaporated to dryness. The products were reduced with $NaBH_4$ and acetylated with acetic anhydride–pyridine.

The resulting mixture was examined by g.l.c. using a fused-silica capillary column (i.d. 0.25 mm; length 30 m), coated with DB-210 and contained in a Model 4000 Finnegan g.l.c.–m.s. unit, interfaced with an Incos 2300 Data System. The electron-impact spectra were obtained repetitively every 2 s by scanning from mass 40 to 420. The injections were carried out in the split mode at 50°, and then programmed (40°/min) to 195° (hold). The carrier gas was helium (linear velocity 35 cm/s). Under these conditions, the *O*-methylxylitol acetates had the following retention times relative to the solvent peak: 2,3-di (7.20), 3-mono (11.35), 2-mono (11.61), and unsubstituted (16.53 min). Other columns (30-m length) tested were of fused silica coated with Durowax-4, and of glass coated with OV-225 or OV-17.

^{13}C-N.m.r. spectroscopy. — The spectra were obtained with spectrometers equipped with a Fourier-transform facility. With a Varian XL-100-15 instrument, the solution (0.85 mL) of each compound (10–100 mg) was contained in a coaxial cylinder fitted snugly within a 12 mm (diam.) × 20 cm tube; the spectral width was 5000 Hz, acquisition time 0.8 s, and the pulse width 9.5 μs. With the Bruker AM-360-WB instrument, the sample in solvent (3 mL) was contained in a 10 mm (diam.) × 20 cm tube; the spectral parameters were: sweep width 20 000 Hz, acquisition time 0.8 s, and pulse width 23 μs. Chemical shifts (δ) are expressed relative to the resonance of Me_4Si, obtained in a separate experiment.

In quantitative experiments where the ratio of area of C-1 signals of **5** and **6** were compared, the just mentioned spectral conditions used with the 100-MHz instrument were found to give the same results as those obtained in a quantitative experiment[15]. In this case, the IPDNA program[23] was used, *i.e.*, a one-pulse experiment with the decoupler on during acquisition, and off during a delay of more than 5 times of the T_1 values of the resonances [1.03 and 1.10 s (±0.01), respectively, for a solution in D_2O at ambient temperature].

Methyl 3-O-benzyl-4-O-methyl-β-D-xylopyranoside (**2**). — Potassium (0.1 g) was dissolved in benzyl alcohol (5 mL), the reaction flask being continuously flushed with N_2 to prevent combustion. Methyl 2,3-anhydro-4-*O*-methyl-β-D-ribopyranoside[16] (**1**; 0.70 g) was added and the mixture maintained for 4 h at 125°. The solution was made neutral by addition of acetic acid, partitioned between chloroform (100 mL) and water (50 mL), and the chloroform layer evaporated to give a residue that crystallized, and was recrystallized from hexane (0.83 g), m.p. 120°, $[\alpha]_D^{25}$ −42° (*c* 1.3, ethanol); ^{13}C-n.m.r.; (100 MHz; $CDCl_3$): δ 138.6,

128.5, 127.9, 127.8 (arom.), 103.9 (C-1), 81.0, 79.0, 74.1 ($CH_2C_6H_5$), 72.2, 62.0 (C-5), 58.4, and 56.7 (OCH_3).

Anal. Calc. for $C_{14}H_{20}O_5$: C, 62.67; H, 7.51. Found: C, 62.78; H, 7.45.

Methyl 2-O-acetyl-3-O-benzyl-4-O-methyl-β-D-xylopyranoside (**3**). — Compound **2** (0.67 g) was dissolved in pyridine (2 mL) containing acetic anhydride (1 mL), and the solution heated to 100° for 15 min and evaporated to give a syrup which crystallized from hexane (0.65 g), m.p. 42°, $[\alpha]_D^{25}$ −18° (*c* 0.8, ethanol); ^{13}C-n.m.r. (100 MHz; $CDCl_3$): δ 169.6 (C=O), 138.5, 128.4, 127.8, 127.6 (arom.), 102.2 (C-1), 80.5, 79.6, 74.2 ($CH_2C_6H_5$), 72.1, 62.9 (C-5), 58.7, 56.4 (OCH_3), and 21.0 (CH_3CO).

Anal. Calc. for $C_{16}H_{22}O_6$: C, 61.92; H, 7.15. Found: C, 61.98; H, 7.15.

Methyl 2-O-acetyl-4-O-methyl-β-D-xylopyranoside (**6**). — Compound **3** (200 mg) was *O*-debenzylated by hydrogenolysis in acetic acid (10 mL) with 5% Pd-on-charcoal as catalyst. The mixture was filtered, and the filtrate lyophilized to give a residue. This was extracted with cold dichloromethane and evaporation gave syrupy **6** (135 mg), $[\alpha]_D^{25}$ −48° (*c* 0.9, ethanol); ^{13}C-n.m.r. [100 MHz; $(CD_3)_2SO$]: δ 169.14 (C=O), 101.44 (C-1), 79.1, 73.4, 72.9, 62.7 (C-5), 58.1, 55.7 (OCH_3), and 20.77 (CH_3CO); ^{13}C-n.m.r. [$(CD_3)_2SO$ at 50°]: δ 169.04 (C=O), 101.50 (C-1), 79.1, 73.5, 72.8, 62.8 (C-5), 58.0, 55.6 (OCH_3), 20.70 (CH_3CO); ^{13}C-n.m.r. (D_2O): δ 174.74 (C=O), 102.8 (C-1), 79.5, 74.6, 73.3, 63.5 (C-5), 59.4, 58.0 (OCH_3), 21.56 (CH_3CO).

Anal. Calc. for $C_9H_{16}O_6$: C, 49.08; H, 7.32. Found: C, 49.22; H, 7.09.

Mixture of **5** *and* **6**. — Methyl 2,3-anhydro-4-*O*-methyl-β-D-ribopyranoside (**1**; 1.5 g) was treated with water (50 mL) containing $Ba(OH)_2$ (2.0 g) for 20 h at 100°. Barium ions were removed with dry ice as barium carbonate, and the filtrate was evaporated. Crystallization of the residue from ether gave methyl 4-*O*-methyl-β-D-xylopyranoside (**4**; 1.04 g), m.p. 93°; ^{13}C-n.m.r. [100 MHz; $(CD_3)_2SO$, 50°]: δ 104.40 (C-1), 79.3 (C-4), 75.3, 73.1, 62.7 (C-5), 57.9, and 55.7 (OCH_3).

Compound **4** (0.54 g) was dissolved in *N,N*-dimethylformamide (3 mL) and silver oxide (3.0 g) added. Benzyl bromide (0.43 mL; 1.2 molar equiv.) was added dropwise to the shaken suspension and agitation continued for 18 h. The mixture was diluted with dichloromethane, the solution filtered, and the filtrate evaporated to give a syrup. Examination on t.l.c. (19:1, v/v, chloroform–ethanol) showed spots corresponding to unchanged material with R_F 0.2, mono-*O*-benzyl derivatives (R_F 0.4), and di-*O*-benzyl derivative (R_F 0.7). Column chromatography on silicic acid provided the di-*O*-benzyl derivative (0.08 g; chloroform eluant) and mono-*O*-benzyl derivatives (0.35 g; 50:1,v/v, chloroform–methanol eluant). The mono-*O*-benzyl fraction contained, according to the area of the C-1 signals, the 2- and 3-*O*-benzyl derivatives in a 2.5:1 ratio. The 3-*O*-benzyl derivative gave signals whose shifts were already known. The ^{13}C-n.m.r. signals (100 MHz) of the 2-*O*-benzyl derivative were as follows ($CDCl_3$): δ 138.56, 128.50, 128.03, 127.81 (arom.), 104.8 (C-1), 82.0, 79.1, 75.1, 74.2, 63.2 (C-5), 58.7, and 56.8 (OCH_3).

The mixture of 2- and 3-*O*-benzyl derivatives of **4** were converted into the

acetates with acetic anhydride–pyridine as described earlier; ^{13}C-n.m.r. (100 MHz; 3-acetate component; $CDCl_3$); δ 170.12 (C=O), 128.3, 127.9, 127.6 (arom.), 105.1 (C-1), 79.0, 77.6, 74.6, 74.2, 63.2 (C-5), 58.5, 56.45 (OCH_3), and 21.07 (CH_3CO); [$(CD_3)_2SO$, 50°]: δ 169.36, 104.1 (C-1), 76.9, 75.8, 70.9, 62.5 (C-5), 57.5, 55.9 (OCH_3), and 20.79 (CH_3CO); (D_2O): δ 174.3 (C=O), 104.8 (C-1), 77.8, 76.9, 72.2, 63.7 (C-5), 59.2, 58.4 (OCH_3), and 21.68 (CH_3CO).

O-Debenzylation with hydrogen in the presence of Pd–C in acetic acid gave a mixture of **5** and **6** in a ratio of 2.3:1 as determined by ^{13}C-n.m.r. spectroscopy.

Migration of O*-acetyl groups of methyl* O*-acetyl-4-*O*-methyl-β-*D*-xylopyranosides.* — Migration was not observed for a solution of **6** in D_2O in a Pyrex tube at room temperature (3 days). It occurred at 50° (8 and 29% after 3 and 21 h, respectively), and at 70°, but without hydrolysis to acetic acid which would give an easily recognizable ^{13}C-n.m.r. signal at δ 177.8, at a field ~3 p.p.m. lower than that given by the carboxyl groups of the *O*-acetyl groups. The equilibrium of the reaction was followed for a solution in D_2O at 70° and the composition at equilibrium was extrapolated as **6** (42%), and **5** (58%).

In di($^2H_3$)methyl sulfoxide **6** did not undergo acetyl group migration at room temperature (3 days) or at 50° after 21 h. At 70°, 6 and 20% of **5** were formed after 3 and 19 h, respectively.

Treatment of **6** *with chlorine.* — Chlorine gas was bubbled into an aqueous solution (5 mL) of **6** (23 mg) which was cooled to 0°. After 15 min, the solution was extracted with ethyl acetate, and the extract washed twice with water and evaporated to a syrup. ^{13}C-N.m.r. spectroscopy (D_2O) at 360 MHz showed the absence of **5**.

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