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ISOLATION FROM A SOFTWOOD XYLAN OF OLIGOSACCHARIDES CONTAINING TWO 4-*O*-METHYL-D-GLUCURONIC ACID RESIDUES*

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ABSTRACT

Partial hydrolysis of a larch arabino(4-*O*-methylglucurono)xylan afforded two series of oligouronides composed of 4-*O*-methyl-D-glucuronic acid and D-xylose residues. The first series included aldouronic acids up to the aldopentaouronic acid. Methylation analysis indicated that the aldopentao- and aldotetrao-uronic acids were mixtures of isomers. One aldotetraouronic acid was isolated and identified as *O*-β-D-Xyl*p*-(1 → 4)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-D-Xyl. The two isomeric aldotriouronic acids were separated from each other. The acids of the second series, which were composed of two uronic acids and 2–4 D-xylose residues, were identified as follows: *O*-β-D-Xyl*p*-(1 → 4)-*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl, *O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl, *O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl, and *O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-D-Xyl. The first three compounds were new acidic oligosaccharides. The 4-*O*-methyl-D-glucuronic acid in the second series was present in a larger proportion than in the first series, indicating that a large proportion of the uronic acid side-chains were located on two contiguous D-xylose residues in the backbone of the softwood xylan.

INTRODUCTION

In a previous paper[1], a dimer of 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl, namely, *O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-D-Xyl, was isolated from a partial hydrolysate of the hemicellulose which was precipitated from the spruce neutral-sulphite liquor. This fact indicated that the 4-*O*-methyl-D-glucuronic acid residues were located on adjacent xylosyl residues in the main chain of the softwood xylan.

In order to ascertain whether this structural feature is a general characteristic

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for softwood xylan, more native xylan was isolated from the holocellulose of larch wood, and its hydrolysate was investigated in detail.

EXPERIMENTAL

General methods. — Optical rotations were measured for aqueous solutions at 25° with an automatic polarimeter (JASCO model DIP-SL). G.l.c. was performed on a Shimadzu model GC-IC chromatograph, with flame-ionisation detectors, glass columns (187.5 × 0.3 cm) containing 3% of OV-17 on Shimalite W (100–120 mesh), and nitrogen as the carrier gas. The column temperature was programmed at 5°/min from 100° → 250°. Mass spectra (75 eV) were recorded on a JEOL JMS-D 100 combined gas chromatograph–mass spectrometer with an ionization current of 300 μA and an ion-source temperature of 270°. Monosaccharides were analysed by a Technicon sugar analyser following the procedure of Kesler[2]. Equivalent weight was determined by automatic titration with 0.01M sodium hydroxide to pH 8 in an atmosphere of nitrogen.

Methylation analysis. — Oligosaccharides (1–2 mg) were methylated with methylsulphinyl carbanion and methyl iodide in methyl sulphoxide, according to a modification[3] of a standard method[4]. Each reaction solution was poured into cold water and extracted with chloroform (5 × 15 ml). The combined extracts were washed five times with water, dried ($CaCl_2$), and concentrated to dryness. The permethylated saccharides were subjected to mass spectrometry and to methanolysis.

Methanolysis was performed with boiling, anhydrous, 2.5% methanolic hydrogen chloride for 8 h. After neutralisation (Ag_2CO_3), filtration, and concentration to dryness, the residues were subjected to g.l.c.–m.s.

Isolation of arabino(4-O-methylglucurono)xylan. — Larch wood (*Larix leptolepis* Gord) was milled to 60–80 mesh in a Wiley mill and exhaustively extracted with methanol (Soxhlet). After removal of arabinogalactan by extraction[5] with water at room temperature for 24 h, the wood meal was delignified with sodium chlorite[6]. The resulting holocellulose (72.5%) was extracted successively with hot water to remove galactoglucomannan[7], and with 5% aqueous potassium hydroxide. The latter extract was adjusted to pH 6 with glacial acetic acid and poured into ethanol (5 vol.). The precipitate (16% based on holocellulose) was collected, washed in succession with 70% ethanol, ethanol, and light petroleum, and dried *in vacuo* over phosphorus pentaoxide. A solution of the crude polysaccharide in water was treated twice with barium hydroxide[8], and then centrifuged. The supernatant solution was neutralised with glacial acetic acid and added to ethanol (5 vol.). The precipitated barium salt of arabino(4-*O*-methylglucurono)xylan (11.3% based on the holocellulose) was recovered, as described above, and treated for a few minutes at 5° with 50% aqueous methanol containing 5% of hydrochloric acid, to remove barium ions[9] and to give the title compound, $[\alpha]_D^{25}$ −31° (*c* 2), d.p. 40 (in cadoxen solution[10]) (Found: OMe, 3.61%; equiv. wt., 901). The ratios of galactose, glucose, mannose, arabinose, and xylose were 0.07:0.06:0.16:0.10:1.00.

The polysaccharide (10 mg) was methylated, and the product was methanolysed for 16 h. G.l.c.–m.s. of the methanolysate revealed methyl 2,3-di-*O*-methyl-D-xylosides[11], methyl 3-*O*-methyl-2-*O*-(methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronate)-D-xylosides as the main products, together with small proportions of methyl 2,3,5-tri-*O*-methyl-L-arabinofuranosides[13] and methyl 2,3,4-tri-*O*-methyl-D-xylosides[13], methyl 2-*O*-methyl-D-xylosides, and contaminants.

Hydrolysis of arabino(4-O-methylglucurono)xylan. — The polysaccharide (8 g) was hydrolysed[1] with 0.125M sulphuric acid at 90° for 15 h. After neutralisation with M sodium hydroxide, the hydrolysate was kept at pH 8 for 4 h at room temperature to hydrolyse the lactones, and then applied to a column of Dowex 1-X8 (AcO^-) resin. The neutral sugars were eluted with water (until the anthrone test was negative), and acidic material (1.77 g) was eluted with 5M acetic acid.

The acidic sugars were separated by chromatography[14] on Aminex A-27 (12–15 μm) and Diaion (23–25 μm) (AcO^-) resins by elution with *A*, 0.08M sodium acetate; *B*, 0.02M sodium acetate; *C*, M acetic acid; and *D*, 0.5M acetic acid. The volume distribution coefficients (D_v) were calculated in the usual way[15]. Eluant *A* gave seven fractions (1–7). Fractions 1–3 were rechromatographed with eluant *B* and *D*, giving five fractions (1-S1, 2-S1, 2-S2, 3-S1, and 3-S2). Fractions 4–7 were purified (eluant *C*) to give 4-S1, 4-S2, 4-S3, 5-S1, 6-S1, and 7-S1, respectively.

Fraction 1-S1 contained 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylotetraoses and had $[\alpha]_D^{25}$ −2° (*c* 0.54) and an equivalent weight of 732 (calc., 736). With eluant *B*, the fraction gave one peak having D_v 0.73 identical with that of *O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl[16]. Hydrolysis of 1-S1 with 2M trifluoroacetic acid at 100° for 2 h gave xylose and 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl. Methanolysis of the permethylated derivatives gave methyl 2,3-di-*O*-methyl-D-xylosides, methyl 2,3,4-tri-*O*-methyl-D-xylosides, methyl 3,4-di-*O*-methyl-2-*O*-(methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronate)-D-xylosides, and methyl 3-*O*-methyl-2-*O*-(methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronate)-D-xylosides, which were identified by g.l.c.–m.s.[11–13,17]. Thus, 1-S1 was a mixture of the aldopentaouronic acids having the uronic acid attached to the nonreducing end of (1 → 4)-β-D-linked xylotetraose and of other possible isomers.

Fraction 2-S1 contained 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylotrioses and had $[\alpha]_D^{25}$ +23° (*c* 1.91) (Found: OMe, 4.88; equiv. wt., 604. $C_{22}H_{36}O_{19}$ calc.: OMe, 5.13%; equiv. wt., 604). 2-S1 gave one peak in eluants *B* and *D*, with D_v 1.66 and 1.98, respectively, which were identical with those of *O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl[16]. Hydrolysis of 2-S1 with 2M trifluoroacetic acid at 100° for 2 h gave xylose and 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl. Methanolysis of the methylated acid gave the same products as those from methylated 1-S1. Thus, 2-S1 is a mixture of aldotetraouronic acids having the uronic acid attached to the nonreducing terminal and central units, respectively, of (1 → 4)-β-D-linked xylotriose. The other possible isomer was present in 2-S2.

Fraction 2-S2 was *O*-β-D-Xyl*p*-(1 → 4)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-D-Xyl, and had $[\alpha]_D^{25}$ +22° (*c* 0.23) and an equivalent weight of 597 (calc., 604). 2-S2 was homogeneous, having D_v 1.66 and 2.20 in eluants *B* and *D*, respectively. Hydrolysis with 2M trifluoroacetic acid at 100° for 2 h gave xylose and 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl. Methanolysis of the methylated acid gave methyl 2,3,4-tri-*O*-methyl-D-xylosides, methyl 2,3-di-*O*-methyl-D-xylosides, and methyl 3-*O*-methyl-2-*O*-(methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronate)-D-xylosides. The structure of 2-S2 was determined by mass spectrometry[18-21].

Fraction 3-S1 was *O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl, and had $[\alpha]_D^{25}$ +52° (*c* 1.67), and D_v 1.04, 4.11, 1.72, and 4.10 in eluants *A*–*D*, respectively (Found: OMe, 6.30; equiv. wt., 458. $C_{17}H_{28}O_{15}$ calc.: OMe, 6.57%; equiv. wt., 472). Hydrolysis with 2M trifluoroacetic acid at 100° for 2 h gave xylose and 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl. Methanolysis of the methylated acid gave methyl 2,3-di-*O*-methyl-D-xylosides and methyl 3,4-di-*O*-methyl-2-*O*-(methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronate)-D-xylosides.

Fraction 3-S2 was *O*-β-D-Xyl*p*-(1→4)-*O*-(4-*O*-Me-α-D-GlcA*p*)-(1→2)-D-Xyl, and had $[\alpha]_D^{25}$ +54° (*c* 0.61), an equivalent weight of 497 (calc., 472), and D_v 1.04, 4.11, and 4.48 in eluants *A*, *B*, and *D*, respectively. Hydrolysis with 2M trifluoroacetic acid at 100° for 2 h gave xylose and 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl. Methanolysis of the methylated acid gave methyl 2,3,4-tri-*O*-methyl-D-xylosides and methyl 3-*O*-methyl-2-*O*-(methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronate)-D-xylosides.

Fraction 4-S1 was 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl, and had $[\alpha]_D^{25}$ +111° (*c* 1.38), D_v 2.85 and 4.52 in eluants *A* and *C*, respectively (Found: OMe, 8.98; equiv. wt., 356. $C_{12}H_{20}O_{11}$ calc.: OMe, 9.12%; equiv. wt., 340). Hydrolysis with 2M trifluoroacetic acid at 120° for 2 h was partial and gave xylose and 4-*O*-methyl-D-glucuronic acid. The mass spectrum of the permethylated acid was identical with that reported by Kováčik *et al.*[17].

Fraction 4-S2 was *O*-β-D-Xyl*p*-(1 → 4)-*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xylp-*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl, and had $[\alpha]_D^{25}$ +37° (*c* 1.07), an equivalent weight of 460 (calc.: 463), and D_v 2.90 and 7.69 in eluants *A* and *C*, respectively. Hydrolysis with 2M trifluoroacetic acid at 100° for 2 h gave xylose and 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl. Methanolysis of the methylated acid gave methyl 2,3,4-tri-*O*-methyl-D-xylosides, methyl 2,3-di-*O*-methyl-D-xylosides, and methyl 3-*O*-methyl-2-*O*-(methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronate)-D-xylosides. When the sodium salt of 4-S2 was reduced with sodium borohydride and the product hydrolysed with M trifluoroacetic acid at 100° for 1 h, it gave *O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-D-xylitol (reduced 3-S1), 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl, 7-S1, and reduced 5-S1. These acids were identified on the basis of their D_v values. Reduced 3-S1 had D_v 0.81, 3.11, and 1.49 in eluants *A*–*C*, respectively. The D_v values of 7-S1 and reduced 5-S1 are described later.

Fraction 4-S3 was *O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl, and had $[\alpha]_D^{25}$ +35° (*c* 1.07), an equivalent weight of 453 (calc., 463), and D_v 3.09 and

8.66 in eluants *A* and *C*, respectively. Hydrolysis with 2M trifluoroacetic acid at 100° for 2 h gave xylose and 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl. Methanolysis of the methylated acid gave methyl 2,3-di-*O*-methyl-D-xylosides, methyl 3,4-di-*O*-methyl-2-*O*-(methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronate)-D-xylosides, and methyl 3-*O*-methyl-2-*O*-(methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronate)-D-xylosides. Reduction of the sodium salt of 4-S3 with sodium borohydride, followed by hydrolysis of the product with M trifluoroacetic acid at 100° for 1 h, gave *O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl, 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl, 5-S1, and 7-S1.

Fraction 5-S1 was *O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl, and had $[\alpha]_D^{25}$ +55° (*c* 2.35), and D_v 7.01 and 17.77 in eluants *A* and *C*, respectively (Found: OMe, 7.02; equiv. wt., 405. $C_{29}H_{46}O_{25}$ calc.: OMe, 7.81%; equiv. wt., 397). Hydrolysis with 2M trifluoroacetic acid at 100° for 2 h gave xylose and 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl. Methanolysis of the methylated acid gave methyl 2,3-di-*O*-methyl-D-xylosides, methyl 3,4-di-*O*-methyl-2-*O*-(methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronate)-D-xylosides, and methyl 3-*O*-methyl-2-*O*-(methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronate)-D-xylosides. Reduction of the sodium salt of 5-S1 with sodium borohydride, followed by hydrolysis of the product with M trifluoroacetic acid at 100° for 1 h, gave *O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-D-xylitol, 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl, 7-S1, and the unhydrolysed reduced 5-S1, namely *O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-D-xylitol (D_v 5.96 and 16.78 in eluants *A* and *C*, respectively).

Fraction 6-S1 was 4-*O*-methyl-D-glucuronic acid[16], $[\alpha]_D^{25}$ +43° (*c* 0.71), D_v 7.96 and 11.53 in eluants *A* and *C*, respectively, and was identified by g.l.c.–m.s. of the trimethylsilyl derivative of its methyl ester methyl glycosides.

Fraction 7-S1 was *O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-D-Xyl, and had $[\alpha]_D^{25}$ +72° (*c* 0.94), and D_v 17.51 in eluant *A* (Found: OMe, 9.38; equiv. wt., 325. $C_{24}H_{38}O_{21}$ calc.: OMe, 9.37%; equiv. wt. 331). Hydrolysis of 7-S1 with 2M trifluoroacetic acid at 100° for 2 h gave only 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl. Methanolysis of the methylated acid gave methyl 3,4-di-*O*-methyl-2-*O*-(methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronate)-D-xylosides and methyl 3-*O*-methyl-2-*O*-(methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronate)-D-xylosides. Reduction of the sodium salt of 7-S1 with sodium borohydride and hydrolysis of the product with M trifluoroacetic acid at 100° for 1 h gave 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl and 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-xylitol, D_v 1.99 and 4.26 in eluants *A* and *C*, respectively.

RESULTS AND DISCUSSION

The extractive-free wood meal was first treated with water, which removed an arabinogalactan[5], and subsequently delignified with acid chlorite[6], giving 72.5% of

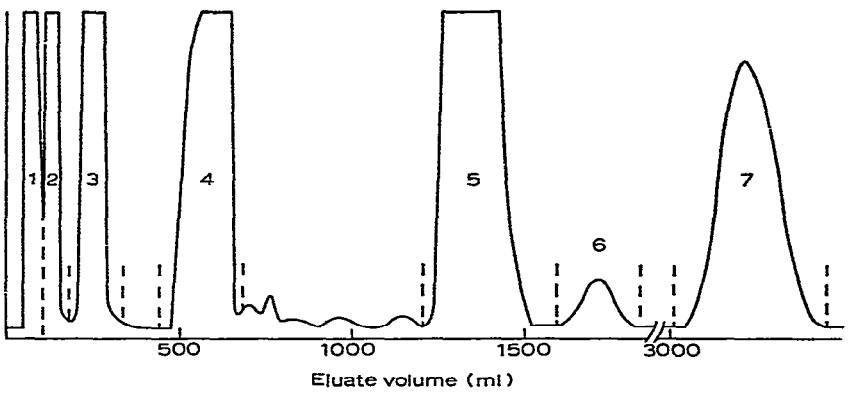


Fig. 1. Fractionation of the uronic acids formed on hydrolysis of arabino(4-*O*-methylglucurono)-xylan; column (15 × 930 mm) of Diaion (AcO^-) resin eluted with 0.08M NaOAc at 2 ml/min.

holocellulose. After removal of galactoglucomannan by extraction with hot water[7], the arabino(4-*O*-methylglucurono)xylan was extracted from the holocellulose with 5% potassium hydroxide and purified by repeated treatment with aqueous barium hydroxide[8]. The analytical data showed that this polysaccharide had the structure of typical softwood xylan[22], containing one 4-*O*-methyl-D-glucuronic acid residue per 5–6 xylose residues and one arabinose residue per 10 xylose residues of a (1 → 4)-β-D-linked framework.

The arabino(4-*O*-methylglucurono)xylan was partially hydrolysed with 0.125M sulphuric acid at 90° for 15 h. The neutral and acidic products were separated, and the latter were fractionated by ion-exchange chromatography using 0.08M sodium acetate as eluant. As shown in Fig. 1, seven main bands were obtained, together with many small bands which were neglected in the present study. The first three bands were rechromatographed in eluants *B* and *D*, giving fractions 1-S1, 2-S1, 2-S2, 3-S1, and 3-S2. The last four bands were purified in eluant *C*, giving fractions 4-S1, 4-S2, 4-S3, 5-S1, 6-S1, and 7-S1.

The fractions were characterised by optical rotation, methoxyl content, equivalent weight, and D_v value. Their structures were determined by hydrolysis, methylation analysis, and mass spectrometry.

Fractions 4-S1 and 6-S1 were 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl and 4-*O*-methyl-D-glucuronic acid, respectively. All other acids afforded, on hydrolysis, xylose and 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl as the main acidic product.

Fractions 1-S1 and 2-S1 corresponded to aldopentao- and aldotetrao-uronic acids, respectively. Methanolysis of the methylated acids yielded methyl 2,3,4-tri-*O*-methyl-D-xylosides, methyl 2,3-di-*O*-methyl-D-xylosides, methyl 3,4-di-*O*-methyl-2-*O*-(methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronate)-D-xylosides, and methyl 3-*O*-methyl-2-*O*-(methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronate)-D-xylosides, which were identified by g.l.c.–m.s.[11–13]. Thus, 1-S1 and 2-S1 were mixtures of

isomeric aldopentao- and aldotetrao-uronic acids, respectively, having the uronic acid groups attached to the nonreducing terminal and other xylose residues.

Fraction 2-S2 was obtained in very small yield and identified as *O*-β-D-Xyl*p*-(1 → 4)-*O*-β-D-Xyl*p*-*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-D-Xyl by mass spectrometry. The mass spectrum of the permethylated derivative showed the characteristic fragmentation of oligosaccharides[18–21]. The presence of 2-S2 indicated that the aldotetraouronic acids 2-S1 were a mixture of the two isomers having the uronic acid group attached to the nonreducing terminal and central xylose residues of xylotriose, respectively.

The aldotriouronic acids were fractionated in eluant *D*. 3-S1 was one of the main products and was identified as *O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl, whereas 3-S2 was obtained in a small proportion and identified as *O*-β-D-Xyl*p*-(1 → 4)-*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-D-Xyl.

These results were in good agreement with those previously obtained for partial hydrolysis of 4-*O*-methylglucurono-[23–25] and arabino(4-*O*-methylglucurono)-xylans[26], and need not be discussed in detail here.

The aldouronic acids consisting of two 4-*O*-methyl-D-glucuronic acid residues and 2–4 xylose residues were present in bands 4-S2, 4-S3, 5-S1, and 7-S1. These acids afforded xylose and 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl on hydrolysis, and showed characteristic behaviour on ion-exchange chromatography, having D_v values higher than those of the conventional aldouronic acids described above in sodium acetate and especially in acetic acid. They also showed severe tailing in chromatography on elution with acetic acid. For example, when fraction 4 (Fig. 1) was rechromatographed with acetic acid, it gave three separated acids, 4-S2 and 4-S3 in addition to 4-S1 (Fig. 2).

7-S1 was previously isolated from the partial hydrolysate of the hemicellulose which was precipitated from the spruce neutral-sulphite liquor[1]. The other three

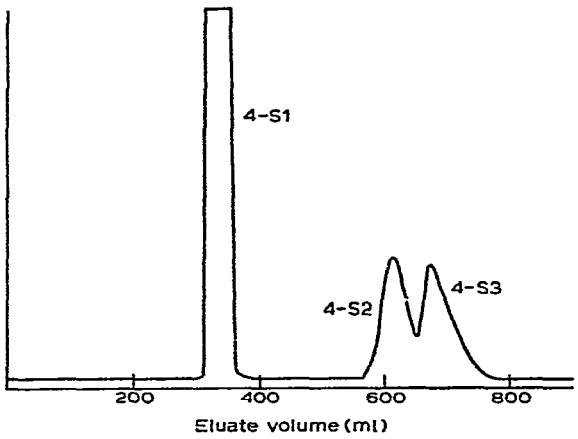
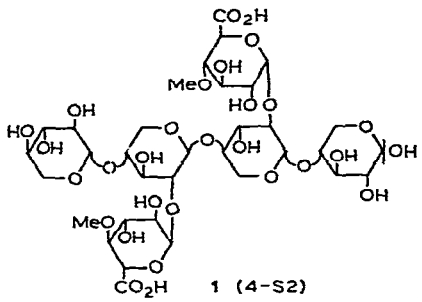
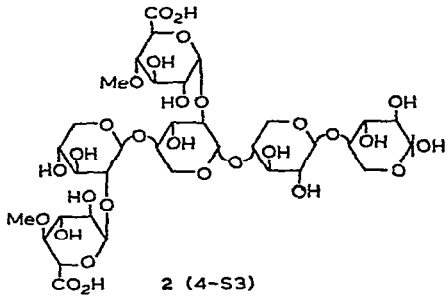
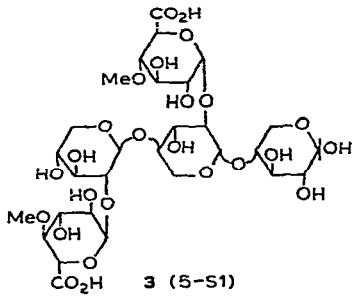
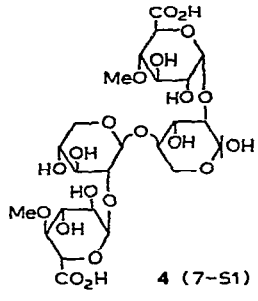


Fig. 2. Rechromatography of band 4 in Fig. 1 on a column (10 × 830 mm) of Aminex 27 (AcO^-) resin with M acetic acid at 0.67 ml/min.


1 (4-S2)


2 (4-S3)


3 (5-S1)


4 (7-S1)

acids, 4-S2, 4-S3, and 5-S1, were new compounds, and their structures are shown in **1–4**. Methylation analyses of 4-S3 and 5-S1 gave methyl 2,3-di-*O*-methyl-D-xylosides, methyl 3,4-di-*O*-methyl-2-*O*-(methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronate)-D-xylosides, and methyl 3-*O*-methyl-2-*O*-(methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronate)-D-xylosides. For 7-S1, methyl 2,3-di-*O*-methyl-D-xylosides were absent, and 4-S2 gave methyl 2,3,4-tri-*O*-methyl-D-xylosides, methyl 2,3-di-*O*-methyl-D-xylosides, and methyl 3-*O*-methyl-2-*O*-(methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronate)-D-xylosides.

Reduction of 7-S1 with sodium borohydride, followed by partial hydrolysis of the product with M trifluoroacetic acid, gave 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl, 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-xylitol, and reduced 7-S1. Likewise, 4-S2 and 5-S1 gave *O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-D-xylitol, 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl, 7-S1, and reduced 5-S1. On the other hand, 4-S3 gave *O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl, 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl, 5-S1, and 7-S1.

The structures of 5-S1 and 7-S1 were further confirmed by mass spectrometry of the permethylated derivatives[27].

The yields of these acids are summarised in Table I, and although the data are only semi-quantitative, it appears that more than half of the 4-*O*-methyl-D-glucuronic acid residues were located on adjacent xylose residues in the main chain.

Recently, Havlicek and Samuelson isolated oligosaccharides consisting of 2–18 xylose residues from the partial hydrolysate of birch xylan, and stated that the 4-*O*-methyl-D-glucuronic acid groups were randomly attached to the xylan backbone[28]. This finding was confirmed by Rosell and Svensson by using specific degradation techniques[29]. However, oligosaccharides containing more than one 4-*O*-methyl-D-

TABLE I

YIELDS OF ACIDIC OLIGOSACCHARIDES OBTAINED FROM A PARTIAL HYDROLYSATE OF ARABINO(4-*O*-METHYLGLUCURONO)XYLAN (8 g)

Band number	*Acidic oligosaccharide*	*Yield (mg)*	*Content of "anhydro" 4-O-Me-GlcA*p *(mg)*
1-S1	Aldopentaouronic acids	10.9	2.8
2-S1	Aldotetraouronic acid	37.9	11.9
2-S2	*O*-β-D-Xyl*p*-(1 → 4)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-D-Xyl	4.5	1.4
3-S1	*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl	121.1	48.7
3-S2	*O*-β-D-Xyl*p*-(1 → 4)-*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-D-Xyl	12.2	4.9
4-S1	2-*O*-(4-*O*-Me-α-D-GlcA*p*)-D-Xyl	141.9	79.3
6-S1	4-*O*-Me-D-GlcA*p*	14.1	12.9
	Total amount	342 6	161.9
4-S2	*O*-β-D-Xyl*p*-(1 → 4)-*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl	31.8	13.0
4-S3	*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl	33.6	13.8
5-S1	*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl	249.3	119.3
7-S1	*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-*O*-β-D-Xyl*p*-(1 → 4)-*O*-(4-*O*-Me-α-D-GlcA*p*)-(1 → 2)-D-Xyl	114.3	69.6
	Total amount	436.0	215.7

glucuronic acid residue have never been isolated from the partial hydrolysates of hardwood xylan, suggesting that few of the acid groups occur on contiguous xylose residues[22]. This fact was confirmed in previous reports[16,30].

4-*O*-Methyl-D-glucuronic acid side-chains are more numerous in softwood than in hardwood xylans, 5–6 xylose residues being present per acid group in the former, compared to 10 in the latter[22]. From the results reported herein, it can be concluded that the distribution mode of uronic acid side-chains is different for hard- and soft-wood xylans. In softwood xylans, the large portion of the uronic acid residues are located on adjacent xylose residues.

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