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STUDIES OF THE POLYSACCHARIDES OF SUGAR CANE *(Saccharum officinarum)*: STRUCTURAL FEATURES OF THE WATER-INSOLUBLE D-XYLANS*

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ABSTRACT

Polysaccharides extracted from the cell-wall material of *Saccharum officinarum* were fractionated into pectic polysaccharides, hemicellulose, and cellulose components. Structural features of the alkali- and methyl sulfoxide-soluble, water-insoluble arabinoglucuronoxylan, arabinoxylan, and *O*-acetylarabinoglucuronoxylan were established by methylation analysis and ^{13}C-n.m.r. spectroscopy. The results show that the D-xylans exhibit heterogeneity and have structural features similar to those found in non-endospermic tissues of Gramineae.

INTRODUCTION

Sugar cane *(Saccharum officinarum)* is a widely distributed member of the Gramineae and an important source of foodstuff. Moreover, its fibrous residue, bagasse, is a raw material for the production of cellulose, paper, boards and 2-furaldehyde, but there have been few studies of its content of polysaccharides[1–3]. We now report on the water-insoluble polysaccharides.

RESULTS AND DISCUSSION

Extractive-free cell walls were depectinated with hot water and ammonium oxalate and then delignified[4]. Treatment of the resulting holocellulose (81.7% of the wall) with alkaline solutions solubilised the hemicelluloses, which were fractionated into water-soluble and water-insoluble materials. The proportions of the polysaccharide fractions and their chemical composition are given in Table I.

The water-insoluble polysaccharide fractions *C-1* and *D-1*, which constitute

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TABLE I

EXTRACTION OF POLYSACCHARIDES FROM *Saccharum officinarum* CELL-WALL MATERIAL (CWM)

Fraction	*Extractant*	*Yield*[a] *(%)*	$[\alpha]_D$[b] *(°)*	*Uronic acid (%)*	*Molar ratios of monosaccharides*						
					Gal	*Glc*	*Man*	*Ara*	*Xyl*	*Rha*	*Fuc*
CWM[c]				1.06	0.6	18.1	0.6	1.4	10.0	0.1	0.1
A	Water	1.31	+21	1.20	14.1	18.4	12.1	5.5	10.0	18.6	12.3
B	aq. 0.5% ammonium oxalate	1.01	+61	1.86	21.5	113.4	20.0	6.6	10.0	24.5	7.2
C-1[d]	aq. 15% potassium hydroxide	30.6	−136	1.08	0.1	1.3	0.1	0.6	10.0		
C-2[e]		6.1	−76	1.80	0.5	1.3	0.1	2.3	10.0	0.1	
D-1[d]	aq. 17.5% sodium hydroxide +	1.1	−111	5.30	tr	1.0	0.3	0.7	10.0		
D-2[e]	4% of boric acid	0.4	−45	2.76	1.1	6.3	4.5	3.1	10.0	0.2	
E	Residue	39.1				100.4		0.6	1.0		

[a]Based on CWM. [b]Aqueous 4% potassium hydroxide. [c]Cellulose, 44.9; lignin (Klason), 20.5; acetyl, 0.3%. [d]Water-insoluble material. [e]Water-soluble material.

TABLE II

O-ACETYLATED POLYSACCHARIDES FROM *Saccharum officinarum* CWM

Fraction	*Extractant*	*Yield*[a] *(%)*	$[\alpha]_D$[b] *(°)*	*Acetyl (%)*	*Molar ratios of monosaccharides*				
					Gal	*Glc*	*Man*	*Xyl*	*Ara*
P-1	Me_2SO	3.3	−100	6.75		0.1		10.0	0.7
P-2	water	1.1	−79	3.87	0.4	0.5	0.1	10.0	1.5

[a]Based on CWM. [b]Aqueous 4% potassium hydroxide.

TABLE III

CHEMICAL COMPOSITION OF NEUTRAL OLIGOSACCHARIDES ISOLATED FROM ARABINOGLUCURONOXYLAN (AGX)

Saccharide	*Yield (mg)*	*D.p.*	$[\alpha]_D$[a] *(°)*	R_{xyl}[b]	*Methylation analysis (mol %)*		
					2,3,4-Me_3-Xyl	*2,3-Me_2-Xyl*	*2,4-Me_2-Xyl*
1[c]	20.2	2	−26	0.70	44.6	49.6	5.8
2	29.0	3	−47	0.44	34.3	65.7	
3	9.5	4	−59	0.32	19.9	80.1	
4	7.0	5	−70	0.15	nd[d]	nd	nd
5	9.2	6	−78	0.07	nd	nd	nd

[a]Water. [b]P.c. (solvent *D*). [c]This compound also contained small amounts (<0.15 mol%) of (1→3)-linked xylobiose. [d]Not determined.

31.4% of the cell wall, were fractionated repeatedly by treatment with Fehling's solution[5]. This process yielded an arabinoglucuronoxylan *AGX*, $[\alpha]_D$ −143° (aqueous KOH), which consisted of xylose and arabinose in the molar proportions 10.0:0.6 together with 1% of uronic acid. A neutral arabinoxylan *AX* was also obtained with $[\alpha]_D$ −132° and a molar ratio of xylose and arabinose of 10.0:0.5.

Treatment of the holocellulose with methyl sulfoxide[6] and subsequently with water gave the partially acetylated hemicellulose fractions *P-1*, $[\alpha]_D$ −100° (xylose–arabinose 10:0.7; OAc, 6.8%), and *P-2*, $[\alpha]_D$ −41° (xylose–arabinose 10:1.5; OAc, 3.9%) (see Table II for yields and analytical data).

Partial hydrolysis of *AGX* with acid gave acidic and neutral fractions. The neutral fraction contained the saccharides **1–5** (Tables III and IV), which were isolated using Sephadex G-10 and by p.c. and identified on the basis of methylation analysis, mass spectrometry, and ^{13}C-n.m.r. spectroscopy as 4-*O*-β-D-xylopyranosyl-D-xylose (**1**) and the corresponding tri- (**2**), tetra- (**3**), penta- (**4**), and hexa-saccharide (**5**). Compounds **1–5** were also identified in the partial hydrolysate of *AX*.

The acidic fraction contained the aldouronic acids **6–9** (Table V) which were characterised by ^{13}C-n.m.r. spectroscopy as 2-*O*-(4-*O*-methyl-α-D-glucopyranosyl-

TABLE IV

^{13}C-N.M.R. DATA FOR NEUTRAL OLIGOSACCHARIDES ISOLATED FROM ARABINOGLUCURONOXYLAN (AGX)

Saccharide[a]	*Residue*	*Chemical shifts (δ, p.p.m.)*				
		C-1	*C-2*	*C-3*	*C-4*	*C-5*
1 β-D-Xyl*p*-(1→4)-D-Xyl*p*	A	α 93.2	72.6	72.1	77.8	60.1
B A		β 97.7	75.1	75.2	72.7	64.2
	B	103.0	74.0	76.8	70.4	66.4
2 β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→4)-D-Xyl*p*	A	α 93.2	72.6	72.1	77.8	60.1
C B A		β 97.7	75.1	75.2	77.6	64.2
	B	102.9	73.9	74.9	77.6	64.2
	C	103.0	74.0	76.8	70.4	66.4
3 β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→4)-β-D-Xyl*p*	A	α 93.2	72.6	72.1	77.8	60.1
C B B A		β 97.7	75.1	75.2	77.6	64.2
	B	102.8	73.9	74.9	77.6	64.2
	C	103.0	74.0	76.8	70.4	66.4

[a]Compounds **4** and **5** showed the same ^{13}C-n.m.r. spectra as **3**.

TABLE V

ACIDIC OLIGOSACCHARIDES FROM ARABINOGLUCURONOXYLAN (AGX)

Saccharide	*Yield (mg)*	*D.p.*	$[\alpha]_D$[a] (°)	R_{GlcA}[b]	*Residue*[c]	*Chemical shifts (δ, p.p.m.)*[d]						
						C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*MeO-4*
6	18.7	2	+86.5	0.67	t-Xyl*p*	α 91.0	77.7	72.6	70.6	62.0		
						β 97.3	79.5	75.6	70.6	66.1		
					GlcA	98.7	72.6	73.4	83.6	70.6	172.3	61.1
7	24.0	3	+57	0.34	t-Xyl*p*	α 93.1	72.5	72.5	77.6	59.8		
						β 97.7	75.2	75.2	77.6	64.0		
					s-Xyl*p*	102.7	78.0	75.6	70.6	66.1		
					GlcA	98.6	72.5	73.5	83.6	70.6	172.3	61.1
8	22.6	4	+25	0.15	t-Xyl*p*	α 93.1	72.5	72.5	77.6	59.8		
						β 97.7	75.2	75.2	77.6	64.0		
					i-Xyl*p*	102.8	74.9	73.9	77.6	64.0		
					s-Xyl*p*	102.8	78.4	77.6	70.6	66.1		
					GlcA	98.7	72.5	73.2	83.6	70.6	172.3	61.1
9	10.8	5	+0.6	0.05								

[a]Water. [b]P.c. (solvent *E*). [c]t, terminal; s, substituted; i, internal. [d]Downfield from the signal for Me_4Si, measured against internal methanol (δ 50.15).

TABLE VI

METHYLATED SUGARS FROM HYDROLYSATES OF THE METHYLATED XYLANS AND *O*-ACETYLARABINOGLUCURONOXYLAN

Sugar (as alditol acetate)	T[b]	*Molar ratios*			*Linkage indicated*
		AGX	*AX*	*P-1*	
2,3,5-Me_3-Ara*f*[a]	0.68	0.93	0.53	1.60	L-Ara*f*-(1→
2,3,4-Me_3-Xyl*p*	0.83	1.00	1.00	1.00	D-Xyl*p*-(1→
2,3-Me_2-Xyl*p*	1.16	13.50	9.52	20.00	→4-D-Xyl*p*-(1→
3,4-Me_2-Xyl*p*	1.03	0.64			→2-D-Xyl*p*-(1→
3-Me-Xyl*p*	1.43	0.20	0.18	0.30	→4,2-D-Xyl*p*-(1→
2-Me-Xyl*p*	1.43	1.83	1.59	2.70	→4,3-D-Xyl*p*-(1→

[a]2,3,5-Me_3-Ara*f* = 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylarabinitol, *etc.* [b]Retention times of the corresponding alditol acetates with respect to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol on column *B* (see Experimental).

uronic acid)-D-xylose (**6**), and (1→4)-linked D-xylobiose (**7**), D-xylotriose (**8**), and D-xylotetraose (**9**) with 4-*O*-methyl-α-D-glucuronic acid linked to O-2 of the non-reducing terminal unit. The aldobiouronic acid **6** has been isolated from various Gramineae xylans, *e.g.*, corn cob[7], cocksfoot grass[8], and jute fibre[9]; in addition to **6**, 2-*O*-(α-D-glucopyranosyluronic acid)-D-xylose has been found in hydrolysates of xylans from wheat straw[10] and oat straw[11].

Methylation of *AGX* by the Hakomori procedure[12] and twice by the Purdie method[13], followed by g.l.c.–m.s.[14] of the derived alditol acetates, revealed derivatives of 2,3,5-tri-*O*-methylarabinose, 2,3,4-tri-*O*-methylxylose, 2,3-di-*O*-methylxylose, and 2(3)-*O*-methylxylose (see Table VI). These results suggested that *AGX* contained a backbone of (1→4)-linked xylopyranosyl residues with more than two of every seventeen residues substituted by terminal arabinosyl and xylosyl groups mainly at O-3. Small amounts of 3,4-di-*O*-methylxylose (Table VI, column 1) indicated the presence of some (1→2) linkages. The average *AGX* molecule contained 168 glycosyl residues, thus suggesting the presence of 18 branch points.

Linkage analysis of *AX* and *P-1* revealed structural features the same as those reported for *AGX* (Table VI), but with different proportions of constituent sugars and pattern of substitution. The average *AX* and *P-1* molecules contained 160 and 142 glycosyl residues, respectively, and thus it is concluded that they have 20 and 16.6 branching points, respectively.

The ^{13}C-n.m.r. spectra of the partially acetylated and deacetylated *P-1* and *AGX* (see Table VII) differ by the presence, in the former, of signals characteristic of acetyl groups (20.1, 20.8, 169.2, and 168.9 p.p.m.). The precise assignments were effected on the basis of the literature data[15–18] and the spectral characteristics of **1**–**9**. The positions of the *O*-acetyl groups were confirmed using methyl 3- and 2-*O*-acetyl-β-D-xylopyranosides as model compounds to represent the (1→4)-linked β-D-xylopyranosyl residues in the native acetylated xylan macromolecule[19].

TABLE VII

^{13}C-N.M.R. DATA FOR SUGAR-CANE D-XYLANS

Arabinoglucuronoxylan			*O-Acetylarabinoglucuronoxylan*		
Chemical shift (p.p.m.)[a]		*Assignment*[b]	*Chemical shift (p.p.m.)*[a]		*Assignment*[b]
171.6	C=O	4-*O*-Methyl-α-D-Glc*p*A-	169.2	C=O	Ac and 4-*O*-methyl-α-D-Glc*p*A-
107.4	C-1	α-L-Ara*f*-	168.9	C=O	Ac
103.1		β-D-Xyl*p*-	106.9	C-1	α-L-Ara*f*-
102.2	C-1	4)-β-D-Xyl*p*-	103.1		β-D-Xyl*p*-
	C-1	4)-β-D-Xyl*p*-2-substituted	101.6	C-1	4)-β-D-Xyl*p*-
99.3	C-1	4-*O*-Methyl-α-D-Glc*p*A-		C-1	4)-β-D-Xyl*p*-2-substituted
86.6	C-4	α-L-Ara*f*-	99.3	C-1	4-*O*-Methyl-α-D-Glc*p*A-
82.1	C-3	4)-β-D-Xyl*p*-3-substituted	86.0	C-4	α-L-Ara*f*-
80.7	C-2	α-L-Ara*f*-	82.6	C-3	4)-β-D-Xyl*p*-3-substituted
	C-4	4-*O*-Methyl-α-D-Glc*p*A-	81.2	C-2	α-L-Ara*f*-
78.4	C-3	α-L-Ara*f*-	81.1	C-4	4-*O*-Methyl-α-D-Glc*p*A-
77.2	C-2	4)-β-D-Xyl*p*-2-substituted	77.7	C-3	α-L-Ara*f*-
76.9	C-3	β-D-Xyl*p*-	76.7	C-2	4)-β-D-Xyl*p*-2-substituted
76.3	C-4	4)-β-D-Xyl*p*-		C-3	β-D-Xyl*p*-
74.4	C-3	4)-β-D-Xyl*p*-	75.3	C-4	4)-β-D-Xyl*p*-
73.9	C-2	β-D-Xyl*p*-	73.9	C-3	4)-β-D-Xyl*p*-
73.2	C-2	4)-β-D-Xyl*p*-	73.3	C-2	β-D-Xyl*p*-
72.5	C-3	4-*O*-Methyl-α-D-Glc*p*A-	72.5	C-2	4)-β-D-Xyl*p*-
70.2	C-4	β-D-Xyl*p*-	71.5	C-3	4-*O*-Methyl-α-D-Glc*p*A-
65.7	C-5	β-D-Xyl*p*-	70.2	C-4	β-D-Xyl*p*-
63.6	C-5	4)-β-D-Xyl*p*-	65.7	C-5	β-D-Xyl*p*-
61.9	C-5	α-L-Ara*f*-	63.1	C-5	4)-β-D-Xyl*p*-
61.1	OCH_3		61.9	C-5	α-L-Ara*f*-
			61.2	OCH_3	
			20.8	CH_3	of AcO-3-β-D-Xyl*p*-
			20.1	CH_3	of AcO-2-β-D-Xyl*p*-

[a]Downfield from the signal for Me_4Si, measured against internal methanol (δ 50.15). [b]The assignments were made by comparison with literature values (refs. 16–19) and data for **1–9**.

The native *O*-acetylarabinoglucuronoxylan contained one acetyl group per 4.2 xylopyranosyl residues. Signals at 20.1 and 20.8 p.p.m. in the spectrum of *P-1* and the ratio of their intensities suggest that the acetyl groups are located on O-3 and O-2 of the β-D-xylopyranosyl residues in the relative proportions 1.3:1.0. Similar patterns of substitution have been found in xylans from several tree species[20,21]. Migration of acetyl groups during polysaccharide isolation cannot be excluded[22].

The above results indicate sugar cane to contain water-insoluble substituted xylans in which there is a small variation in the proportions of the constituent sugars and in the pattern of substitution, although they have similar linkages.

Xylans from *Saccharum officinarum* have the same structural features as those reported for other Gramineae xylans. There are differences in chemical composition as well as size and shape of the macromolecules associated with side chains[23–25]. Xylans present in sugar can exhibit heterogeneity as has been reported for primary cell-wall constituents such as xyloglucans[26,27] and pectic polymers[28,29].

EXPERIMENTAL

General. — All evaporations were conducted under diminished pressure at <40°. Optical rotations (1-mL cell) were measured, unless stated otherwise, for solutions in aqueous 4% potassium hydroxide at 20 ±1° with a Perkin–Elmer Model 141 polarimeter. G.l.c. was performed on a Hewlett–Packard Model 5711 A instrument with *A*, a column (305 × 0.3 cm) of 1% of XE-60 on Gas Chrom Z (80–100 mesh) at 130→150° at 1°/min, with N_2 as the carrier gas at 36 mL/min; *B*, a column (200 × 0.3 cm) of 3% of SP-2340 on Chromosorb WAW-DMCS (80–100 mesh) for 4 min at 180° then →210° at 2°/min, with N_2 at 30 mL/min; and *C* a column (400 × 0.45 cm) of 10% of Carbowax 400 on Chromosorb WAW (80–100 mesh) at 45°, with a N_2 flow rate of 17 mL/min. Column *A* was used for quantitative analysis of sugar trifluoroacetates[30].

G.l.c.–m.s. was carried out with a JGC-20 K gas chromatograph fitted with column *B*, and with helium (inlet pressure, 101.3 kPa) as the carrier gas. Mass spectra were obtained at 23 eV and an emission current of 300 μA, using a JMS D (JEOL) spectrometer. The inlet temperature was 220° and that of the ionising chamber 200°.

Descending p.c. was conducted on Whatman No. 1 and 3MM papers with *D*, ethyl acetate–pyridine–water (8:2:1); *E*, ethyl acetate–acetic acid–water (18:7:8); *F*, 1-butanol–ethanol–water–ammonia (4:1:5:trace). Aniline hydrogen phthalate was used for detection. The mobilities (R_{Xyl} and R_{GlcA}, respectively) are expressed relative to those of D-xylose and D-glucuronic acid. The retention times (*T*) of the methylated alditol acetates are given relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. Uronic acids were determined by the modified carbazole method[31] in the fraction eluted from Dowex 1 (AcO^-) resin with 6M acetic acid after total hydrolysis, and acetyl contents by g.l.c. on column *C*.

^{13}C-N.m.r. spectra were recorded for solutions in D_2O and $(CD_3)_2SO$ with a Bruker AM-300 FT-spectrometer operating at 75.46 MHz in the noise-decoupled mode with deuterium lock. A spectral width of 17.241 Hz was used, 16k was used for the acquisition, and F.t. was with 32k (zero filling), an acquisition time of 0.48 s, and a relaxation delay of 3 s. Spectra were calibrated in D_2O (internal MeOH, *vs.* Me_4Si at 50.15 p.p.m.) and $(CD_3)_2SO$ (signal of the solvent *vs.* Me_4Si at 39.4 p.p.m.).

Determination of molecular weight. — The number-average molecular weights ($\overline{M}_n$) were determined by osmotic pressure measurements on solutions in Me_2SO with a Knauer Membrane Osmometer and with Schleicher–Schüll membranes at 35°. The value for $(h/w)_{w=0}$ for *AGX* was 10.6 where *h* was the osmotic height in cm of the solution and *w* was the concentration in g/kg of the solution, giving an $\overline{M}_n$ value of 22,300 (average d.p., 168). The $\overline{M}_n$ values for *AX* and *P-I* were 21,180 and 18,980, respectively.

Hydrolysis of the polysaccharides. — Complete hydrolysis of the polysaccharides was achieved by heating the sample (10 mg) with aqueous 90% formic acid (1 mL) for 8 h at 100°.After evaporation of the formic acid, the hydrolysate was

applied to a column (1 × 10 cm) of Dowex 1 (AcO^-) resin and eluted with water (200 mL) and then 6M acetic acid (100 mL). Each eluate was concentrated to dryness. The sample *E* and bagasse were treated with aqueous 72% sulfuric acid for 1 h at 20°, and the hydrolysate was then diluted to 3% acid, heated for 4 h at 100°, cooled, neutralised ($BaCO_3$), filtered, treated with Dowex 50 (H^+) resin, and concentrated. The monosaccharides in the hydrolysates were converted into the corresponding alditol trifluoroacetates which were analysed by g.l.c. (column *A*).

The oligosaccharides (0.1–2 mg) were hydrolysed completely with aqueous 90% formic acid (0.1–0.5 mL) for 4 h at 100°. The products in the hydrolysates were examined by p.c. (solvent *D* and *E*) and g.l.c. of the derived alditol acetates.

The acidic oligosaccharides were treated with methanolic 3% HCl (12 h, 90°), and the products were reduced ($NaBH_4$ in dry methanol, 16 h), then hydrolysed with aqueous 90% formic acid (4 h, 100°), converted into the corresponding alditol trifluoroacetates, and analysed by g.l.c.

Plant material. — Sugar cane (*Saccharum officinarum*, var. J-60-5; 23.2 kg) cultivated in the Sugar Cane Experimental Centre, Bauta, Havana, was cut and the juice was separated by rolling and then extraction with water (twice, 24 h, 40°). The fibrous residue was defibrated in a Sproud Waldrom disk refinator to yield 3.5 kg of air-dried cell-wall material (CWM). Standard analytical methods showed that the CWM contained (%) cellulose (44.9), lignin (Klason; 20.5), uronic anhydride (1.0), acetyl (0.3), galactose (0.4), glucose (50.4), mannose (1.7), arabinose (3.3), and xylose (23.2).

Fractionation of CWM. — Sawdust (40–60 mesh) obtained from freshly cut sugar-cane stalks was extracted with benzene–ethanol (35:65) for 4 h, then stirred (30 mL/g) with (*a*) water [4 h, 80° (bath)], and (*b*) aqueous 0.5% ammonium oxalate (4 h, 80°) to remove pectic substances. The depectinated residue (60 g) was treated with acidified sodium chlorite solution[4] for 2 h at 25°, and the resulting holocellulose (49.02 g, 81.7% of the CWM) was extracted as follows. (*a*) The holocellulose (20.0 g) was extracted (4 h, 25°, under N_2) successively with aqueous 15% potassium hydroxide and then aqueous 17.5% sodium hydroxide containing 4% of boric acid. Each extract was filtered and neutralised with acetic acid to give the insoluble fractions *C-1* and *D-1*, respectively, which were collected by centrifugation at 1.8×10^4 r.p.m. The soluble fractions, *C-2* and *D-2*, were obtained by precipitation with ethanol (10 vol.) following exhaustive dialysis against distilled water. (*b*) Holocellulose (28.0 g) was extracted (30 mL/g) twice with methyl sulfoxide[6] (72 h, 25°) to give polysaccharide *P-1* (1.13 g, 3.3% of the CWM), $[\alpha]_D$ −100° (*c* 1) (Found: uronic acid, 0.7; acetyl, 6.8%; $\overline{M}_n$ 18,980), which, on hydrolysis, gave xylose, arabinose, and glucose in the molar ratios 10.0:0.7:0.1. The Me_2SO-insoluble residue (26.8 g) was further extracted with water (30 mL/g, 72 h, 25°) to yield an insoluble residue (26.3 g) and soluble material which was dialysed exhaustively against distilled water and then freeze-dried to yield polysaccharide *P-2* (377 mg, 1.1% of the CWM), $[\alpha]_D$ −79° (*c* 1) (Found: uronic acid, traces; acetyl, 3.9%), which, on hydrolysis, gave xylose, arabinose, glucose, galactose,

and mannose in the molar ratios 10.0:1.5:0.5:0.4:0.1. Data on these fractions are given in Table II.

Arabinoglucuronoxylan. — (*a*) *Isolation*. Fraction *C-1* (1 g), $[\alpha]_D$ −136° (*c* 1), was fractionated by precipitation from solution in aqueous 4% potassium hydroxide (100 mL) by addition of Fehling's solution[5] (10–15 mL). The resulting precipitate was dispersed in water (50 mL), and 0.2M hydrochloric acid was added, followed by precipitation with ethanol (5 vol.). Repetition of this procedure twice gave no further change in the sugar composition of the arabinoglucuronoxylan *AGX* (807 mg, 24.7% of the CWM), $[\alpha]_D$ −143° (*c* 1), which, on hydrolysis, gave xylose and arabinose in the molar ratio 10.0:0.6 (Found: uronic acid, 1.0%; $\overline{M}_n$ 22,300).

Fraction *D-1* (200 mg), $[\alpha]_D$ −111° (*c* 1), was treated, as described above for *C-1*, to give arabinoxylan *AX* (169.7 mg, 0.7% of the CWM), $[\alpha]_D$ −132° (*c* 1), which, on hydrolysis, gave xylose and arabinose in the molar ratio 10.0:0.5 (Found: uronic acid, 0%; $\overline{M}_n$ 21,180).

(*b*) *Partial hydrolysis*. *AGX* (2.0 g) was treated twice with 0.25M trifluoroacetic acid (60 mL) for 2 h at 100°. The hydrolysate was concentrated after each treatment and the part to be hydrolysed further was collected by centrifugation. The combined hydrolysates were concentrated and the residue was resolved into neutral and acidic fractions on a column of Dowex 1 (AcO^-) resin.

The low-molecular-weight neutral fragments (684 mg), after fractionation on a column (2.5 × 100 cm) of Sephadex G-10 with water, gave xylose, arabinose, and a mixture (174.9 mg) of oligosaccharides. Preparative p.c. on Whatman No. 3MM paper (solvent *D*) gave **1–5** (Tables III and IV). The acidic fragments (82 mg) were subjected to preparative p.c. (solvent *E*) to give **6–9** (Table V). ^{13}C-N.m.r. data were recorded for each oligosaccharide. The neutral oligosaccharides (4 mg) were each methylated with methyl iodide (2 mL) and sodium hydride (20 mg) in *N*,*N*-dimethylformamide (2 mL). The products were hydrolysed with 0.1M hydrochloric acid, the hydrolysate was neutralised, and the products were analysed as alditol acetates by g.l.c.[14] (column *B*).

Oligosaccharides **6–9** were also methylated (Hakomori), and the products were reduced ($NaBH_4$), hydrolysed, and analysed by g.l.c. as the partially methylated alditol acetates.

(*c*) *Methylation analysis*. A portion (55 mg) of *AGX* was methylated once by the Hakomori method[12], then twice by the Purdie method[13], to give a product (53 mg), $[\alpha]_D$ −74° (*c* 1, chloroform), showing no i.r. absorption for hydroxyl (Found: methoxyl, 37.6%; $\overline{P}_n$ 76). Likewise, *AX* (30 mg) was methylalted to give a product (25 mg) having $[\alpha]_D$ −69° (*c* 0.5, chloroform) (Found: methoxyl, 38.1%; $\overline{P}_n$ 84).

The methylated polysaccharides (5 mg of each) were treated with aqueous 90% formic acid (2 mL) for 3 h at 100°, the hydrolysates were concentrated to dryness, and each residue was hydrolysed with 2M hydrochloric acid (2 mL) for 6 h at 100°. P.c. then revealed mono-, di-, and tri-*O*-methyl saccharides with R_{Xyl} 1.00, 0.94, 0.89, 0.82, and 0.52 (solvent *F*), which were converted into their alditol acetates. Analysis by g.l.c.–m.s.[14] gave the results in Table VI.

O-Acetylarabinoglucuronoxylan. —(*a*) *Methylation. P-1* (10 mg), $[\alpha]_D$ −100° (*c* 1), was methylated (Hakomori) to give a product (8 mg) having $[\alpha]_D$ −78° (*c* 1, chloroform) (Found: OMe, 37.8%) (see Table VI, column 3).

(*b*) *Location of the O-acetyl groups.* The location of the acetyl groups in *P-1* was determined qualitatively by ^{13}C-n.m.r. spectroscopy, employing the data for methyl 2-*O*- and 3-*O*-acetyl-β-D-xylopyranosides[19].

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