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Studies on Hemicelluloses in Tension Wood

II. Structural Studies on Xylans from Tension, Opposite and Side Woods of Japanese Beech (*Fagus crenata* Blume)

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Abstract——Structural differences in three 4-*O*-methyl-glucuronoxylans from tension, opposite and side woods of Japanese beech (*Fagus crenata* Blume) were investigated. Each 4-*O*-methyl-glucuronoxylan was isolated from the 1% potassium hydroxide extract and purified by gel filtration on Sepharose 4B. The weight average molecular weights of three xylans were similar and estimated to be in the range of 20,000–25,000 by gel filtration. A structural analysis by the methylation method and ^{13}C-n.m.r. spectroscopy showed that no substantial differences were detected between these three xylans. ^{13}C-N.m.r. spectroscopy was used in determination of the ring size, anomeric configuration, the position of *O*-glycosidation and the purity of these xylans, suggesting the effectiveness of this technique for characterization of polysaccharides in wood.

1. Introduction

Tension wood is peculiar in its low lignin and high cellulose contents[1~11]. As for hemicellulose, tension wood contains much more galactose than does normal wood[12~15]. Kuo and Timell[16], and Meier[17] have shown that the origin of galactose in tension wood is exclusively ascribed to galactan which is unique among wood polysaccharides both in its structural complexity and its high degree of branching. The structural studies on hemicelluloses in tension wood are, however, confined to the galactan. The problem of the structure of the other hemicelluloses in tension wood is remained to be solved. We have previously shown the similarity in the molecular weights of the hemicelluloses extracted from three different types of woods, tension, opposite and side woods of Japanese beech (*Fagus crenata* Blume), particularly those included in the Sepharose 4B gel matrices[18]. We have also shown that the extracts with 1% and 24% potassium hydroxide solutions are rich in xylan and are attractive source for elucidation of xylan.

In this work, we extended the previous studies on hemicelluloses in tension wood. We isolated and characterized three xylans from the 1% potassium hydroxide extracts of tension, opposite and side woods of Japanese beech (*Fagus crenata* Blume). The ^{13}C-n.m.r. spectroscopic analysis of these xylans was also undertaken.

* Research Section of Wood Chemistry.

2. Experimental

2.1 Materials

Japanese beech (*Fagus crenata* Blume) having several bands of tension wood in the 130–150th growth rings from the pith was obtained from Kyoto University Forest and separated into tension wood (T), opposite wood (O) and side wood (S) as previously described[18]. Dextran T fractions (T-10, T-20, T-40, T-70 and T-500), FITC Dextran (FITC-3) and Sepharose 4B were obtained from Pharmacia Fine Chemicals. Xylo-oligosaccharides were obtained by gel filtration of the partial acid hydrolysate of normal beech 4-*O*-methyl glucuronoxylan on Toyopearl HW40-S. An aldobiouronic acid (2-*O*-(4-*O*-methyl-*α*-D-glucopyranosyluronic acid)-D-xylose) was prepared following the procedure of Roy and Timell[19]. All other reagents used were analytical reagent grade.

2.2 General methods

Total carbohydrate and uronic acid contents were determined by the phenol-sulfuric acid method[20] and the modified carbazole method[21], respectively. The neutral sugar compositions were analyzed after hydrolysis with 1 N H_2SO_4 for 6 hr at 100°C. The hydrolysate was neutralised with barium carbonate and deionised with Dowex 50 W×8 (H^+) and Dowex 1×8 (AcO^-). The neutral sugars were converted into corresponding alditol acetates and separated by g.l.c. on a column (2 m×0.3 cm) of 3% ECNSS-M on Gas Chrom Q at 180°C using methyl *β*-D-glucopyranoside as an internal standard[22]. Configurations of the monosaccharides were determined by g.l.c. on a S.C.O.T. column of SP-1000 at 200°C after conversion into acetylated (+)-2-octyl D- and L-glycosides[23]. Partially methylated alditol acetates were separated by g.l.c. on a column of ECNSS-M as described above at 170°C and 150°C, and analyzed by g.l.c.-m.s. with a Shimadzu LKB-9000 system, using a column of 3% OV-225 on Gas Chrom Q at 170°C[24]. The ionization potential was 70 eV, the ionization current was 30 *μ*A and the temperature of the ion-source was 210°C. The purity of xylo-oligosaccharides was analyzed by high performance liquid chromatography on Toyo soda HLC-803B with a Toyo soda RI-8 and a JASCO Finepac Sil NH_2 (25 cm×4.6 mm I.D.) using 75% aqueous acetonitrile as an eluent. ^{13}C-N.m.r. spectra were obtained at 80°C for solutions in deuterium oxide with a Varian XL-200 spectrometer operating at 50.3 MHz. Chemical shifts in p.p.m. are given as relative values to that of internal 1,4-dioxane, which was taken as 67.40 p.p.m. downfield from tetramethylsilane. Coupling constants ($^1J_{^{13}C-^1H}$) were determined by the gated-decoupling technique. I.r. spectra were determined for KBr discs, using a JASCO IR-S spectrophotometer.

2.3 Isolation of xylan

Each wood area was milled to 42–60 mesh, extracted stepwisely with ethanol-benzene (1:2, v/v), cold water, hot water and 0.25% potassium acetate, and delignified as previously described[18]. The delignified wood meal was subjected to fractional extractions with dimethylsulfoxide, hot water, 1% potassium hydroxide and 24% potassium hydroxide as previously described[18]. The fractions E and F extracted with 1% potassium hydroxide and 24% potassium hydroxide, respectively, were rich in xylan. Since all E fractions, however, had lower molecular weight than the F fractions and contained only a small amount of substances which were eluted at the void volume by analytical gel filtration, it seems relatively easy to isolate xylan from these E fractions. Purification of xylan was performed on a column of Sepharose 4B (6 × 100 cm) equilibrated with 0.25 M sodium phosphate buffer (pH 6.8). About 200 mg of each fraction E was solubilized in the same buffer and applied on the column. Twenty ml fractions were collected at a flow rate of 60 ml/hr at room temperature. The sugar content of each fraction was monitored by the phenol-sulfuric acid method. The carbohydrate-containing fractions were combined and dialyzed throughly against distilled water and lyophilized.

2.4 Methylation analysis

The sample (20 mg) was methylated by the method of Hakomori[25] followed by the method of Kuhn[26]. The product showed no absorption for free hydroxyl groups in its i.r. spectrum. The fully methylated xylan was treated with 90% formic acid for 2 hr at 100°C followed by 0.5 N H_2SO_4 for 12 hr at 100°C[27]. The partially methylated monosaccharides were analyzed by g.l.c. and g.l.c.-m.s. after conversion into the corresponding alditol acetates.

3. Results and Discussion

3.1 Purification and chemical properties of xylans

The E fractions from three parts of woods, tension wood (T), opposite wood (O), and side wood (S), were separately subjected to gel filtration on Sepharose 4B. The elution profiles were shown in Fig. 1. Each fraction E was separated into three subfractions (I, II and III). These three fractions were pooled and throughly dialyzed against distilled water and lyophilized. The yields and chemical properties were listed in Table I. A trace amount of contaminating rhamnose, mannose and glucose residues in the original E fractions were concentrated in the fractions I and II, and removed effectively by gel filtration. Although the fraction III is almost free from these sugars, a small amount of arabinose and galactose residues are still remained especially in the tension wood (TE-III). Since these contaminating

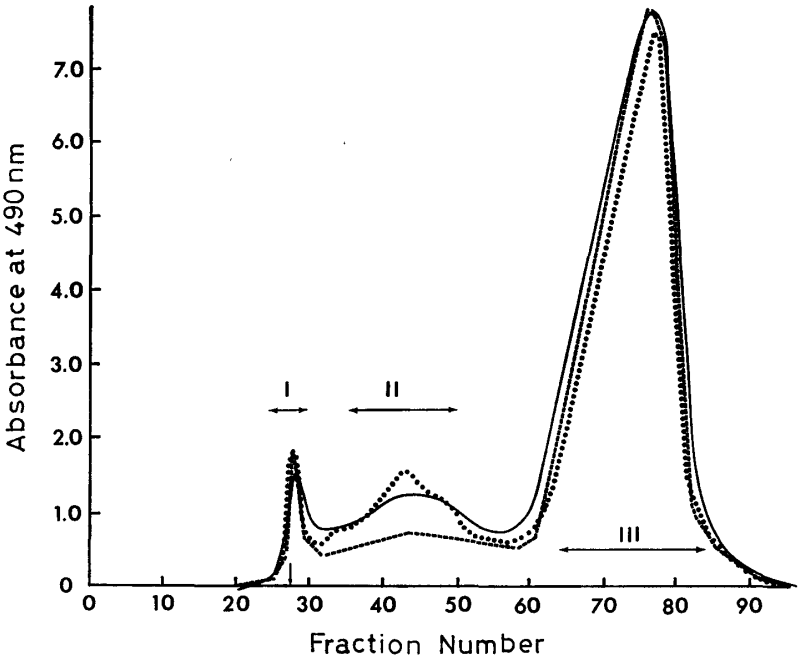

Fig. 1. Preparative gel filtration of the E fractions on Sepharose 4B. The arrow indicates the void volume determined with Blue Dextran. Each fraction was analysed for carbohydrate in tension wood (——), opposite wood (- - -) and side wood (……). The fractions designated by the double arrows were pooled to obtain I, II and III fractions.

Table I. Chemical properties of the purified fractions.

	TE			OE			SE		
	I	II	III	I	II	III	I	II	III
Yield (%)	19.1	15.4	61.6	21.6	10.4	66.2	9.1	20.2	73.1
Neutral sugar composition*									
L-Rhamnose	4.2	9.1	0.9	6.6	6.5	0.0	1.0	0.0	0.0
D-Mannose	3.8	0.6	0.0	4.8	2.3	t	0.7	3.2	0.0
L-Arabinose	0.8	2.9	1.8	t	0.8	0.0	3.5	3.3	3.6
D-Galactose	1.0	33.9	15.9	t	9.5	3.3	t	3.6	0.0
D-Xylose	82.5	51.1	81.4	78.4	77.7	96.7	87.5	84.3	96.4
D-Glucose	7.8	2.5	t	10.2	3.2	t	7.4	5.6	0.0
Uronic acid content**	/	/	9.18	/	/	9.21	/	/	9.56
$[\alpha]_D^{20}$	/	/	-63.6°	/	/	-71.3°	/	/	-69.4°

*Values in per cent of neutral sugars. **Values in per cent of purified materials.

sugars could not be removed by rechromatography, we used the fractions III for further structural analyses. The weight average molecular weights of all III fractions

were similar ranging from 20,000 to 25,000. Timell has reported that tension wood may contain less uronic acid than normal wood, assuming that the chemical structure of xylan in tension wood is the same as that of xylan in normal wood[12]. Present results supported this posturation. The differences between tension and normal wood xylans were not so evident as in the case of compression wood xylan[28].

3.2 Methylation and i.r. spectroscopic analyses

The fully methylated all III fractions were individually converted into partially methylated alditol acetates and analyzed by g.l.c. and g.l.c.-m.s. as shown in Table

Table II. Methyl ethers from the hydrolysate of the methylated xylans*

Methylated sugars**	TE-III	OE-III	SE-III
2, 3, 4, 6-Gal	1.5	—	—
2, 3, 5-Ara	0.7	—	—
2, 3, 4-Xyl	0.9	1.0	1.0
2, 3, 4-Gal	6.6	—	—
2, 3, 6-Gal	5.7	—	—
2, 3-Xyl	75.3	88.8	90.0
3-Xyl	9.3	10.2	8.8

*Values in molar per cent of partially methylated sugars.
**2, 3, 4, 6-Gal=2, 3, 4, 6-tetra-*O*-methyl-D-galactose, *etc.*

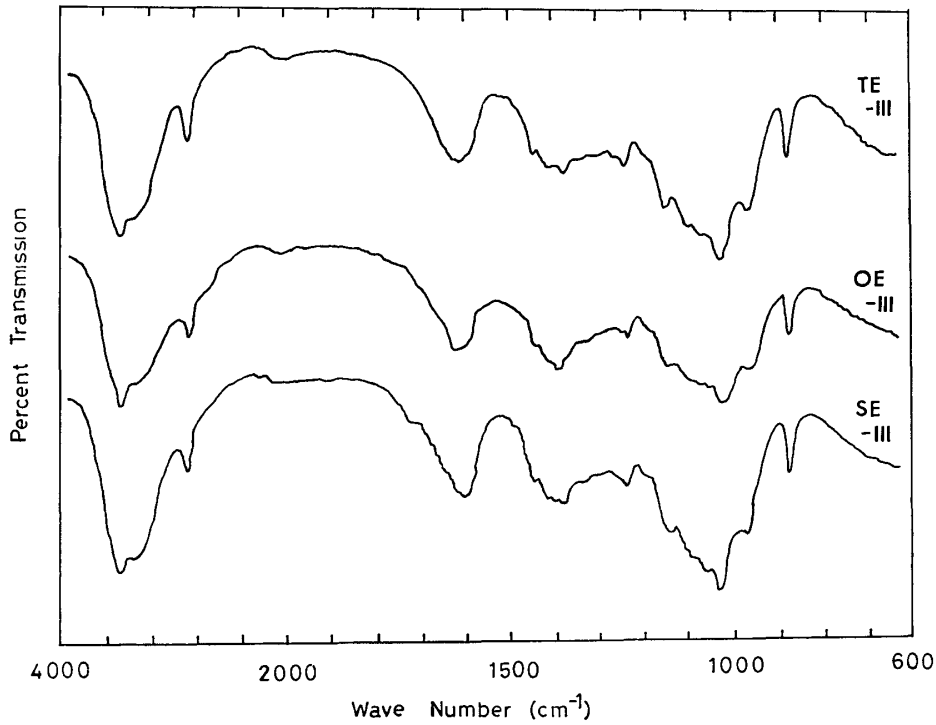

Fig. 2. I.r. spectra of the E-III fractions. Details of the procedure are given in the Experimental section.

II. 2,3-Di-*O*-methyl-D-xylose was the most prominent partially methylated monosaccharide in each III fraction. This result indicates the backbone structure of all xylans is composed of (1→4)-xylopyranosidic linkages. The negative specific rotations of all xylans and i.r. absorption at 895 cm^{-1} (Fig. 2) indicate that the principal mode of linkage is β-D-glycosidic bond. This was further supported by ^{13}C-n.m.r. spectroscopic analysis as described later. The 2,3,4-tri-*O*-methyl-D-xylose must be derived from, terminal, non-reducing residues of xylose. The 3-*O*-methyl-D-xylose must be derived from branching residues of xylose. The major branching sugar may be 4-*O*-methyl-D-glucuronic acid. The xylans in tension, opposite and side woods are found to contain one 4-*O*-methylglucuronic acid side chain per 9, 10 and 11 xylopyranose residues. These results are consistent with differences in uronic acid content as shown in Table I. It was suggested that xylans of tension, opposite and side woods might contain a similar number of 4-*O*-methylglucuronic acid side chains. The fraction III in tension wood (TE-III) contains an appreciable amount of partially methylated galactose residues. Presence of 2,3,6-tri-*O*-methyl- and 2,3,4-tri-*O*-methyl-D-galactopyranoses indicates that galactan is composed of

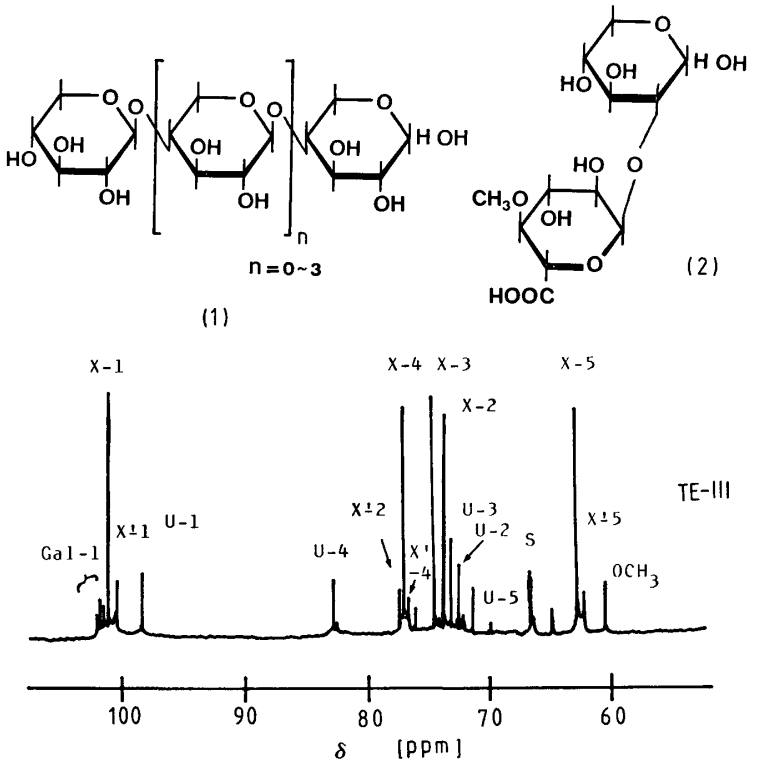

Fig. 3-(a) ^{13}C-N.m.r. spectrum of the TE-III fraction. X, U and Gal indicate xylose, 4-*O*-methylglucuronic acid and galactose residues, respectively. X′ indicates xylose residue substituted at C-2. S indicates 1,4-dioxane as an internal standard.

(1→4)- and (1→6)-galactopyranosidic linkages. 2,3,4,6-Tetra-*O*-methyl-D-galactopyranose residues may be derived from non-reducing terminal or branching points in galactan. Kuo and Timell[16], and Meier[15] have previously reported the presence of a highly branched galactan having a backbone of (1→4)-β-D-galactopyranose residues with (1→6)-linked β-D-galactopyranose oligomers as major side chains. Present results suggest the existence of similar galactan in tension wood of Japanese beech. I.r. spectra of all III fractions (Fig. 2) were similar and had adsorptions at 895, 990, 1040–1050, 1100, 1150, 1160, 1205, 1250 and 1375 cm^{-1}. These results together with chemical analysis are in good agreement with those of xylan from normal beech wood[29,30].

3.3 ^{13}C-N.m.r. spectroscopic analysis

^{13}C-N.m.r. spectra of the three fractions (TE-III, OE-III and SE-III) at 52-108 p.p.m. are shown in Fig. 3, and the ^{13}C-chemical shifts of the individual carbon atoms are listed in Table III. Signals due to xylopyranose residues could be readily assigned based on comparisons with the chemical shifts of β-D-xylo-oligosaccharides (1) (Table III) and 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose (2)[31]

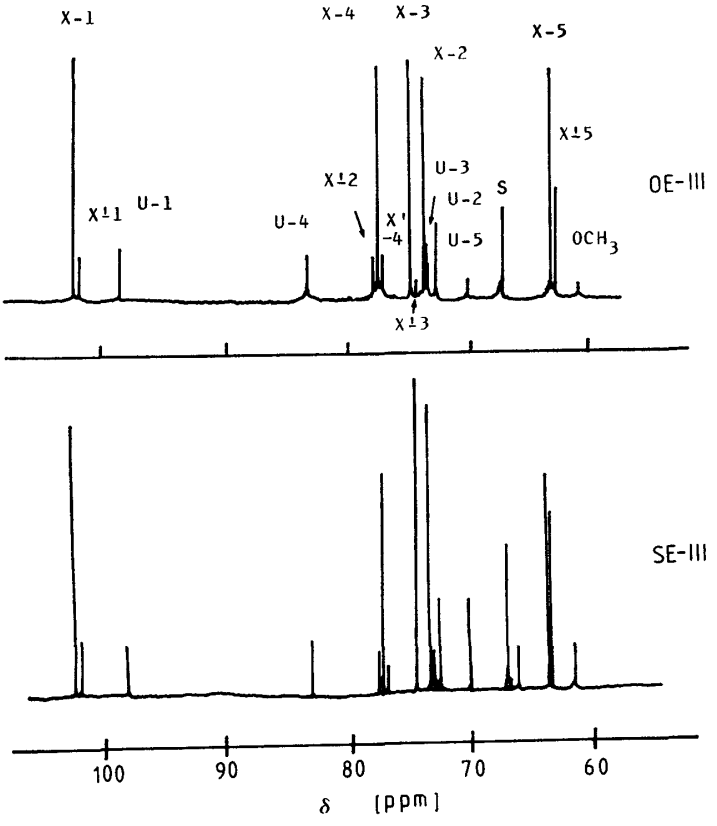

Fig. 3-(b) ^{13}C-N.m.r. spectra of the OE-III and SE-III fractions. X and U indicate xylose and 4-*O*-methylglucuronic acid, respectively. X′ indicates xylose residue substituted at C-2.

Table III ^{13}C-N.m.r. spectral data for xylans from tension, opposite and side woods, and aldobiouronic acid (2)*.

Component		Tension TE-III	Opposite OE-III	Side SE-III	(2)	
4-*O*-methyl glucuronic acid residue	C-1	98.42	98.45	98.45	98.42	
	C-2	72.29	72.58	72.34	72.27	
	C-3	73.26	73.58	73.28	73.36	
	C-4	83.01	83.06	83.08	83.15	
	C-5	70.10	70.42	70.30	70.23	
	C-6	177.01	177.35	177.03	173.16	
	O-CH_3	60.24	60.57	60.23	60.32	
Unsubstituted xylose residue	C-1	102.47	102.49	102.50		
	C-2	73.55	73.57	73.55		
	C-3	74.59	74.62	74.60		
	C-4	77.27	77.30	77.26		
	C-5	63.87	63.89	63.89		
					α	β
Substituted xylose residue	C-1	101.99	101.99	101.99	90.63	97.30
	C-2	77.71	77.91	77.77	77.32	79.97
	C-3	74.13	74.10	74.08	72.92	76.30
	C-4	76.95	77.08	77.16	70.39	70.28
	C-5	63.59	63.69	63.68	61.75	65.90

*Chemical shifts in p.p.m. relative to that of 1,4-dioxane.

(Table IV). Beta-D-(1→4)-linked D-xylopyranan from wood[32] and 4-*O*-methylglucuronoxylan from red lauan[33] were previously measured in deuterium oxide at 70°C and in 0.3 N NaOH. In the present experiments, however, we selected 80°C in deuterium oxide to enhance the sharpness of peak, since all III fractions were soluble in deuterium oxide. The chemical shift of carbons shifted dependent on temperature and solvent. The ^{13}C-n.m.r. spectra of OE-III and SE-III were essentially identical, indicating the similarity of their structures. The ^{13}C-n.m.r. spectrum of TE-III, however, gives a number of small signals other than the spectra of OE-III and SE-III. On the basis of the ^{13}C-n.m.r. spectra of β-D-(1→4)-linked galactopyranan and galactan from gum arabic[34], these small signals were assigned to be D-galactopyranose residues as shown in Fig. 3-a. Anomeric configurations of the glycopyranosidic nature of the xylopyranose, galactopyranose and 4-*O*-methylglucuronic acid residues were determined to be β, β and α, respectively by the coupling constants $^1J_{^{13}C-^1H}$ since a difference of about 10 Hz was observed between α and β anomeric pairs for these pyranoses[32]. Beta (1→4)-glycosidic structure of xylo-

Table IV. ^{13}C-N.m.r. spectral data for xylo-oligosaccharides (1)*.

(1)		n = 0 $(Xyl)_2$		n = 1 $(Xyl)_3$		n = 2 $(Xyl)_4$		n = 3 $(Xyl)_5$	
		α	β	α	β	α	β	α	β
Reducing residue	C-1	92.86	97.36	92.88	97.41	92.89	97.41	92.91	97.42
	C-2	71.92	74.86	71.91	74.86	71.91	74.86	71.93	74.82
	C-3	72.33	74.95	72.33	74.96	72.34	74.94	72.36	74.93
	C-4	77.40	77.39	77.34	77.34	77.34	77.34	77.31	77.31
	C-5	60.01	63.92	59.97	63.90	59.96	63.89	59.97	63.89
Internal residues	C-1			102.46		102.47		102.50	
	C-2			73.53		73.55		73.56	
	C-3			74.62		74.62		74.63	
	C-4			77.34		77.34		77.31	
	C-5			63.90		63.89		63.89	
Non-reducing residue	C-1	102.63		102.69		102.69		102.71	
	C-2	73.63		73.69		73.63		73.65	
	C-3	76.58		76.57		76.58		76.60	
	C-4	70.13		70.11		70.11		70.12	
	C-5	66.08		66.10		66.08		66.10	

*Chemical shifts in p.p.m. relative to that of 1, 4-dioxane.

pyranose residues in xylan was in agreement with the results of optical rotation and i.r. measurements, and methylation analysis.

Thus, ^{13}C-n.m.r. spectroscopy was found to be useful in determination of the ring size, anomeric configuration, the position of *O*-glycosidation, and the purity of the specimens. This technique must be an indispensable tool in elucidation of the structure of other polysaccharides in wood cell-walls. The details of these experiments will be published elsewhere.

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