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NOTE

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Structural unit of xylans from sugi (*Cryptomeria japonica*) and hinoki (*Chamaecyparis obtusa*)

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Abstract Arabinoglucuronoxylans (AGXs) isolated from the holocellulose of sugi (*Cryptomeria japonica*) and hinoki (*Chamaecyparis obtusa*) contained one 4-*O*-methyl-D-glucopyranosyluronic acid (4-*O*-Me-D-GlcA*p*) residue per 6.2 D-xylopyranose (D-Xyl*p*) residues and one 4-*O*-Me-D-GlcA*p* residue per 3.8 D-Xyl*p* residues. These AGXs were subjected to partial acid hydrolysis. Analyses by size exclusion chromatography and electrospray-ionization mass spectroscopy of the neutral sugar fractions in the hydrolysates showed the presence of xylooligosaccharides having a degree of polymerization of 2–8 in addition to D-Xyl, suggesting that the AGXs from sugi and hinoki contained unsubstituted chains consisting of at least eight D-Xyl residues. The acidic sugars in the hydrolysates were separated into two series of aldouronic acids composed of 4-*O*-Me-D-GlcA*p* and D-Xyl*p* by ion-exchange chromatography. The first series included aldouronic acids from aldobiouronic acid (4-*O*-Me-D-GlcA*p*-Xyl) to aldopentaouronic acids (4-*O*-Me-D-GlcA*p*-Xyl_4). The second series were aldouronic acids composed of two 4-*O*-Me-D-GlcA*p* residues and 2–4 D-Xyl residues. In these acidic sugars, the uronic acid side chains were located on two contiguous D-Xyl residues. These facts indicated that AGXs from sugi and hinoki had a structural unit containing two 4-*O*-Me-D-GlcA*p* residues on two contiguous D-Xyl residues as well as AGXs from spruce and larch.

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Introduction

The chemical structure of arabinoglucuronoxylan (AGX) in the secondary wall of softwood is well established.[1] It has a linear backbone composed of (1→4)-linked β-D-xylopyranose (β-D-Xyl*p*) residues, some of which are substituted at C-2 with a single 4-*O*-methyl-α-D-glucopyranosyluronic acid (4-*O*-Me-α-D-GlcA*p*) or α-D-GlcA*p*. The AGX also contains α-L-arabinofuranose (α-L-Ara*f*) residues directly linked to C-3 of the Xyl*p*. AGX contains generally one 4-*O*-Me-α-D-GlcA*p* residue per 5–6 D-Xyl*p* residues and one L-Ara*f* per 5–12 D-Xyl*p* residues. The distribution of these side chains along the backbone of softwood AGX is, however, still open to discussion. The distribution pattern of side chains in heteroxylans is an important feature affecting their solubility, interactions with other polymeric cell wall substances, degradability by enzymes, solution behavior, and other functional properties.[2]

Based on the results of the hydrolysis by xylanases of AGXs from larch wood and redwood, Comtat and Joseleau[3] and Debeire et al.[4] suggested the irregularity of the distribution of the uronic acid substituents on the xylosyl backbone and the presence of nonhydrolyzable blocks having a higher density of substituents.

On the other hand, based on matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) analysis of oligosaccharide mixtures obtained by partial acid hydrolysis of AGXs isolated from spruce, pine, and larch, Jacobs et al.[5] concluded that the major portion of the 4-*O*-Me-D-GlcA*p* residues are distributed regularly on every seventh or eighth D-Xyl residue, while a minor portion of these residues were attached to adjacent D-Xyl residues located randomly or periodically between larger domains.

In previous articles,[6,7] we isolated xylooligosaccharides [degree of polymerization (DP) 2–4] containing two

4-*O*-Me-D-GlcA*p* residues on contiguous D-Xyl residues from the partial hydrolysates of xylan precipitated from spruce neutral-sulfite liquor[6] and AGX isolated from the holocellulose of larch wood (*Larix leptolepis*).[7] Although the data were only semi-quantitative, it appeared that more than half of the 4-*O*-Me-D-GlcA*p* residues were located on adjacent D-Xyl residues in larch wood AGX.[7]

However, it is only in these two examples that xylooligosaccharides containing two 4-*O*-Me-D-GlcA*p* residues have been isolated from AGX by hydrolysis so far. The purpose of this work was to ascertain whether the AGXs of sugi (*Cryptomeria japonica*, Japanese cedar) and hinoki (*Chamaecyparis obtusa*, hinoki cypress) woods, which are representative species of Japanese plantation forests, have a structural unit with 4-*O*-Me-D-GlcA*p* side chains on two contiguous D-Xyl residues like the AGXs of spruce[6] and larch do.[7]

Experimental

General methods

The relative sugar composition of poly- and oligosaccharides was determined by means of partition chromatography on ion-exchange resin after hydrolysis with 2 M trifluoroacetic acid (TFA) at 120°C for 2 h. Partition chromatography was carried out by using a Shimadzu LC-10AT high-performance liquid chromatograph following the procedure of Nakamura et al.[8] A mixture of monosaccharides was chromatographed in a TSK-gel Sugar AX1 column (TOSOH) with 0.5% borate–1.0% ethanolamine-hydrochloric acid buffer (pH 7.9). Relative percentage amounts were calculated electronically.

Size exclusion chromatography (SEC) for neutral xylooligosaccharides was performed on KS 802 columns [4.6 × 250 mm × 2 (in series), Shodex] at 70°C and a flow rate of 0.7 ml/min using distilled water as an eluent. The eluate was monitored using a differential refractometer (TOSOH, Model RI-8010). Fourier transform infrared spectroscopy (FT-IR) spectra were recorded on a JASCO model FT/IR-410 spectrometer using a KBr disc containing 1% finely ground sample.

Nuclear magnetic resonance (NMR) spectroscopy

NMR spectra of oligosaccharides were recorded at 25°C by taking samples in D_2O with a JEOL ALPHA 500FT-NMR spectrometer. ^{1}H and ^{13}C NMR spectra were obtained at 500.16 and 125.77 MHz. The chemical shifts were referred to acetone at 2.225 (^{1}H) and 31.07 ppm (^{13}C) as internal standards and were reported relative to TSP. Standard pulse sequences were utilized to obtain COSY, HOHAHA, NOESY, HMQC, and HMBC spectra.

Electrospray-ionization mass spectroscopy

Electrospray-ionization mass spectroscopy (ESI-MS) analysis was performed with a Thermo-Quest LCQ DUO mass spectrometer (Thermoelectron, Waltham, MA, USA) operated in positive-ion mode with a spray voltage of 4.55 kV, a capillary temperature of 3.1 V, and a capillary temperature of 180°C. Mass spectra were obtained between m/z 150 and 2000.

Chemical compositions

The chemical compositions of sugi (*C. japonica*) and hinoki (*C. obtusa*) were determined by the standard method of the Japan Wood Research Society (1985, chap. 4).[9] Extractives were removed with a mixture of alcohol–benzene (1:2) by use of the Soxhlet apparatus. The lignin content was determined in extractive-free samples by the Klason method. The neutral sugar composition was determined in the hydrolysate of Klason lignin by chromatography, as described above.

Preparation of xylan

Wood meal (60–80 mesh) of sugi (*C. japonica*) and hinoki (*C. obtusa*) were exhaustively extracted with methanol. Extractive-free meal samples (20 g from each species) were treated[9] at 80°C for 1 h with sodium chlorite (8 g) and acetic acid (1.6 ml) with gentle stirring. After four successive treatments, the solid residue was recovered by filtration, washed with water and acetone, and air dried. The resulting holocelluloses were extracted successively with hot water to remove galactoglucomannan,[10] and with 10% aqueous potassium hydroxide in the presence of barium hydroxide to isolate AGX.[11] The latter extracts were adjusted to pH 6 with glacial acetic acid and poured into ethanol (4 vol.). The precipitates were collected; washed in succession with 80% ethanol, ethanol, and light petroleum; and dried in vacuo over phosphorus pentoxide.

A portion of each AGX isolate was delignified at room temperature with buffered sodium chlorite[3,12] and then precipitated by addition of ethanol. The precipitate collected by centrifugation was treated for a few minutes at 0°C with 50% aqueous methanol containing 5% hydrochloric acid, giving the free acid form of the polysaccharides.[13] The polysaccharides (50 mg) were dissolved in water and titrated with 0.1 M sodium hydroxide to pH 8 in an atmosphere of nitrogen to determine their equivalent weights.

Partial hydrolysis of AGX

The AGX isolates (3 g each) from sugi (*C. japonica*) and hinoki (*C. obtusa*) were hydrolyzed with 0.125 M sulfuric acid at 90°C for 9 h. The precipitates (nonhydrolyzable residue) were removed by centrifugation; the supernatant was maintained at pH 8 for 4 h at room temperature to hydrolyze the lactone and then applied to columns of Dowex 1 × 8 (AcO^-). The neutral sugars were eluted with water until the anthrone test was negative, and acidic sugars were eluted with 5 M acetic acid.

Separation of acidic sugars

The acidic sugars were separated by chromatography on preparative columns of anion-exchange resins Diaion CA08Y (23–25 μm, AcO^-, 15 × 899 mm) and Aminex A-27 (12–15 μm, AcO^-, 10 × 849 mm and 5 × 436 mm) by elution with A, 0.08 M sodium acetate; B, 0.03 M acetic acid; C, 0.15 M acetic acid; D, 0.5 M acetic acid; and E, 1.0 M acetic acid. The eluate was monitored by a differential refractometer (ERC, model 7515A). The volume distribution coefficient (*Dv*) of each acidic sugar was calculated in the usual way.[14]

The acidic sugars obtained from sugi and hinoki AGXs were separated into groups by chromatography on Diaion CA08Y by eluent A and, after removal of sodium ions with a cation exchange resin Dowex 50E-X8 (H^+), the fractions were evaporated under reduced pressure to dryness and weighed. Each group was rechromatographed on Aminex A-27 with eluents B–E.

Each acidic sugar was identified by *Dv*, acid hydrolysis, subsequent identification of the hydrolysis products, and ^1H and ^{13}C NMR spectroscopy. The anomeric configurations of the glycosyl residues were determined by means of ^1H and ^{13}C NMR spectroscopy. Assignments of signals were carried out using 2D NMR spectrometry.

Results

The chemical compositions of original wood meals of sugi and hinoki are shown in Table 1. The extractive-free wood meals of sugi and hinoki were delignified with acid chlorite,[9] giving the holocelluloses at yields of 75.7% and 76.5%., respectively (Table 2). After removal of galactoglucomannan by extraction with hot water,[10] AGXs were extracted from the holocelluloses with 10% potassium hydroxide in the presence of barium hydroxide.[11] The yields were 8.3% from sugi and 9.9% from hinoki based on the extractives-free wood meals.

The sugar compositions of the two AGXs are shown in Table 2. Although the two AGX preparations were contaminated with small amounts of other polysaccharides and appreciable amounts of lignin, as described later, they were used as starting samples without further purification.

For determination of the uronic acid content, a part of each AGX sample was purified by treatment with buffered sodium chlorite[3,12] and converted to the free acid form.[13] As shown in Table 2, the contaminated polysaccharides were removed on purification. Their IR spectra showed absorption at 1735 cm^{-1} due to C=O stretching (acid), indicating the acid form of uronic acid, and did not show any absorption due to aromatic rings, indicating the absence of lignin. The purified AGXs were titrated with 0.1 M sodium hydroxide to pH 8. The equivalent weight was 1014.1 for sugi AGX and 694.4 for hinoki AGX, corresponding to ratios of Xyl residue to uronic acid residue of 6.2 and 3.8, respectively. The ratio of Xyl residue to Ara residue was 21.7 for sugi AGX and 10.8 for hinoki AGX. Rha was detected in only trace amount in both AGXs.

Crude AGXs (3 g each) from sugi and hinoki were subjected to partial hydrolysis with 0.125 M sulfuric acid for 9 h affording precipitates at yields of 41.7% and 44.5%, respectively (Table 2). These precipitates were attributed to lignin.[15,16] The sugars in the supernatants were separated into neutral and acidic sugars. The yields of acidic sugars were 28.3% from sugi AGX and 30.4% from hinoki AGX, based on the hydrolyzed parts.

The fractions of neutral sugars were analyzed by SEC. Both AGXs gave almost the same chromatograms showing the presence of a homologous series of xylooligosaccharides from xylobiose to xylooctaose in addition to monomers (Fig. 1). The sugars eluted at the void volume and those eluted in the 50–62 min region of retention time (from after the void volume up to xylotetraose) in Fig. 1 were separately

Table 1. Chemical composition of wood meals of sugi (*Cryptomeria japonica*) and hinoki (*Chamaecyparis obtusa*)

Species	Extractives (%)	Klason lignin (%)	Sugar composition of original wood (%)					
			Rha	Man	Ara	Gal	Xyl	Glc
Sugi	1.7	34.0	Trace	15.1	2.7	2.7	11.8	67.3
Hinoki	4.1	30.7	Trace	23.9	1.9	3.4	7.6	63.2

Table 2. Yields (%) of holocellulose and arabinoglucuronoxylan (AGX) and sugar composition of AGX

Species	Yield (%) of holocellulose	Yield[a] (%) of AGX	Sugar composition of AGX (%)						Xyl/Ara[b]	Xyl/GlcA[c]	Precipitate[d] (%)
			Rha	Man	Ara	Gal	Xyl	Glc			
Sugi	75.7	8.3	Trace	1.0	9.0	5.0	79.0	6.0			41.7
After purification			Trace		4.4		95.6		21.7	6.2	
Hinoki	76.5	9.9	Trace	5.0	13.0	7.0	63.0	9.0			44.5
After purification			Trace		8.5		91.5		10.8	3.8	

[a] Based on the extractive-free wood meal
[b] Molar ratio of Xyl/Ara in AGX
[c] Molar ratio of Xyl/4-*O*-Me-GlcA in AGX
[d] Nonhydrolyzable part by hydrolysis with 0.125 M H_2SO_4 at 90°C for 9 h

collected and were analyzed by ESI-MS. The positive-ion mode ESI-MS spectrum (Fig. 2) of the neutral sugars showed peaks at m/z 569.2, 701.3, 833.3, 965.3, and 1097, corresponding to $[Xyl_n + Na]^+$ (n = 4–8), respectively, indicating that the longest neutral xylooligosaccharide was xylooctaose. On the other hand, the positive-ion mode ESI-MS spectrum (Fig. 3) of the sugars at void volume showed peaks at m/z 759.3, 891.3, 1023.3, 1155.4, 1287.4, 1419.5, 1551.4, corresponding to $[MeGlcA\text{-}Xyl_n + Na]^+$ (n = 4–10), respectively, indicating that the sugars were a mixture of aldouronic acids higher than aldopentaouronic acid. This means that aldouronic acids higher than aldopentaouronic acids were eluted out from the strong anion-exchange resin column (OAc^- form) with water even though the resins were used at amounts 10 times in excess of those calculated based on the equivalent weights of the hydrolysates.

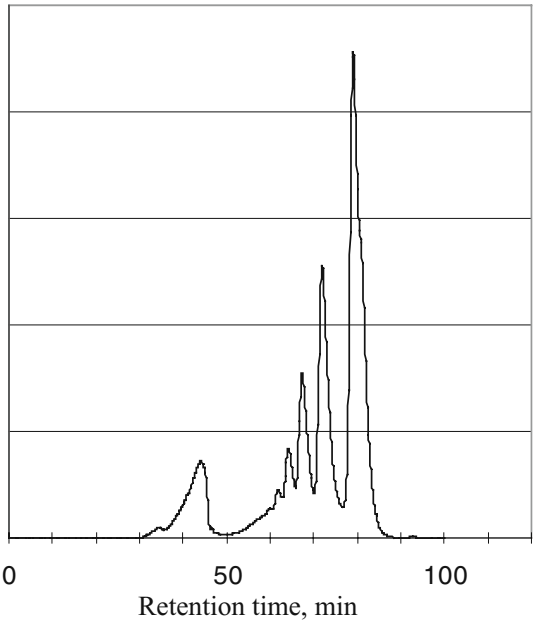

Fig. 1. Size exclusion chromatography (SEC) of neutral sugars in partial hydrolysate of arabinoglucuronoxylan (AGX) from sugi (*Cryptomeria japonica*). The column was a KS 802 (4.6 × 250 mm × 2, Shodex) operating at 70°C. The eluent was distilled water at flow rate of 0.7 ml/min

The acidic sugars were first separated according to their molecular size by means of anion-exchange chromatography using eluent A. Six main groups were obtained together with many small and long-tailing bands which were neglected in the present study. The yield of each group is shown in Tables 3 and 4. Each group was fractionated according to its strength of acid by anion-exchange chromatography using various concentrations of aqueous acetic acid (0.03–1.0 M) as eluent.

Group 1, representing aldopentaouronic acids, gave four fractions (1-S1, 1-S2, 1-S3, and 1-S4) on rechromatography in eluent B, as shown in Fig 4. Their *Dv* values were 17.09, 20.76, 22.45, and 26.12, respectively. These acids gave only Xyl as neutral sugar on acid hydrolysis with 2 M TFA at 120°C for 2 h indicating that they comprised a homogeneous series of acidic oligosaccharides. Fractions 1-S2 and 1-S3 were the main peaks and their *Dv* values and ^{13}C NMR spectra were identical with those of authentic samples of *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 [Xyl-(MeGlcA)-Xyl-Xyl-Xyl][17] and *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*-D-Xyl [(MeGlcA)-Xyl-Xyl-Xyl-Xyl], respectively.[17,18] Fractions 1-S1 and 1-S4 were minor products and may be other possible isomers.

Group 2, representing aldotetraouronic acids, gave three fractions (2-S1, 2-S2, and 2-S3) on rechromatography in eluent C as shown in Fig. 5. Their *Dv* values were 4.54, 4.78, and 5.41, respectively. These acids gave only Xyl as neutral

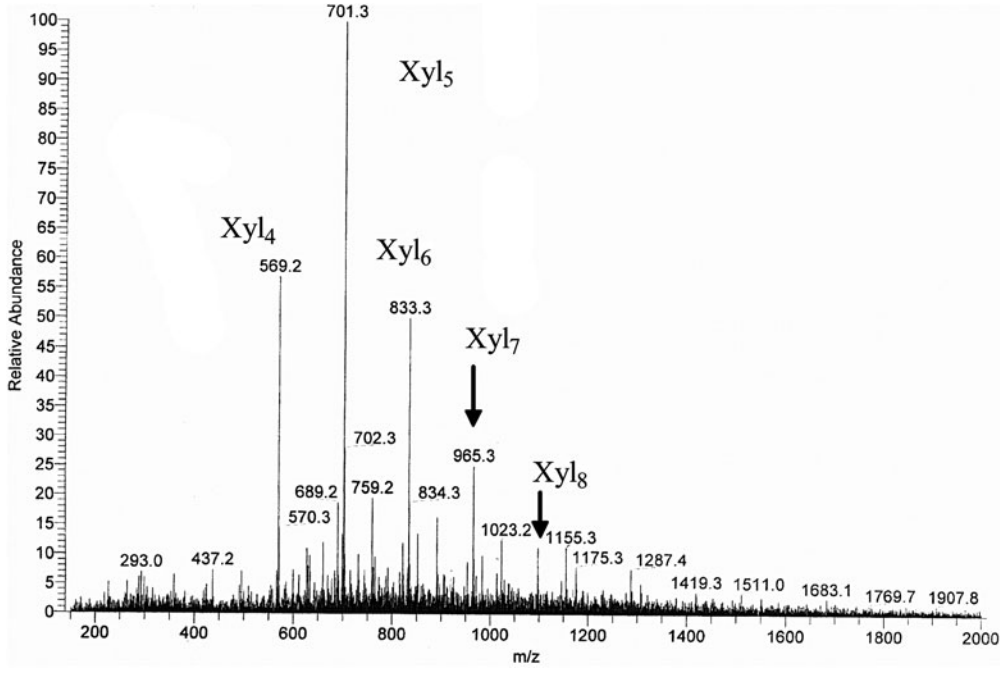

Fig. 2. Positive-ion mode ESI-MS spectrum of neutral xylooligosaccharides from partial hydrolysate of sugi AGX

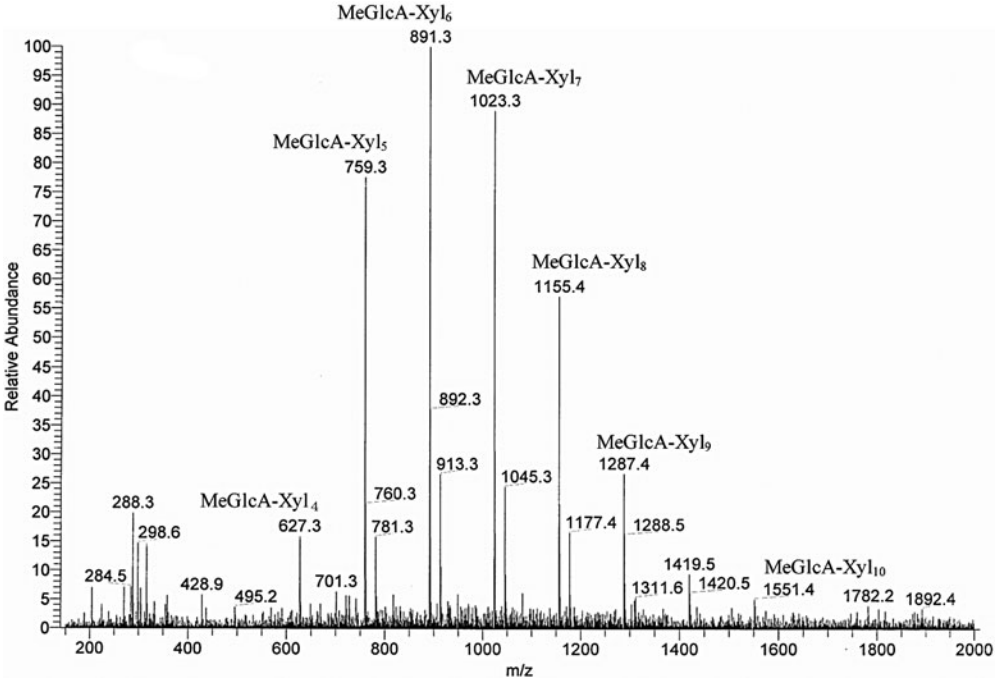

Fig. 3. Positive-ion mode ESI-MS spectrum of the acidic sugars remaining in the neutral sugar fraction obtained from partial hydrolysate of sugi AGX

Table 3. Yields of aldouronic acids composed of 4-*O*-Me-D-GlcA and D-Xyl residues obtained from partial hydrolyzate of arabinoglucuronoxylan from sugi and hinoki

Group	Fr. no.	Aldouronic acid[a]	Yield (mg)			
			Sugi	MeGlcA[b]	Hinoki	MeGlcA[b]
1	1:S1	Isomer	83.8	21.8	40.3	10.5
	1:S2	Xyl-(MeGlcA)-Xyl-Xyl-Xyl				
	1:S3	(MeGlcA)-Xyl-Xyl-Xyl-Xyl				
	1:S4	Isomer				
2	2:S1	Xyl-(MeGlcA)-Xyl-Xyl	48.0	15.4	41.6	17.1
	2:S2	(MeGlcA)-Xyl-Xyl-Xyl				
	2:S3	Xyl-Xyl-(MeGlcA)-Xyl				
3	3:S1	(MeGlcA)-Xyl-Xyl	64.5	25.8	80.9	32.3
	3:S2	Xyl-(MeGlcA)-Xyl				
4	4:S1	MeGlcA-Xyl	14.0	7.8	14.0	7.8
Sum			210.3	70.8	190.5	68.3

Fr. no., fraction number
[a] Aldouronic acids containing one 4-*O*-Me-GlcA
[b] Content of anhydro 4-*O*-Me-GlcA*p*

Table 4. Yields of xylooligosaccharides containing two MeGlcA residues obtained from partial hydrolyzate of arabinoglucuronoxylan from sugi and hinoki

Group	Fr. no.	Aldouronic acid[a]	Yield (mg)			
			Sugi	MeGlcA[b]	Hinoki	MeGlcA[b]
4	4:S2	Xyl-(MeGlcA)-Xyl-(MeGlcA)-Xyl-Xyl	28.0	11.5	41.6	17.1
	4:S3	(MeGlcA)-Xyl-(MeGlcA)-Xyl-Xyl-Xyl				
5	5:S1	(MeGlcA)-Xyl-(MeGlcA)-Xyl-Xyl	30.9	14.0	42.6	20.2
6	6:S1	(MeGlcA)-Xyl-(MeGlcA)-Xyl	11.5	6.7	12.5	7.3
Sum			70.0	32.2	96.7	44.8

[a] Aldouronic acids containing two 4-*O*-Me-GlcA residues
[b] Content of anhydro 4-*O*-Me-GlcA*p*

sugar on acid hydrolysis with 2 M TFA at 120°C for 2 h. Fraction 2-S3 was obtained in a small amount. The *Dv* values of fractions 2-S2 and 2-S3 coincided with those of authentic samples 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[(MeGlcA)-Xyl-Xyl-Xyl][19] and *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*p* [Xyl-Xyl-(MeGlcA)-Xyl], respectively.[7,19] The ^{13}C NMR spectrum of fraction 2-S2 was identical with that reported in a previous article and by Excoffier et al.[20] The structure of fraction 2-S3 was confirmed by ^{13}C NMR spectrum. Its spectrum and carbon shifts are shown in Fig. 6 and Table 5. Fraction 2-S1 could be considered to be another possible isomer and was identified as *O*-β-D-Xyl*p*-(1→4)-(4-*O*-Me-α-D-GlcA*p*)-*O*-β-D-Xyl*p*-(1→4)-D-Xyl*p* [Xyl-(MeGlcA)-Xyl-Xyl] on the basis of ^{13}C NMR spectroscopy. The ^{13}C NMR spectrum and carbon shifts of fraction 2-S1 are also shown in Fig. 6 and Table 5.

Group 3, representing aldotriouronic acid, gave two fractions (3-S1 and 3-S2) on rechromatography in eluent D, as shown in Fig. 7. Their *Dv* values were 4.05 and 4.66, respectively, identical with those of authentic samples of *O*-(4-*O*-Me-α-D-GlcA*p*)-(1→2)-*O*-β-D-Xyl*p*-(1→4)-D-Xyl*p* [(MeGlcA)-Xyl-Xyl] and *O*-β-D-Xyl*p*-(1→4)-*O*-(4-*O*-Me-β-D-GlcA*p*)-(1→2)-D-Xyl*p* [Xyl-(MeGlcA)-Xyl].[7] The ^{13}C NMR spectrum of fraction 3-S1 was identical with that reported in a previous article.[17] The ^{13}C NMR spectrum of fraction 3-S2 was identical with that reported by Cavagna et al.[21]

Group 4 was separated into three fractions (4-S1, 4-S2, and 4-S3) on rechromatography in eluent E. The chromatogram was the same as that reported previously.[7] Their *Dv*

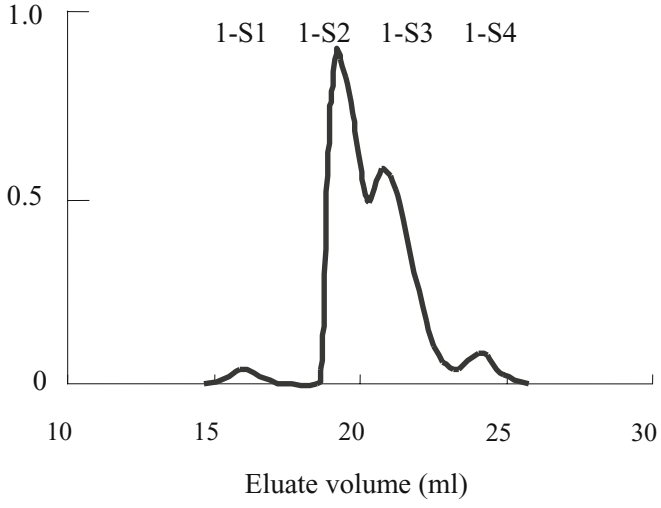

Fig. 4. Rechromatography of group 1 on a column (5 × 436 mm) of Aminex 27 (AcO^-) resin eluted with 0.03 M AcOH at 0.7 ml/min

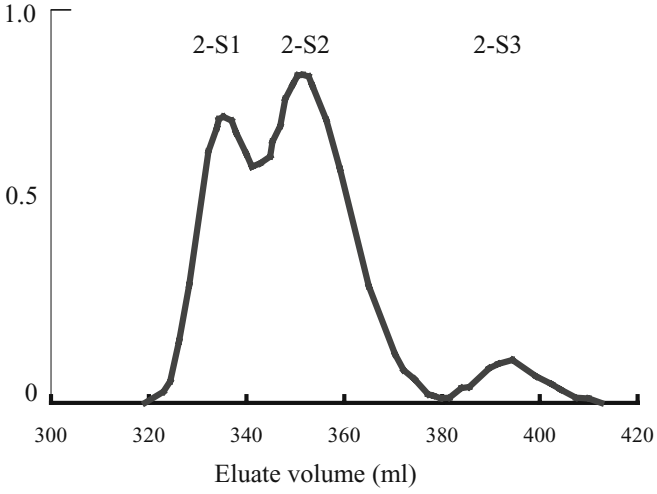

Fig. 5. Rechromatography of group 2 on a column (10 × 830 mm) of Aminex 27 (AcO^-) resin eluted with 0.15 M AcOH at 0.7 ml/min

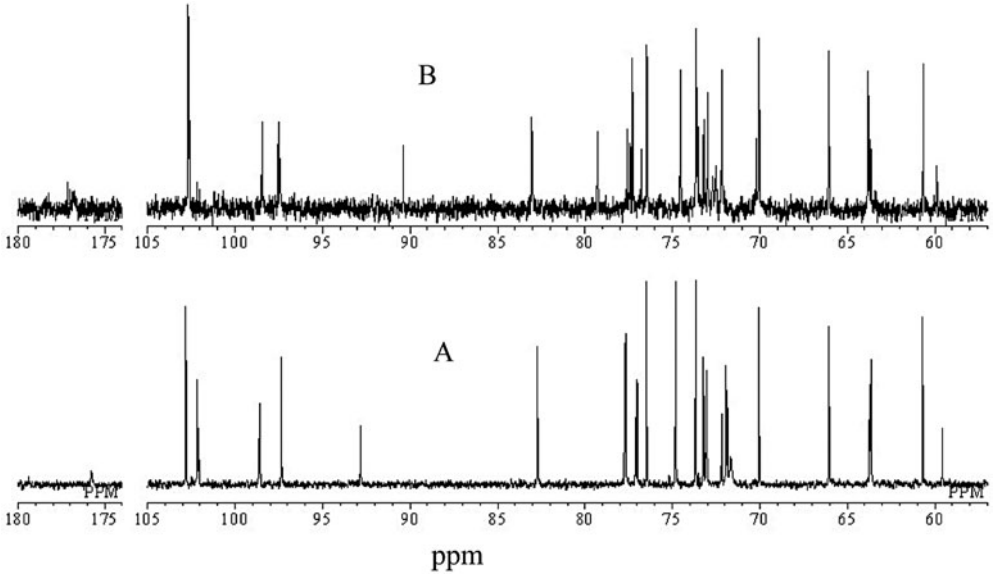

Fig. 6. ^{13}C nuclear magnetic resonance spectra of aldotetraouronic acids: **A** fraction 2-S1, Xyl-(MeGlcA)-Xyl-Xyl; **B** fraction 2-S3, Xyl-Xyl-(MeGlcA)-Xyl

Table 5. Assignments of signals in the ^{13}C nuclear magnetic resonance spectra of the aldouronic acids shown in Fig. 3

		Fr. 2-S1 Xyl″-(MeGlcA)-Xyl′-Xyl		Fr. 2-S3 Xyl″-Xyl′-(MeGlcA)-Xyl	
		β	*α*	*β*	*α*
Xyl	1	97.35	92.82	97.52	90.37
	2	74.82	72.18	79.27	
	3	74.82	71.85	73.21	70.19
	4	77.01	77.11	77.59	77.41
	5	63.72	59.57	63.67	59.88
Xyl′	1	102.15	102.08	102.62	
	2	77.68		73.56	
	3	73.22		74.53	
	4	77.72		77.27	
	5	63.66		63.80	
Xyl″	1	102.81		102.67	
	2	73.67		73.64	
	3	76.47		76.46	
	4	70.05		70.04	
	5	66.04		66.05	
MeGlcA	1	98.59		98.45	97.43
	2	71.93		72.16	
	3	73.05		72.98	
	4	82.71		83.04	82.98
	5	71.65		72.50	72.71
	6	175.78		177.00	177.15
	OMe	60.69		60.65	

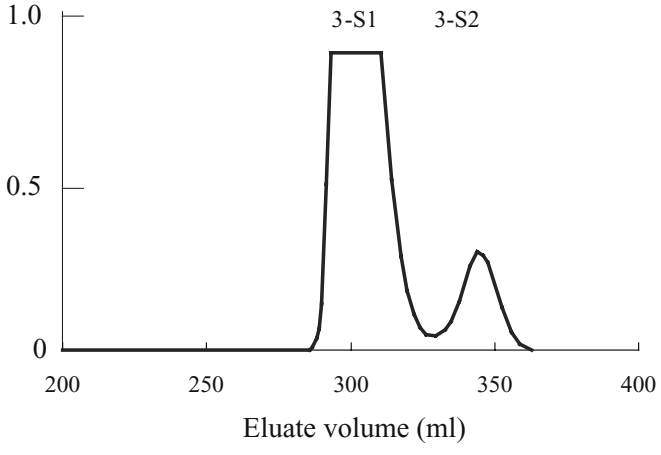

Fig. 7. Rechromatography of fraction 3 on a column (10 × 830 mm) of Aminex 27 (AcO^-) resin eluted with 0.5 M AcOH at 0.7 ml/min

values were 5.01, 9.55, and 10.54, respectively, identical with those of the authentic samples. Fraction 4-S1 was identified as aldobiouronic acid, 2-*O*-(4-*O*-Me-*α*-D-GlcA*p*)-D-Xyl [MeGlcA-Xyl] and its ^{13}C NMR spectrum coincided with that reported in a previous article.[18] It should be noted here that aldobiouronic acid *α*-L-GalA*p*-(1→4)-D-Xyl was not detected in fraction 4-S1. This aldobiouronic acid, the *Dv* values of which are close to those of MeGlcA-Xyl,[22] is expected to be derived from the glycosyl sequence at the reducing end of xylan main chain, if it is present.

Fractions 4-S2 and 4-S3 gave only xylose as neutral sugar on hydrolysis with 2 M TFA at 100°C for 2 h and were identified as *O*-*β*-D-Xyl*p*-(1→4)-*O*-(4-*O*-Me-*α*-D-GlcA*p*)-(1→2)-*O*-*β*-D-Xyl*p*-*O*-(4-*O*-Me-*α*-D-Glc*p*)-(1→2)-*O*-*β*-D-Xyl*p*-(1→4)-D-Xyl [Xyl-(MeGlcA)-Xyl-(MeGlcA)-Xyl-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)-*O*-*β*-D-Xyl*p*-(1→4)-*O*-*β*-D-Xyl*p*-(1→4)-D-Xyl [(MeGlcA)-Xyl-(MeGlcA)-Xyl-Xyl-Xyl], respectively, based on their *Dv* values.[7]

Groups 5 and 6 were purified on rechromatography in eluent E to give one peak each, 5-S1 and 6-S1, respectively. Their *Dv* values were 18.73 and 46.64 and were identical with those of authentic samples.[7] They were identified as *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 [(MeGlcA)-Xyl-(MeGlcA)-Xyl-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 [(MeGlcA)-Xyl-(MeGlcA)-Xyl], respectively.

Discussion

The analytical data in Table 1 indicated that the lignin contents and sugar compositions of sugi (*C. japonica*) and hinoki (*C. obtusa*) were similar to those given by Shimizu.[1] Sugi contained more lignin and xylan and less mannan than hinoki did.[1] Wood meal samples of sugi and hinoki were first delignified according to the method of Wise et al.[9] (Table 2). It is seen from the data shown in Tables 1 and 2 that about a quarter of the lignin still remained in the holocelluloses of sugi and hinoki.

The sugar composition of crude AGXs (Table 2) showed that they were contaminated with other polysaccharides. Partial acid hydrolysis revealed that both AGX prepara-

tions were accompanied by considerable amounts of lignin (Table 2). For the determination of uronic acid contents, a part of each AGX sample was delignified according to the method of Clayton[12] and converted to the acid forms with acidic aqueous methanol.[13]

The hinoki AGX was more highly substituted with 4-*O*-Me-D-GlcA and L-Ara than sugi AGX was. The level of substitution with the acid in the hinoki AGX was the same as that of redwood AGX.[3] The degree of substitution with L-Ara in the sugi AGX was extraordinarily low, i.e., around the same level as that of redwood AGX.[3]

It is well known that softwood AGX cannot be isolated in reasonable yield and purity without delignification, but delignification seriously affects the structure.[23] We previously[24] isolated a unique glycosyl sequence -*O*-*β*-D-Xyl*p*-(1→3)-*O*-*α*-L-Rha*p*-(1→2)-*O*-*α*-L-GalA*p*-(1→4)-D-xylitol from the enzymatic hydrolysate of holocellulose. In that study,[24] the holocellulose was prepared from the in situ reduced wood meal by the modified chlorite method. However, in the present study, special care was not taken in the preparation of the holocelluloses.[9] Rha residue was detected in only trace amounts in both AGX samples. As described in the Results section, the aldobiouronic acid 4-*O*-(*α*-D-GalA*p*)-D-Xyl was found not to be present in the partial hydrolysates of either AGX, as was also the case for larch AGX[7], suggesting that most of the glycosyl sequence was possibly cleaved off from the reducing terminal during the delignification. On the other hand, the glycosyl sequence survived in the eucalyptus glucuronoxylan[25] that was extracted from holocellulose prepared in the same way as that used in the present study. This discrepancy may be due to the differences in the conditions during holocellulose preparation between hardwood and softwood, which is severer in the latter than in the former.

The two AGXs in this study were subjected to milder partial acid hydrolysis than that applied to larch AGX in a previous article[7] in order to minimize the formation of aldobiouronic acid (MeGlcA-Xyl).

Analyses by SEC (Fig. 1) and ESI-MS (Fig. 2) of the neutral sugar fractions in the hydrolysates showed the presence of xylooligosaccharides having a DP value of 2–8 in addition to D-Xyl. It is known[26] that the *α*-(1→2) linkages between the D-Xyl units and 4-*O*-Me-D-GlcA substituents are more stable toward acid hydrolysis than are the *β*-(1→4) linkages between the D-Xyl residues in the xylan backbone. 4-*O*-Me-GlcA was not detected in the hydrolysate (Tables 3 and 4). These facts suggested that the AGXs from sugi and hinoki contained unsubstituted chains consisting of at least eight D-Xyl residues. Although hinoki AGX was more highly substituted with uronic acid, xylooctaose was also found to be present in the hydrolysate.

The acidic sugars in the hydrolysates were separated into two series of acidic oligosaccharides composed of 4-*O*-Me-D-GlcA and D-Xyl by ion-exchange chromatography, as was also the case for AGX isolated from larch.[7]

The first series included the aldouronic acids from aldobio- (MeGlcA-Xyl_2) to aldopentao- (MeGlcA-Xyl_5) uronic acids. Most of the possible isomers from aldotrio- to aldopentaouronic acids were separated by anion-exchange chromatography using 0.08 M NaOAc and 0.03–0.5 M acetic acid as eluent (Figs. 2, 3, 5). Aldouronic acids higher than aldohexaouronic acids and part of the aldopentaouronic acids were missed from this fraction (Fig. 3).

The second series was composed of two 4-*O*-Me-D-GlcA and 2-4 D-Xyl residues, and in these acidic xylooligosaccharides, 4-*O*-Me-D-GlcA residues were located on adjacent D-Xyl residues, as has been reported in the case of larch AGX.[7] As the yields of the aldobiouronic acid (fraction 4-S1, MeGlcA-Xyl) were low, it was assumed that most of xylosidic linkages between contiguous D-Xyl residues carrying 4-*O*-Me-D-GlcA survived the partial acid hydrolysis. These acidic xylooligosaccharides were identified based on their *Dv* values. Their structures were confirmed by ^{1}H and ^{13}C NMR spectra after labeling of the reducing end with 2-aminobenzamide (2AB).[27]

The anhydro 4-*O*-Me-GlcA residue content in each group of acidic sugars was calculated and shown in Tables 3 and 4. The ratios of 4-*O*-Me-D-GlcA residues belonging to the aldouronic acids carrying one 4-*O*-Me-GlcA residue to those belonging to the aldouronic acids carrying two 4-*O*-Me-GlcA residues were 7:3 for sugi AGX and 3:2 for hinoki AGX. As described above, ESI-MS analysis revealed that the aldouronic acids eluted at the void volume were a mixture of aldouronic acids from aldopentao- (MeGlcA-Xyl_4) to aldodecaouronic acids (MeGlcA-Xyl_{10}) (Fig. 3). As the amounts of these aldouronic acids were missed in the above calculations results, the ratio of the structural unit having two 4-*O*-Me-D-GlcA residues on two contiguous D-Xyl residues must actually be smaller than the values calculated. The results in this study are compatible with the findings by Jacobs et al.[5] that the major portion of the 4-*O*-Me-D-GlcA*p* residues are distributed regularly on every seventh or eighth D-Xyl residue, whereas a minor portion of these residues were attached to adjacent D-Xyl residues located randomly or periodically between larger domains.[5]

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