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Short communication

NMR characterization of acidic xylo-oligosaccharides containing two methylglucuronic acid residues from Japanese cedar and Hinoki cypress

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ABSTRACT

Xylo-oligosaccharides containing 4-*O*-methyl glucuronic acid (MeGlcA) residues attached to two adjacent xylosyl residues and those with a single MeGlcA residue were isolated from partial acid hydrolyzates of the arabinoglucuronoxylans (AGX) from Japanese cedar (Sugi, *Cryptomaria japonica*) and Hinoki cypress (*Chamaecyparis obtusa*). The ratio of xylo-oligosaccharides with two MeGlcA residues and those with a single MeGlcA residue was about 1:3 for both Sugi and Hinoki. Four xylo-oligosaccharides with two MeGlcA residues were labeled at their reducing end with 2-aminobenzamide (2AB). The 2AB-labeled oligosaccharides (**1**–**4**) were structurally characterized using normal-phase liquid chromatography, electrospray-ionization mass spectrometry (ESI-MS), and nuclear magnetic resonance (NMR) spectroscopy. The signals in the ^{1}H and ^{13}C NMR spectra of the 2AB-labeled oligosaccharides were fully assigned using one- and two-dimensional NMR spectroscopy.

1. Introduction

Glucomannan and arabinoglucuronoxylan (AGX) are quantitatively major components of the secondary cell walls of softwood. AGX has a linear backbone composed of (1 → 4) linked β-D-xylosyl (Xyl) residues, some of which are substituted at *O*-2 with a single α-D-glucosyluronic acid (GlcA) or 4-*O*-methyl-α-D-glucosyluronic acid (MeGlcA) residue. The Xyl residues may also be substituted with α-L-arabinofuranosyl (Ara*f*) and *O*-acetyl residues (O'Neill & York, 2003; Shimizu, 1991).

Acidic xylo-oligosaccharides with MeGlcA residues attached to adjacent D-Xyl residues have been isolated from partial acid hydrolyzates of the xylan precipitated from spruce neutral-sulfite liquor (Shimizu & Samuelson, 1973) and AGX extracted from holocellulose of larch wood (Shimizu, Hashi, & Sakurai, 1978) and characterized on the basis of their glycosyl composition and glycosyl-linkage analyses. Jacobs, Larsson, and Dahlman (2001) have also reported that a small portion of the MeGlcA residues are located on adjacent Xyl residues in the β-(1 → 4)-D-Xyl*p* chains of AGX from spruce, larch, and pine. In hardwood xylans, a single MeGlcA residue is attached at *O*-2 of xylan backbone. As far as we know acidic xylo-oligosaccharides with MeGlcA residues attached to adjacent D-Xyl residues have been isolated from hardwood xylans. To determine if AGX from other softwoods also has this characteristic feature, AGXs were isolated from the holocellulose of Japanese cedar (Sugi, *Cryptomaria japonica*) and Hinoki cypress (*Chamaecyparis obtusa*). Acidic xylo-oligosaccharides containing two MeGlcA residues and 2–4 Xyl residues ($MeGlcA_2Xyl_{2-4}$) were isolated in addition to the aldouronic acids composed of a single MeGlcA residue and 1–4 Xyl residues ($MeGlcAXyl_{1-4}$) from the partial acid hydrolyzates of these AGXs. In this report four acidic xylo-oligosaccharides ($MeGlcA_2Xyl_{2-4}$) containing two MeGlcA residues were labeled at their reducing ends with 2-aminobenzamide (2AB) and their structure was characterized by electrospray-ionization mass spectrometry (ESI-MS) and ^{1}H and ^{13}C NMR spectroscopy. We report the assignment of NMR spectra of these oligosaccharides.

2. Experimental

2.1. Material

2AB and $NaBH_3CN$ were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Other chemicals were purchased from Wako Pure Chemicals (Osaka, Japan).

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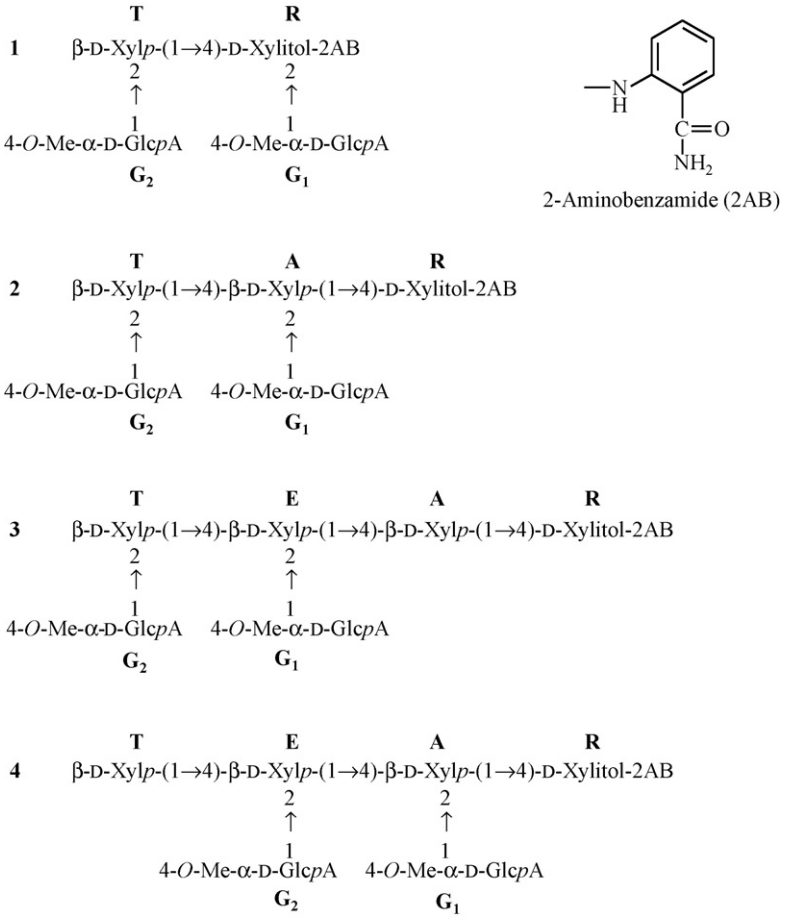

Fig. 1. Structures of compounds **1**–**4**. **1**, Tetrasaccharide consisting of a xylitol (**R**), a terminal Xyl residue (**T**), and two 4-*O*-Me-Glc*p*A residues ($\mathbf{G_1}$ and $\mathbf{G_2}$); **2**, pentasaccharide consisting of a xylitol (**R**), internal Xyl residue (**A**), a terminal Xyl residue (**T**) and two 4-*O*-Me-Glc*p*A residues ($\mathbf{G_1}$ and $\mathbf{G_2}$); **3**, hexasaccharide consisting of a xylitol (**R**), internal Xyl residues (**A** and **E**), a terminal Xyl residue (**T**) and two 4-*O*-Me-Glc*p*A residues ($\mathbf{G_1}$ and $\mathbf{G_2}$); **4**, hexasaccharide consisting of a xylitol (**R**), internal Xyl residues (**A** and **E**), a terminal Xyl residue (**T**), and two 4-*O*-Me-Glc*p*A residues ($\mathbf{G_1}$ and $\mathbf{G_2}$).

2.2. Preparation of acidic xylo-oligosaccharides

The following four acidic xylo-oligosaccharides were isolated in addition to the aldouronic acids composed of a single MeGlcA residue and 1–4 Xyl residues ($MeGlcAXyl_{1-4}$) from the partial hydrolyzates of AGXs extracted from the holocelluloses of Japanese cedar (Sugi, *C. japonica*) and Hinoki cypress (*C. obtusa*) in the same way as described in previous papers (Komiyama, Kato, Aimi, Ogihara, & Shimizu, 2008; Shimizu et al., 1978; Shimizu & Samuelson, 1973). They were identified on the basis of their volume distribution coefficients (*Dv*) in ion exchange chromatography compared with those of authentic samples, which were calculated in the usual way (Samuelson, 1963).

2.3. 2AB labeling of oligosaccharides

The oligosaccharides were labeled with 2AB and purified as described (Ishii, Ichita, Matsue, Ono, & Maeda, 2002) and analyzed by normal-phase liquid chromatography (LC).

2.4. Analytical methods

LC was performed using a Shimadzu LC system (Shimadzu LC-20AD, Kyoto, Japan), a fluorescence detector (Shimadzu RF-$10A_{XL}$, Kyoto, Japan) at λ_{ex} = 330 nm and λ_{em} = 420 nm and an Amide-80 column (4.6 mm × 250 mm, TOSOH, Tokyo, Japan) eluted at 1.0 mL min^{-1} at 30 °C (Ishii et al., 2008). ESI-MS analysis was per-

Table 1
Electrospray ionization mass spectrometry data for compounds **1**–**4**.

Nominal mass	Molecular ion	Compound[a]	Molecular weight	Observed fragment ions m/z[b]	
				Y_n ions	C_n ions
805	$[M+Na]^+$	**1**	782	615, 483, 293	
937	$[M+Na]^+$	**2**	914	747, 615, 425	667, 477
1,069	$[M+Na]^+$	**3**	1,046	879, 747, 557	799, 667, 477
1,069	$[M+Na]^+$	**4**	1,046	937, 879, 747, 615, 425	799, 609, 477

[a] Structures shown in Fig. 1.
[b] Y_n ions and C_n ions (Costello & Vath, 1993).

Table 2
^{1}H chemical shifts and first-order coupling constants (Hz) for compounds **1**–**4**.

Compound	Residue	^{1}H chemical shifts (ppm)								First-order coupling constants (Hz)							
		H-1a	H-1b	H-2	H-3	H-4	H-5a	H-5b	CH_3	$^{3}J_{1a,2}$	$^{3}J_{1b,2}$	$^{2}J_{1a,1b}$	$^{3}J_{2,3}$	$^{3}J_{3,4}$	$^{3}J_{4,5a}$	$^{3}J_{4,5b}$	$^{2}J_{5a,5b}$
1	**R**	3.565	3.555	4.002	3.922	3.907	3.843	3.683		4.5	5.0	11.0	8.0	9.0	6.0	12.0	11.0
	T	4.668	–	3.348	3.463	3.589	3.915	3.262		7.8	–	–	9.0	9.0	6.0	11.0	11.0
	G_1	5.118	–	3.583	3.745	3.222	3.928	–	3.458[a]	4.0	–	–	9.0	6.0	10.0	–	–
	G_2	5.364	–	3.594	3.737	3.210	4.295	–	3.456[a]	3.4	–	–	9.0	6.0	10.0	–	–
2	**R**	3.467	3.268	3.990	3.767	3.921	3.825	3.779		4.5	5.0	11.0	8.0	9.0	6.0	12.0	11.0
	A	4.668	–	3.265	3.634	3.709	4.002	3.376		7.6	–	–	9.0	9.0	6.0	11.0	11.0
	T	4.541	–	3.391	3.473	3.645	3.975	3.285		7.6	–	–	9.0	9.0	6.0	11.0	11.0
	G_1	5.332	–	3.579	3.750	3.208	4.292	–	3.451	3.9	–	–	9.0	6.0	10.0	–	–
	G_2	5.266	–	3.540	3.736	3.206	4.280	–	3.455	3.8	–	–	9.0	6.0	10.0	–	–
3	**R**	3.474	3.253	3.991	3.769	3.949	3.832	3.780		4.5	5.0	11.0	8.0	9.0	6.0	12.0	11.0
	A	4.536	–	3.270	3.554	3.715	4.012	3.360		7.7	–	–	9.0	9.0	6.0	11.0	11.0
	E	4.567	–	3.411	3.641	3.786	4.141	3.486		7.4	–	–	9.0	9.0	6.0	11.0	11.0
	T	4.593	–	3.408	3.469	3.630	3.996	3.289		7.6	–	–	9.0	9.0	10.0	11.0	11.0
	G_1	5.242	–	3.535	3.753	3.203	4.304	–	3.455	3.9	–	–	9.0	6.0	10.0	–	–
	G_2	5.298	–	3.753	3.749	3.210	4.291	–	3.455	3.9	–	–	9.0	6.0	10.0	–	–
4	**R**	3.478	3.277	4.108	3.778	3.923	3.818	3.783		4.5	5.0	11.0	8.0	9.0	6.0	12.0	11.0
	A	4.667	–	3.368	3.623	3.705	3.993	3.285		7.5	–	–	9.0	9.0	6.0	11.0	11.0
	E	4.564	–	3.406	3.586	3.790	4.105	3.357		7.5	–	–	9.0	9.0	6.0	11.0	11.0
	T	4.455	–	3.243	3.422	3.604	3.966	3.299		7.8	–	–	9.0	9.0	6.0	11.0	11.0
	G_1	5.338	–	3.574	3.750	3.221	4.288	–	3.451	3.9	–	–	9.0	6.0	10.0	–	–
	G_2	5.330	–	3.541	3.738	3.208	4.310	–	3.451	4.0	–	–	9.0	6.0	10.0	–	–

[a] Interchangable, uncertain.

formed with a Thermo-Quest LCQ DUO mass spectrometer (Thermo Electron, Waltham, MA, USA) (Ishii et al., 2002). 1D, and 2D-double quantum filtered correlation spectroscopy (DQF-COSY), 2D-total correlation spectroscopy (TOCSY) with 100 msec mixing time, 2D $\{^{1}H–^{13}C\}$ ^{1}H-detected heteronuclear single quantum coherence spectroscopy (HSQC), and ^{1}H-detected multiple-bond heteronuclear multiple quantum coherence spectroscopy (HMBC) were performed at 303 K and 800 MHz with a Bruker Avance 800 NMR spectrometer (Bruker Biospin, Karlsruhe, Germany) as described (Ishii et al., 2008).

3. Results and discussion

3.1. 2-Aminobenzamide labeling of oligosaccharides

Four acidic xylo-oligosaccharides consisting of two MeGlcA residues and 2–4 Xyl residues ($MeGlcA_2Xyl_{2-4}$) were isolated from the partial acid hydrolyzates of Sugi and Hinoki AGX. They were labeled at their reducing ends with 2AB (Ishii et al., 2002) and eluted as a single peak when analyzed by normal-phase LC (data not shown). The molecular weight and sequence of glycosyl residues

Table 3
^{13}C chemical shifts for compounds **1**–**4**.

Compound	Residue	^{13}C chemical shifts (ppm)						
		C-1	C-2	C-3	C-4	C-5	C-6	CH_3
1	**R**	43.98	76.85	73.99	80.69	61.33	–	
	T	103.32	78.96	76.13	71.25	66.66	–	
	G_1	98.91	72.97[a]	73.92	83.94	71.78	177.63	61.63
	G_2	99.38	73.07[a]	73.92	84.05	73.67	178.24	61.63
2	**R**	46.95	70.60	72.66	81.06	62.18	–	
	A	103.91	78.56	73.99	78.29	64.26	–	
	T	103.32	78.32	76.02	71.08	66.62	–	
	G_1	99.27	73.01	73.90	84.11	73.89	178.46	61.51
	G_2	99.24	72.97	73.97	84.16	73.84	178.46	61.59
3	**R**	46.93	70.84	72.75	81.37	62.33	–	
	A	104.03	74.71	75.36	77.65	64.39	–	
	E	102.90	77.96	73.97	78.29	64.37	–	
	T	103.48	78.32	76.05	71.11	66.62	–	
	G_1	99.17	72.99	73.86	84.12[a]	73.86	178.49	61.48[a]
	G_2	99.25	72.99	73.94	84.17[a]	73.86	178.49	61.59[a]
4	**R**	46.98	70.07	72.66	81.14	62.17	–	
	A	103.89	78.56	73.89[a]	78.10	64.23	–	
	E	103.10	78.30	73.95[a]	78.54	64.51	–	
	T	103.67	74.56	77.31	71.11	66.90	–	
	G_1	99.28	73.00[a]	73.94[a]	84.12[a]	73.84	178.43	61.54[a]
	G_2	99.28	72.96[a]	73.88[a]	84.09[a]	73.84	178.43	61.52[a]

[a] Interchangable, uncertain.

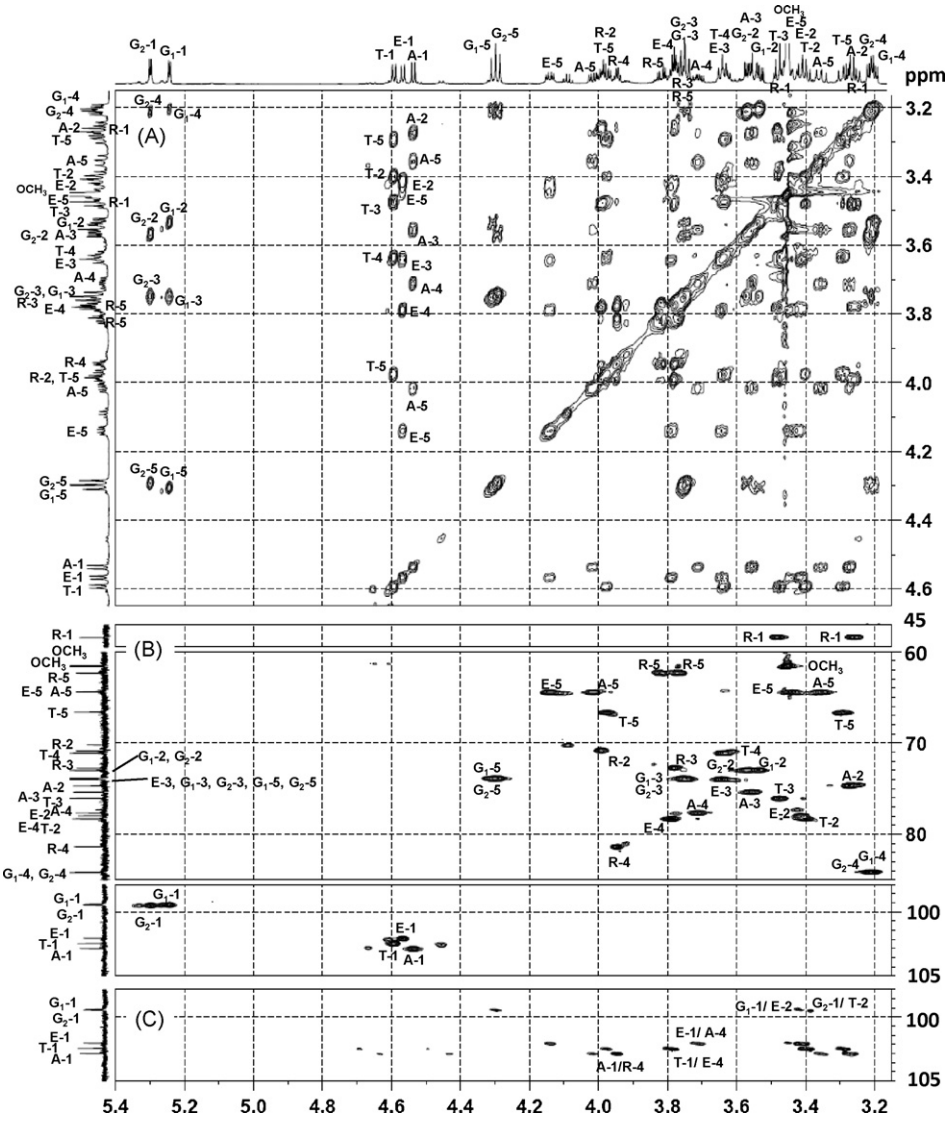

Fig. 2. TOCSY (A), HSQC (B), and HMBC (C) spectra of compound **3**.

of each derivative (**1**–**4**, Fig. 1) were deduced from their positive- and negative-ion mode ESI-MS spectra (Table 1). For example, the positive-ion mode ESI-MS spectrum of **3** contained an ion at *m*/*z* 1,069 that corresponds to the $[M+Na]^+$ ion of an oligosaccharide derivative that is composed of three xylosyl residues, two MeGlcA residues, a xylitol residue, and 2AB. The product ion spectrum of the ion at *m*/*z* 1,069 contained Y_n ions (Costello & Vath, 1993) at *m*/*z* 879, 747, and 557 that correspond to the sequential loss of MeGlcA, xylose, and MeGlcA residues, respectively. The product ion spectrum also contained C_n ions (Costello & Vath, 1993) at *m*/*z* 799, 667, and 447 that correspond to the sequential loss of xylitol-2AB, xylose, and a disaccharide composed of MeGlcA and xylose, respectively. Such results are consistent with the existence of the oligosaccharide derivative **3**.

3.2. Assignment of ^{1}H and ^{13}C NMR spectra of the 2AB-labeled oligosaccharides

The ^{1}H and ^{13}C NMR signals of compounds (**1**–**4** in Fig. 1) were assigned using DQF-COSY, TOCSY, HSQC, and HMBC spectroscopy. All of the signals in the NMR spectra were assigned to the oligosaccharides or 2AB (Table 2), thereby confirming that the derivatives were homogeneous. Herein we describe the complete assignment of the ^{1}H NMR spectrum (see Fig. 2A) of compound **3**. The xylitol residue (the former reducing end of the oligosaccharide) was clearly no longer in the pyranose ring form because C-1 was substituted with two protons rather than one. These two protons gave double doublets at δ 3.474 and 3.251 (**R** H-1 in upper column of Fig. 2A), with coupling constants of 4.5 and 5.0 Hz. The doublet at δ 4.593 (J 7.6 Hz) was assigned to the resonance of the H-1 of the terminal non-reducing Xyl residue **T**, whereas the H-1 resonance of the residue next to the former reducing end (residue **A**) was at δ 4.536. The remaining doublet at δ 4.567 was the resonances of the H-1 of the internal sugar residue (residue **E**). The chemical shift values of H-1s of the non-reducing terminal Xyl residues and the magnitude of the coupling constants (J 7.4–7.7 Hz) were consistent with those of a β-linkage (Utille, Kováč, Sauriol, & Perlin, 1986). The doublet (J 3.9 Hz) at δ 5.242 and 5.298were assigned to the resonance of the H-1 of the MeGlcA residues (**G$_1$** and **G$_2$**). The anomeric resonances were all well resolved from the non-anomeric sugar proton signals. The DQF-COSY and TOCSY spectra allowed the assignment of the proton signals from H-1 to H-5 (Fig. 2A). The proton signals of H-5 were assigned by HSQC (Fig. 2B). The ^{13}C NMR spectra of the 2AB-labeled oligosaccharides were analyzed by HSQC and HMBC spectroscopy. The HMBC spectra gave extensive intramolecular correlations between the proton and carbon atoms of each residue

(Fig. 2C), confirming the connectivity of each glycosyl residue in the oligomers. All the ^{13}C assignments are shown in Table 3. Assignment of 2AB of the 2AB-labeled xylo-oligosaccharides was reported previously (Ishii et al., 2008). NMR assignment of four 2AB-labeled oligosaccharides confirmed the structure previously characterized by glycosyl composition and glycosyl-linkage analyses (Shimizu et al., 1978). Usually proton and carbon signals of the Xyl residue substituted with MeGlcA residue shift down-fielded, about 0.15 ppm and about 4 ppm compared with those of non-substituted one. In the case of compound **2**, the presence of MeGlcA residue ($\mathbf{G_1}$) did not affect the H-2 signal of Xyl residue (**A**) substituted with MeGlcA residue. H-2 signal of residue **A** in compound **2** was at δ 3.265, while H-2 signal of residue **T** in compound **2** was at δ 3.391. On the other hand, C-2 signals of Xyl residues substituted with MeGlcA residues (C-2 of residues **A** and **T** in compound **2**) were down-fielded at δ 78.56 and 78.32. C-2 of the non-substituted Xyl residue in the 2AB-labeled aldotriouronic acid (MeGlcA-Xyl-Xyl-Xylitol-2AB) was observed at δ 74.71 (Ishii et al., 2008).

The ratio of xylo-oligosaccharides with two MeGlcA residues and those with a single MeGlcA residue was about 1:3 for both Sugi and Hinoki AGX. Jacobs et al. (2001) reported that xylo-oligosaccharides with MeGlcA on adjacent Xyl residues from softwoods are minor components. Softwood xylans were hydrolyzed with 50% trifluoroacetic acid at room temperature for 30 days. In the present experiments AGXs were hydrolyzed with 0.125 M sulfuric acid at 90 °C for 9 h. The difference in ratio of acidic xylo-oligosaccharides might be due to acid hydrolysis conditions used.

4. Conclusion

We have generated in high yield (~90%) 2AB-labeled xylo-oligosaccharides that contain two MeGlcA residues and 2–4 Xyl residues, and have assigned all of the signals in their ^{1}H and ^{13}C NMR spectra. In a previous paper (Ishii et al., 2008) we reported the NMR assignments of 2AB-labeled neutral and acidic xylo-oligosaccharides. Together these data present data provide information that will faciltate the structural characterization of softwood and hardwood xylans by NMR spectroscopy.

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