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Note

Characterization of *O*-acetyl-(4-*O*-methylglucurono)xylan isolated from birch and beech

Anita Teleman,[a,*] Maija Tenkanen,[b,†] Anna Jacobs,[a] Olof Dahlman[a]

[a] *Swedish Pulp and Paper Research Institute, STFI, Box 5604, SE-114 86 Stockholm, Sweden*
[b] *VTT Biotechnology, PO Box 1500, FIN-02044 VTT, Finland*

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Abstract

The structures of water-soluble birch and beech xylans, extracted from holocellulose using dimethyl sulfoxide, were determined employing ^{1}H and ^{13}C NMR spectroscopy together with chemical analysis. These polysaccharides were found to be *O*-acetyl-(4-*O*-methylglucurono)xylans containing one 4-*O*-methylglucuronic acid substituent for approximately every 15 D-xylose residues. The average degree of acetylation of the xylose residues in these polymers was 0.4. The presence of the structural element →4)[4-*O*-Me-α-D-Glc*p*A-(1→2)][3-*O*-Ac]-β-D-Xyl*p*-(1→ was demonstrated. Additional acetyl groups were present as substituents at C-2 and/or C-3 of the xylopyranosyl residues. Utilizing size-exclusion chromatography in combination with mass spectroscopy, the weight-average molar masses (and polydispersities) were shown to be 8000 (1.09) and 11,100 (1.08) for birch and beech xylan, respectively.

Keywords: Hardwood; Hemicellulose, extraction; Xylan, structure; *O*-Acetylation; ^{1}H and ^{13}C NMR spectroscopy; MALDI-MS

The most abundant hemicellulose constituent of hardwoods is *O*-acetyl-(4-*O*-methylglucurono)xylan.[1] The backbone of this hardwood xylan consists of β-(1→4)-D-xylopyranosyl residues, with, on average, one α-(1→2)-linked 4-*O*-methylglucuronic acid (MeGlcA) substituent per 10–20 such residues.[1,2] Many of the xylose residues also contain an *O*-acetyl group at position C-2 and/or C-3. The content of such *O*-acetyl groups ranges from 9 to 17 weight%, which corresponds to approximately four to seven acetyl groups per ten xylopyranosyl residues.[3–10]

Osmometry, sedimentation-equilibrium, solvent fractionation, and size-exclusion chromatography (SEC) have all been applied to determine the average molar-mass and degree of polymerization (DP) of hardwood xylans.[2,3,5,11–14] Average molar-masses of 5600–40,000 and average degrees of polymerization of 110–220 have been reported. These wide ranges probably reflect differences between various species of wood, modes of isolation and analytical procedures.

Recently, a new procedure for determination of the molar mass of lignins and polysaccharides involving SEC in combination with off-line matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (MALDI–MS), has been described.[15,16] This approach has been used to determine the molar mass of xylans extracted with 24% (w/w) aqueous potassium hydroxide (which removes acetyl substituents) from birch and aspen holocellulose (defatted and delignified wood). The weight-average molar-masses of the xylans extracted from birch and aspen holocellulose were 13,700 (DP = 101) and 17,100 (DP = 122), respectively.[16]

In an earlier study, we described a new structural element present in *O*-acetyl-(4-*O*-methylglucurono)-xylan extracted from aspen chips by treatment in a microwave oven.[17] This structural element, →4)[4-*O*-Me-α-D-Glc*p*A-(1→2)][3-*O*-Ac]-β-D-Xyl*p*-(1→ (**XG3**),

* Corresponding author.
E-mail address: anita.teleman@stfi.se (A. Teleman).
† Present address: Oy Keskuslaboratorio, KCL, PO Box 70, FIN-02151 Espoo, Finland.

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Table 1
Relative contents of acetyl groups and monosaccharide residues in glucuronoxylans extracted from birch and beech wood with DMSO

Sample	Method	Structural element (mol%)			DS_{Ac} [a]
		Xyl	MeGlcA	Ac	
Birch xylan	chemical analysis [b]	67.8	4.0	28.2	0.39
	NMR [c]	68	3	29 [d]	0.40
Beech xylan	chemical analysis [b,e]	64.1	4.5	29.0	0.42
	NMR [c]	70	4	26 [d]	0.35

[a] DS_{Ac}, degree of substitution with acetyl groups.
[b] See Section 1.
[c] Determined by integration of quantitative 1D NMR spectra.
[d] Of the *O*-acetyl substituents on the xylose residues, approximately 1/3 and 2/3 are at the C-2 and C-3 positions, respectively.
[e] Minor quantities of arabinose (0.9 mol%) and glucose (1.5 mol%) were also detected.

has not been found in other xylans. Since it is not known what happens to acetyl groups upon treatment in a microwave oven at 180 °C, it was of interest to examine whether **XG3** is a common structural element of hardwood xylans. A procedure commonly used to obtain *O*-acetyl-(4-*O*-methylglucurono)xylan involves extraction of the holocellulose with dimethyl sulfoxide (DMSO). In the present investigation, water-soluble xylans from birch and beech holocellulose were thus extracted into DMSO and characterized by chemical analysis, NMR spectroscopy and SEC/MALDI–MS.

Xylans were extracted from the holocelluloses of two hardwood species, birch and beech, employing DMSO. Carbohydrate analysis of the xylans, thus obtained, revealed the presence of one 4-*O*-methylglucuronic acid group per approximately every 15 D-xylose residues (Table 1). The acetic acid released from these xylans upon treatment with sodium hydroxide was quantified utilizing an enzymatic/spectrophotometric procedure (Table 1).

The xylans obtained were subsequently examined by NMR spectroscopy. The samples were first dissolved in deuterated water (after neutralization of the pD) and NMR spectra thereafter run at 27 and 70 °C. The signals observed at approximately 2.2 ppm confirm that the polysaccharides in these samples are indeed acetylated (Fig. 1). The ^{1}H and ^{13}C chemical shifts for the xylans extracted from both birch and beech (Figs. 1 and 2) are in excellent agreement with the values reported earlier[17] for *O*-acetyl-(4-*O*-methylglucurono)xylan isolated from aspen wood (depicted as crosses in Fig. 2). Thus, both signals and cross-peaks characteristic of the structural element →4)[4-*O*-Me-α-D-Glc*p*A-(1→2)][*O*-Ac-(1→3)]-β-D-Xyl*p*-(1→ (**XG3**) were detected (Figs. 2 and 3). Two additional cross-peaks (indicated by arrows in Fig. 2(B)) were present in the two-dimensional (2D) HSQC spectrum. The ^{1}H and ^{13}C chemical shifts of these additional cross-peaks are in agreement with corresponding values reported for the structural element →4)[4-*O*-Me-α-D-Glc*p*A-(1→2)]-β-D-Xyl*p*-(1→ (**XG**, i.e., $^{1}H/^{13}C$ chemical shifts of 3.51/77.9 and 3.69/73.3 ppm[18]). Moreover, the cross-peak originating from the methyl group of MeGlcA was split into two (Fig. 2(B)).

However, no well-resolved signal for the structural element **XG** could be detected in the corresponding one-dimensional (1D) ^{1}H NMR spectra. The doublet originating from MeGlcA H-1 arises from both **XG** and **XG3**. The ratio between the integrated values for the

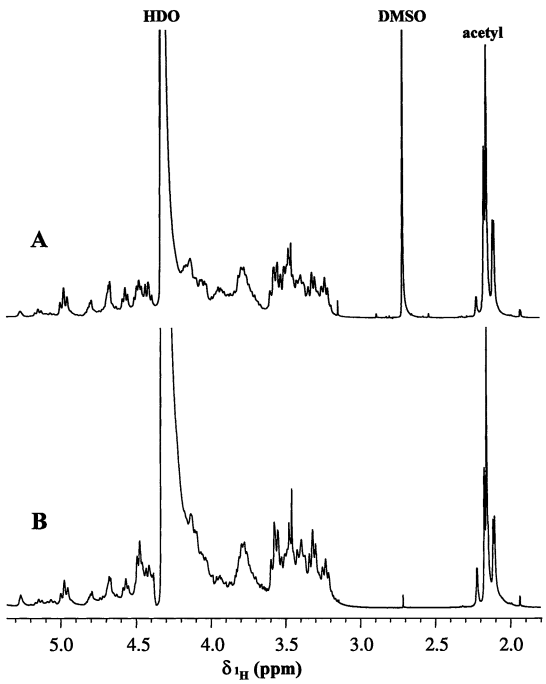

Fig. 1. ^{1}H NMR spectra of the xylans extracted from two hardwood species, birch (A) and beech (B), with DMSO.

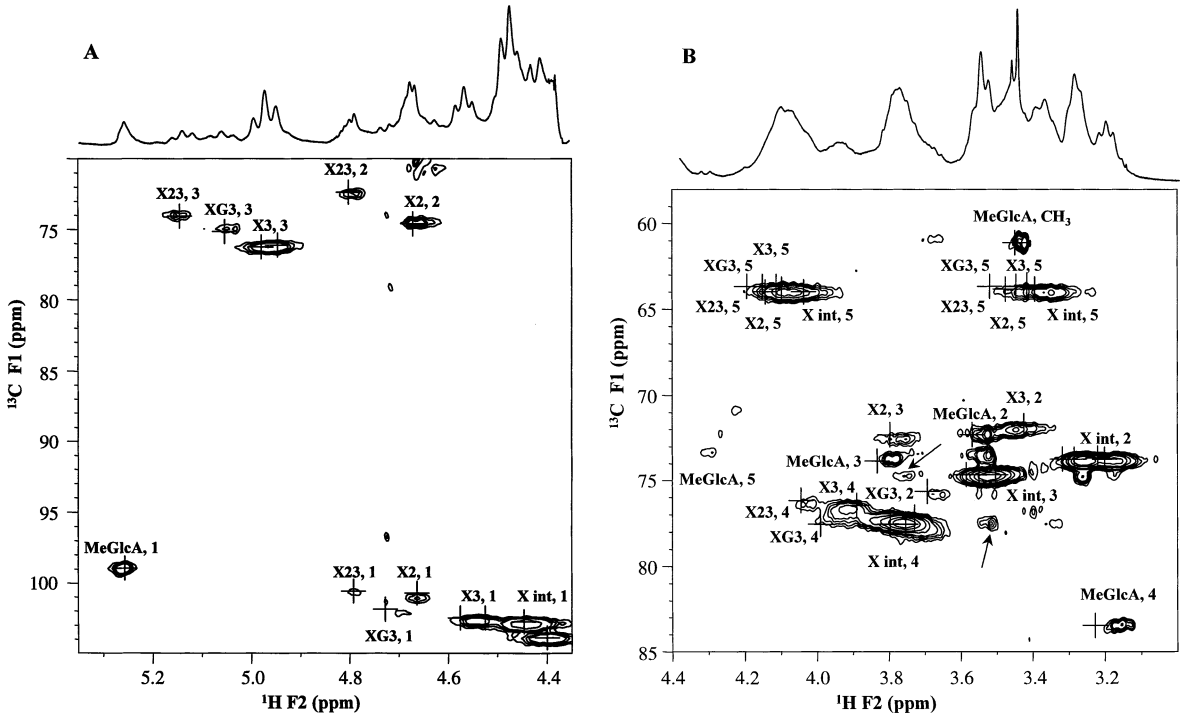

Fig. 2. The 2D HSQC spectrum of beech wood xylan obtained at 27 °C, illustrating the *O*-acetylated xylose and anomeric (A) and other (B) regions. The spectra aligned at the top of these figures are the 1D ^{1}H NMR spectra of beech wood xylan obtained at 70 (A) and 27 °C (B). The following designations are used: X int, non-acetylated internal Xyl residue; X2, 2-*O*-acetylated Xyl; X23, 2,3-di-*O*-acetylated Xyl; X3, 3-*O*-acetylated Xyl; **XG3**, MeGlcA 2-*O*-substituted and 3-*O*-acetylated Xyl; MeGlcA, 4-*O*-methylglucuronic acid. The last number in the cross-peak designations refers to the H- and C-atom. The crosses (+) illustrate the chemical shifts reported earlier for *O*-acetyl-(4-*O*-methylglucurono)xylan extracted from aspen.[17] The arrows (in B) indicate cross-peaks not observed in the HSQC spectrum of *O*-acetyl-(4-*O*-methylglucurono)xylan from aspen.[17]

triplet of **XG3** H-3 and the doublet of MeGlcA H-1 indicates that there is somewhat more **XG3** than **XG** present in the xylans extracted from both birch and beech xylan (Table 2 and Fig. 3). At present, it is not possible to conclude whether the native hardwood xylan contains both the structural elements **XG3** and **XG** or only **XG3**. The xylans studied here may have been partially deacetylated during the extraction procedure, since the precipitate was slightly acidic (pH 3). Previously, only the structural element **XG3** was detected in two fractions of xylans extracted from aspen chips by treatment in a microwave oven.[17] However, **XG** may be present in other fractions of the native aspen xylan.

The relative contents of acetyl and sugar residues were determined by integration of the signals from the anomeric protons, the H-2 signals from 2-*O*-acetylated xylose residues and the H-3 signals from 3-*O*-acetylated xylose residues, observed in the fingerprint region (Table 1). The average degree of substitution of the xylose residues with acetyl groups (DS_{Ac}) was subsequently calculated (Table 1). Similar DS_{Ac} values are obtained by integration of the signals assigned to acetyl groups (at 2.2 ppm) and of all of the carbohydrate signals. Furthermore, NMR spectroscopy and chemical analysis resulted in very similar values for the relative

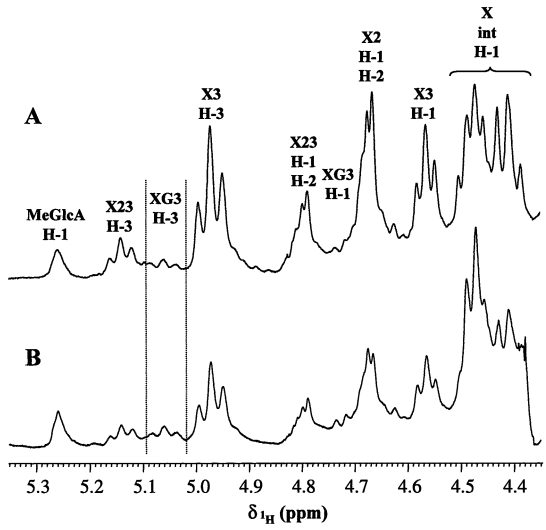

Fig. 3. The fingerprint regions of the ^{1}H NMR spectra of *O*-acetyl-(4-*O*-methylglucurono)xylans, extracted from birch (A) and beechwood (B) using DMSO. See Fig. 2 for an explanation of the peak designations. The triplet of H-3 originating from the xylose residues in the structural element **XG3** is indicated by the vertical dotted lines.

Table 2
Relative integrated values for the doublet of MeGlcA H-1 and the triplet of **XG3** H-3 observed in 1D ^{1}H NMR spectra of xylans extracted from two hardwood species with DMSO

Sample	NMR frequency (MHz)	Relative integrated value	
		MeGlcA H-1	**XG3** H-3
Birch xylan	400	1.0	0.8
	600	1.0	0.6
Beech xylan	400	1.0	0.8
	600	1.0	0.6

Table 3
The weight and number-average molar mass values (M_w and M_n) and the polydispersity indices (M_w/M_n) of the xylans investigated here

Sample	M_w [a]	M_n [a]	M_w/M_n
Birch xylan	8000	7400	1.09
Beech xylan	11,100	10,300	1.08

[a] The estimated standard deviation is 5%.[16]

contents of acetyl and sugar residues (Table 1). These values obtained for the xylans from birch and beech wood examined here are typical for hardwood xylans.[1,19]

The average molar-mass values were determined by SEC in combination with MALDI–MS calibrations for *O*-acetyl-(4-*O*-methylglucurono)xylans (Table 3). The values obtained here are somewhat lower than the weight-average molar-masses of 13,700–17,100 reported earlier for xylans extracted from hardwood holocelluloses with aqueous alkaline solutions.[16] This may reflect the fact that the present values of 8000 and 11,100 for the birch and beech xylans, respectively, are not valid for the entire native polymer, since dimethyl sulfoxide is known to extract only a minor portion of hardwood xylans.[20] Furthermore, slight depolymerization of the native xylan may occur during the delignification process.[21] The average molar-mass values determined here correspond to DP-values of approximately 50 and 60 for the birch and beech xylans, respectively. No signals originating from reducing end or terminal-end residues were observed in the ^{1}H NMR spectra (Fig. 3), confirming that the DP is >40.

In conclusion, →4)[4-*O*-Me-α-D-Glc*p*A-(1→2)][*O*-Ac-(1→3)]-β-D-Xyl*p*-(1→ appears to be a structural element common to xylans isolated from different hardwood species, including aspen, beech and birch. Xylose residues carrying a MeGlcA substituent and without 3-*O*-acetylation were also detected in these xylans, extracted with DMSO.

1. Experimental

Isolation of O-*acetyl-(4*-O-*methylglucurono)xylan.*—Birch and beech wood were delignified by treatment with chlorite[22] and the holocellulose subsequently extracted with Me_2SO and further purified as described by Hägglund et al.[20]

Chemical analysis.—The carbohydrate compositions of the isolated *O*-acetyl-(4-*O*-methylglucurono)xylans were determined employing enzymatic hydrolysis[23] followed by HPAEC-PAD analysis.[24]

The content of acetyl groups was determined by quantifying the AcOH released from *O*-acetyl-(4-*O*-methylglucurono)xylans upon treatment with NaOH. To this purpose, 50 mg of xylan was incubated overnight with 2 mL 0.1 M NaOH, after which the pH was adjusted to approximately 7 with 1 M HCl. The amount of AcOH liberated was then analyzed utilizing an enzymatic/spectrophotometric procedure (Boehringer Test Combination 148 261).

SEC/MALDI–MS.—The SEC/MALDI–MS analysis was performed employing a procedure developed earlier in our laboratory,[16] with the modification that in the present case, 50 mM ammonium acetate was used as the mobile phase. The SEC system consisted of three columns containing Ultrahydrogel 120, 250 and 500 (Waters Assoc., USA) and linked in series with a refractive index detector (Waters Assoc., USA). The signal from this detector was processed using the PL CALIBER SEC software and interface (Polymer Laboratories Ltd., UK) on a standard PC. Fractions were collected sequentially from the outlet of the refractometer. The peak-average molar mass (M_p) values of these fractions were determined on the basis of their MALDI–MS spectra. The logarithms of these M_p values were then plotted as a function of the corresponding times required for elution from the SEC system and the linear relationship thus obtained used to calculate the number and weight-average molar masses (M_n and M_w) of the eluted polysaccharides.

NMR spectroscopy.—For NMR analysis, 3 mg of each of the dried samples was dissolved in 0.35 mL D_2O (99.9 atom%D, Cambridge Isotope Laboratories). The pD of these solutions was then adjusted to 7 by addition of NaOD. The ^{1}H NMR (^{13}C NMR) spectra were obtained employing either a Bruker DPX 400 MHz spectrometer operating at 400.13 MHz (100.61 MHz) or a Bruker DRX600 operating at 600.13 MHz. 1D ^{1}H NMR spectra were recorded using a 90° pulse, a spectral width corresponding to 10 ppm and a repetition time of 19 s. All spectra were run at a probe temperature of 27 or 70 °C. The chemical shifts reported were calibrated relative to the signals from

acetone, used as an internal standard, at 2.225 ppm and 31.55 ppm for the ^{1}H and ^{13}C NMR spectra, respectively.

The proton-detected heteronuclear single quantum (HSQC) spectra[25–27] were acquired over a t_1 spectral width of 11,000 Hz and a t_2 width of 1800 Hz, with a 750×1024 matrix (zero-filled to 1024 and 2048 in t_1 and t_2, respectively) and 32 transients per increment. The delay between transients was 2.6 s and the delay for polarization transfer was set to correspond to an estimated average $^{1}H-^{13}C$ coupling constant of 150 Hz. Data processing was performed using standard Bruker XWIN-NMR software.

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