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Carbohydrate Research 329 (2000) 807–815

CARBOHYDRATE RESEARCH

# Characterization of acetylated 4-*O*-methylglucuronoxylan isolated from aspen employing $^{1}H$ and $^{13}C$ NMR spectroscopy

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Received 26 May 2000; accepted 14 August 2000

**Abstract**

Water-soluble hemicelluloses were extracted from milled aspen wood (*Populus tremula*) employing microwave oven treatment at 180 °C for 10 min. The final pH of this extract was 3.5. From this extract oligo- and polysaccharides were isolated and subsequently fractionated by size-exclusion chromatography. The structures of the saccharides in three of the fractions obtained were determined by $^{1}H$ and $^{13}C$ NMR spectroscopy, using homonuclear and heteronuclear two-dimensional techniques. The polysaccharides present in the two fractions eluted first were *O*-acetyl-(4-*O*-methylglucurono)xylans. The average degree of acetylation of the xylose residues in these compounds was 0.6. The structural element →4)[4-*O*-Me-α-D-Glc*p*A-(1→2)][3-*O*-Ac]-β-D-Xyl*p*-(1→ could also be identified. On the average, these two xylans were composed of the following (1→4)-linked β-D-xylopyranosyl structural elements: unsubstituted (50 mol%), 2-O-acetylated (13 mol%), 3-O-acetylated (21 mol%), 2,3-di-O-acetylated (6 mol%) and [MeGlcA α-(1→2)][3-O-acetylated] (10 mol%). Most of the 4-*O*-methylglucuronyl and acetyl substituents in the isolated polysaccharides survived the microwave oven treatment. The third fraction, eluted last, contained acetylated *xylo*-oligosaccharides, with minor contamination by an acetylated mannan. In the case of these *xylo*-oligosaccharides, the average degree of acetylation was 0.3. 

*Keywords:* Aspen wood (*Populus tremula*); Hemicellulose, extraction; Xylan, structure; O-Acetylation; Microwave oven, fractionation

## 1. Introduction

The major hemicellulose present in hardwood is *O*-acetyl-(4-*O*-methylglucurono)-xylan, sometimes referred to as acetylated 4-*O*-methylglucuronoxylan [1,2]. The backbone consists of this polymer of approximately 200 β-(1→4)-linked xylopyranosyl residues. Most of the hardwood xylans isolated to date contain, on the average, one 4-*O*-methylglucuronic acid (MeGlcA) side-group per ten xylopyranosyl residues, α-(1→2)-linked to the xylan chain. Many of the xylose residues contain an *O*-acetyl substituent at position C-2 and/or C-3. Small amounts of glucomannan can also be found in hardwood [1,2].

Hardwood xylans can be extracted directly from fully lignified wood with aqueous potassium hydroxide. The yields thus obtained vary widely for different types of wood, probably

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0008-6215/00/$ - see front matter 
PII: S0008-6215(00)00249-4

reflecting differences in the structure of the cell wall [1,2]. The structure of the alkali-extracted xylan is thought to be quite similar to that of the native polysaccharide, except that under alkaline conditions the *O*-acetyl groups are removed. Thus, in order to obtain the acetylated 4-*O*-methylglucuronoxylan, the wood must be pretreated in some way prior to extraction.

A general method utilized for this purpose is to isolate the hardwood xylan from defatted and delignified wood, so-called holocellulose, by extraction with dimethyl sulfoxide [1,3–8]. During the last two decades, extraction of acetylated 4-*O*-methylglucuronoxylan after subjecting wood to a water–steam environment, at high temperature for a short time, has come into use. Different procedures, including steam treatment with or without explosion [9–12], thermochemical treatment in water [13] and microwave oven treatment [14] have been developed. The majority of the acetyl substituents in the 4-*O*-methylglucuronoxylan are stable to such steam treatment [10,13].

The positions of the *O*-acetyl substituents in acetylated 4-*O*-methylglucuronoxylan can be studied with the help of various methylation techniques [3–5,7,8]. The free hydroxyl groups in the polymer are protected first, prior to removal of the acetate groups, subsequent methylation and removal of protecting groups. Finally, the modified polymer is degraded into monomers by acid hydrolysis and the resulting mixture of sugars and partially methylated sugars analyzed by gas chromatography. The main focus in this connection has been on the *O*-acetyl substituents on xylose residues. For (4-*O*-methyl-D-glucurono)-D-xylan from quince tree, periodate oxidation has shown that the *O*-acetyl groups are located on xylose residues only [15]. No attempts to determine whether the xylopyranosyl residue with a linked MeGlcA can be 3-O-acetylated as well have been reported. Precaution must be taken when interpreting the relative degrees of 2-O- and 3-O-acetylation, since *O*-acetyl groups may migrate [8,16].

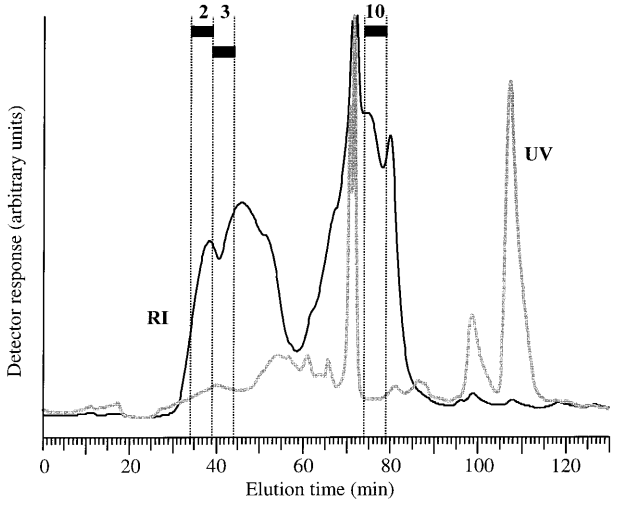


Fig. 1. Elution profile of the water-soluble oligomers and polymers from aspen wood after size-exclusion chromatography. The refractive index (filled) and the UV absorption at 280 nm (hatched) were monitored during the elution. Fractions **2**, **3** and **10** were collected at the elution times indicated by the vertical dotted lines.

Several studies concerning the distribution of *O*-acetyl groups have been conducted directly on the polysaccharides [7,17] employing NMR analysis. Acetyl groups in xylan within acetylated wood have also been analyzed by $^{13}C$ NMR spectroscopy [18]. In addition, structural two-dimensional (2D) NMR studies on the deacetylated forms of 4-*O*-methylglucuronoxylans, or fragment thereof, isolated from wood or pulp have been described [19–21].

In the present study we have extracted acetylated 4-*O*-methylglucuronoxylan from aspen wood (*Populus tremula*) using treatment in a microwave oven. A structural study based on NMR spectroscopy of the isolated oligo- and polysaccharides was subsequently performed.

## 2. Results and discussion

Milled aspen wood was separated into water-soluble (pH 3.5) and water-insoluble fractions by treatment in a microwave oven at 180 °C for 10 min. The water-soluble oligomers and polymers were then further fractionated using size-exclusion chromatography (SEC) (Fig. 1). Three of the fractions thus obtained denoted **2**, **3** and **10**, were subsequently studied by NMR spectroscopy.

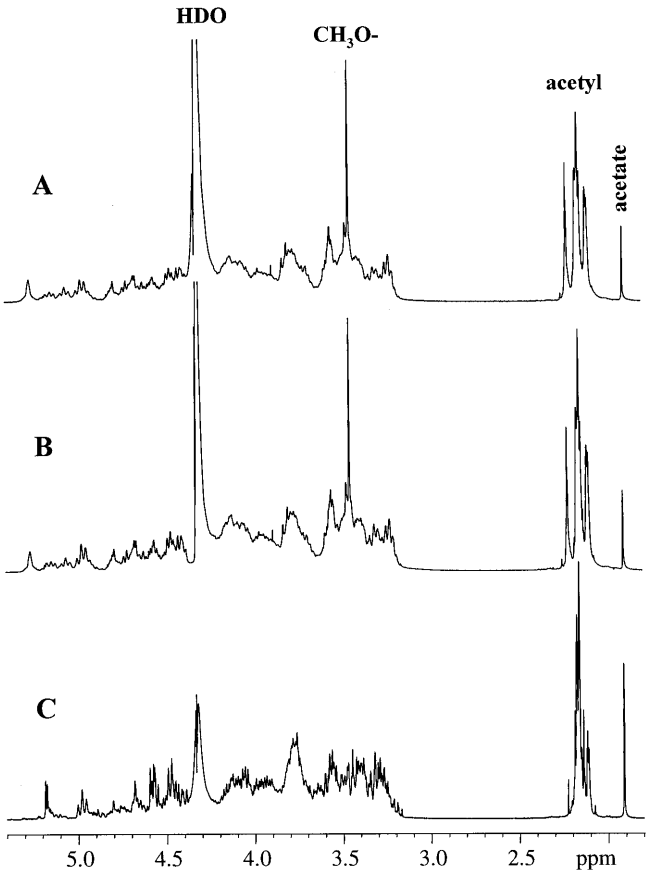


Fig. 2. $^{1}$H NMR spectra of acetylated (4-*O*-methylglucurono)xylans from aspen wood. (A) Fraction **2**; (B) Fraction **3**; and (C) Fraction **10**.

The three samples could easily be dissolved in $D_2O$ at neutral pD. $^{1}$H NMR spectra were then obtained at 27 and 70 °C. The signals at about 2.2 ppm indicated that the oligo- and polysaccharides in these samples are highly acetylated (Fig. 2). These spectra resemble corresponding spectra for acetylated xylan described earlier [17]. van Hazendonk et al. [17] were unable to assign minor signals in these spectra due to insufficient information. Therefore, the hemicelluloses isolated here were further characterized employing 2D NMR procedures. The one-dimensional (1D) proton NMR spectra demonstrated that the acetylated xylans recovered in Fractions **2** and **3** are almost identical (Figs. 2 and 3). Fractions **3** and **10** were selected for more detailed structural analysis. Their proton NMR resonances were assigned on the basis of phase-sensitive COSY and TOCSY experiments, starting with the anomeric protons (Table 1). Subsequent assignment of $^{13}$C resonances was based on heteronuclear correlation spectroscopy employing the assigned proton signals (Table 2).

*Structures of the oligosaccharides present in Fraction* **10**.—In the $^{1}$H NMR spectrum obtained with Fraction **10** several groups of resonances are observed in the fingerprint region between 4.4 and 5.5 ppm (Fig. 3(C)). Some of these groups of resonances appeared to be triplets, rather than the characteristic anomeric proton doublets. The protons in this region were separated into anomeric and other ring protons in the HSQC spectrum (Fig. 4), since the corresponding carbon atoms resonate at characteristic frequencies. The anomeric region in the HSQC spectrum is $^{1}$H 4.4–5.5 ppm/$^{13}$C 90–105 ppm; while the O-acetylated xylose region is $^{1}$H 4.4–5.5 ppm/$^{13}$C 70–80 ppm (Fig. 4). On the basis of this NMR analysis, it could be concluded that Fraction **10** consists of *O*-acetyl-β-D-*xylo*-oligosaccharides (Fig. 5).

With respect to the xylose residues, several different chemical environments were observed. The anomeric proton resonances originating from these groups could be divided into six types those originating from: (i) reducing end residues exhibiting an equilibrium be-

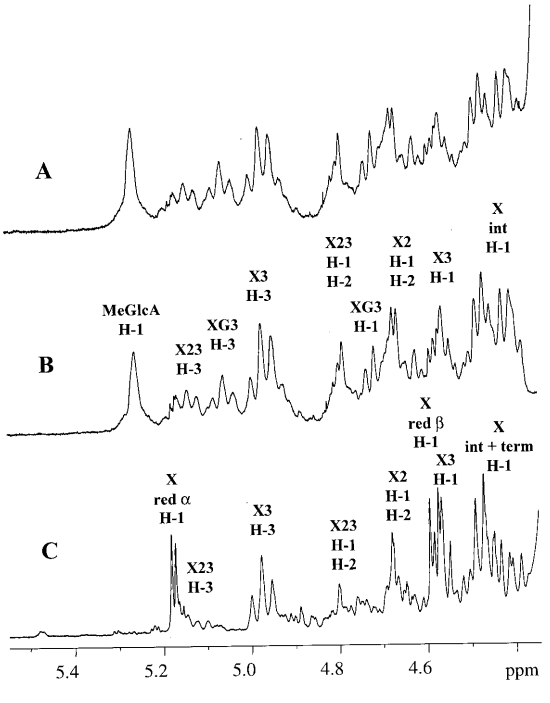


Fig. 3. The fingerprint region of $^{1}$H NMR spectra of acetylated (4-*O*-methylglucurono)xylans. (A) Fraction **2**; (B) Fraction **3**; and (C) Fraction **10**. See footnote [a] in Table 1 for an explanation of the peak designations.

Table 1
$^1$H NMR data on the constituent monosaccharide residues of the oligo- and polysaccharides present in Fractions **3** and **10**

| Fraction | Residue [a] | $^1$H chemical shifts in ppm [b] ($^3J_{H,H}$ in Hz) | | | | | |
|---|---|---|---|---|---|---|---|
| | | H-1 | H-2 | H-3 | H-4 | H-5ax | H-5eq |
| **3** | X red α | 5.18 (3.8) | 3.55 | 3.78 | 3.78 | 3.78 | 3.78 |
| | X red β | 4.58 (8.0) | 3.27 | 3.58 | 3.78 | 3.42 | 4.07 |
| | X int | 4.48 (8) | 3.32 | 3.58 | 3.79 | 3.40 | 4.10 |
| | X int | 4.46 (8) | 3.30 | n.d. [c] | n.d. | n.d. | n.d. |
| | X int | 4.42 (8) | 3.23 | 3.55 | 3.75 | 3.38 | 4.05 |
| | X int | 4.40 (8) | 3.21 | 3.50 | 3.71 | n.d. | 3.98 |
| | X2 | 4.67 | 4.68 | 3.80 | 3.86 | 3.44 | 4.16 |
| | X2 | 4.63 | 4.63 | 3.76 | 3.66 | 3.43 | 4.10 |
| | X23 | 4.79 | 4.81 | 5.14 (9.0) | 4.05 | 3.54 | 4.21 |
| | X3 | 4.58 (8.0) | 3.49 | 4.98 (9.3) | 3.93 | 3.48 | 4.14 |
| | X3 | 4.53 (8) | 3.42 | 4.95 (9.3) | 3.89 | 3.42 | 4.10 |
| | XG3 | 4.73 (7.5) | 3.69 | 5.06 (9.2) | 3.98 | 3.48 | 4.14 |
| | MeGlcA | 5.26 (<5) | 3.57 | 3.83 | 3.24 | 4.30 | – [d] |
| **10** | X red α | 5.18 (3.7) | 3.56 | 3.76 [e] | 3.78 [e] | 3.78 [e] | 3.78 |
| | X red α | 5.16 | 3.53 | 3.76 | 3.78 | 3.78 | 3.78 |
| | X red β | 4.59 (8.0) | 3.27 | 3.55 | 3.78 | 3.41 | 4.06 |
| | X red β | 4.56 | 3.24 | 3.55 | 3.78 | 3.41 | 4.06 |
| | X int | 4.48 (7.8) | 3.22 | 3.58 | 3.79 | 3.40 | 4.11 |
| | X term | 4.463 (7.8) | 3.29 | 3.45 | 3.63 | 3.32 | 3.99 |
| | X term | 4.460 (7.8) | 3.29 | 3.45 | 3.63 | 3.32 | 3.99 |
| | X term | 4.456 (7.8) | 3.29 | 3.45 | 3.63 | 3.32 | 3.99 |
| | X int | 4.43 (7.8) | 3.24 | 3.57 | 3.75 | 3.41 | 4.04 |
| | X term | 4.40 (7.8) | 3.19 | 3.42 | 3.59 | 3.29 | 3.92 |
| | X2 | 4.69 | 4.69 | 3.78 | 3.87 | 3.44 | 4.17 |
| | X23 | 4.82 | 4.81 | 5.14 | 4.05 | 3.54 | 4.22 |
| | X3 | 4.58 (7.7) | 3.49 | 4.98 (9.2) | 3.96 | 3.48 | 4.15 |

[a] The following designations are used: X red, non-acetylated Xyl reducing end; X int, non-acetylated Xyl internal; X term, non-acetylated Xyl terminal end; X2, 2-O-acetylated Xyl; X23, 2,3-di-O-acetylated Xyl; X3, 3-O-acetylated Xyl; XG3, MeGlcA 2-*O*-linked and 3-O-acetylated Xyl; MeGlcA, 4-*O*-methylglucuronic acid.

[b] Relative to an internal acetone standard at 2.225 ppm ($D_2O$, 70 °C, pD 7) and acquired at 400 MHz. The following $^1$H and $^{13}$C chemical shifts were observed for the $CH_3$ groups ($^1$H chemical shift in ppm/$^{13}$C chemical shift in ppm): for 2-O-acetylated Xyl, 2.11/21.20 and 2.11/21.42; for 3-O-acetylated Xyl, 2.22/22.12, 2.17/21.42 and 2.16/21.64; for MeGlcA, 3.46/61.10.

[c] n.d., not detected.

[d] –, not relevant.

[e] These assignments might be interchanged.

tween the α and β conformations; (ii) unsubstituted internal residues; (iii) terminal (non-reducing end) residues; (iv) 2-O-acetylated internal residues; (v) 3-O-acetylated internal residues; and (vi) 2,3-di-O-acetylated internal residues (Fig. 3(C)). The $^1$H and $^{13}$C chemical shifts reported here for reducing end, unsubstituted internal and terminal xylose residues are in good agreement with values published earlier [21–27]. Both the $^1$H and $^{13}$C chemical shifts of 2-O-acetylated and 3-O-acetylated xylopyranosyl residues are also in good agreement with literature values [17].

A number of minor peaks, well separated from the other resonances in the fingerprint region, were also observed (Fig. 3(C)). Three structural elements could be identified from the cross-peaks in the COSY and TOCSY spectra: a reducing-end mannose residue (α H-1,2: $\delta$ 5.18, 3.99), a 2-O-acetylated mannopyranosyl residue (H-1,2,3: $\delta$ 4.92, 5.48, 4.00) and a 3-O-acetylated mannopyranosyl residue (H-1,2,3,4: $\delta$ 4.84, 4.19, 5.18, 4.13). These peaks probably originate from a minor contamination by acetylated mannan.

*Structures of the polysaccharides present in Fraction* **3**.—In comparison with the $^1$H NMR spectrum of Fraction **10**, additional peaks were observed in the spectra of Fractions **2** and **3** (Figs. 2 and 3). A sharp singlet at 3.46 ppm and an anomeric proton at 5.26 ppm are characteristic for 4-*O*-methylglucuronic acid attached via α-(1→2) linkage to xylose [19–21]. Assignment of the resonances (Tables 1 and 2), revealed that Fractions **2** and **3** contain *O*-acetyl-4-*O*-methylglucurono-β-D-xylan (Fig. 5). These intra-residue interactions and assignments were confirmed by the NOESY experiment (Table 3). Taking into account pH effects, the $^1$H and $^{13}$C chemical shifts reported here for the MeGlcA group α-(1→2)-linked to the xylan chain are in agreement with literature values [19–21].

To C-3 of the same β-D-xylopyranosyl residue is linked an *O*-acetyl group (Table 3). Signals or cross-peaks to be expected for the structural element →4)[4-*O*-Me-α-D-Glc*p*A-(1→2)]-β-D-Xyl*p*-(1→ (i.e., $^1$H/$^{13}$C chemical shifts of 4.61/102.0 and 3.39/78.0 ppm [20]) were not detected. The *O*-acetyl groups are linked to C-2 and/or C-3 of the β-D-xylopyranosyl residues. No evidence for the presence of O-acetylated MeGlcA groups was found in agreement with literature [15]. Signals to be expected for glucuronic acid groups (i.e., $^1$H chemical shifts of 5.38 [15], 4.40, 3.75–3.85 and 3.51 ppm [28]) were not detected.

*Relative amounts of the different moieties.*—The relative amounts of acetyl and sugar residues were determined by integration of the anomeric protons signals, the H-2 signals of

Table 2
$^{13}$C NMR data on the constituent monosaccharide residues of the oligo- and polysaccharides present in Fractions **3** and **10**

| Fraction | Residue [a] | $^{13}$C chemical shifts (ppm) [b] | | | | |
|---|---|---|---|---|---|---|
| | | C-1 | C-2 | C-3 | C-4 | C-5 |
| **3** | X red α | n.d. [c] | n.d. | n.d. | n.d. | n.d. |
| | X red β | 97.55 | 75.0 | n.d. | n.d. | n.d. |
| | X int | 102.72 | 73.7 | 74.6 | 77.4 | 64.0 |
| | X int | 102.72 | 73.7 | 74.6 | 77.4 | 64.0 |
| | X int | 103.69 | 73.8 | 74.6 [d] | 77.4 | 64.0 |
| | X int | 103.69 | 73.8 | 74.5 [d] | 77.4 | 64.0 |
| | X2 | 100.90 | 74.49 | 72.5 | 77.2 | 63.9 |
| | X2 | n.d. | n.d. | n.d. | n.d. | 66.3 |
| | X23 | 100.42 | 72.46 | 73.98 | 76.24 | 63.9 |
| | X3 | 102.47 | 71.9 | 76.28 | 76.5 | 63.9 |
| | X3 | 102.47 | 71.8 | 76.04 | 76.5 | 63.9 |
| | XG3 | 101.99 | 75.7 | 74.95 | 77.5 | 63.9 |
| | MeGlcA | 98.83 | 72.3 [d] | 73.7 | 83.34 | n.d. |
| **10** | X red α | 93.32 | 72.71 [e] | 72.26 [f] | 77.7 | 60.1 |
| | X red α | 93.32 | 72.71 [e] | 72.26 [f] | 77.7 | 60.1 |
| | X red β | 97.78 | 75.3 | 75.2 [e] | 77.7 | 64.3 |
| | X red β | 97.78 | 75.3 | 75.2 [e] | 77.7 | 63.4 |
| | X int | 102.97 | 74.0 | 74.9 [e] | 77.7 | 64.3 |
| | X term | 103.14 | 74.0 | 76.9 | 70.5 | 66.6 |
| | X term | 103.14 | 74.0 | 76.9 | 70.5 | 66.6 |
| | X term | 103.14 | 74.0 | 76.9 | 70.5 | 66.6 |
| | X int | 103.93 | 74.0 | 74.9 [e] | 77.7 | 64.3 |
| | X term | 104.07 | 74.1 | 76.9 | 70.5 | 66.6 |
| | X2 | 101.12 | 74.76 | 72.77 [f] | 77.45 | 64.3 |
| | X23 | 100.68 | 72.72 | 74.25 | 76.50 | 64.07 |
| | X3 | 102.71 | 72.23 | 76.54 | 76.79 | 64.3 |

[a] See footnote [a] in Table 1 for an explanation of the designations used.
[b] Relative to an internal acetone standard at 31.55 ppm ($D_2O$, 27 °C, pD 7) and acquired at 100 MHz.
[c] n.d., not detected
[d,e,f] These assignments might be interchanged.

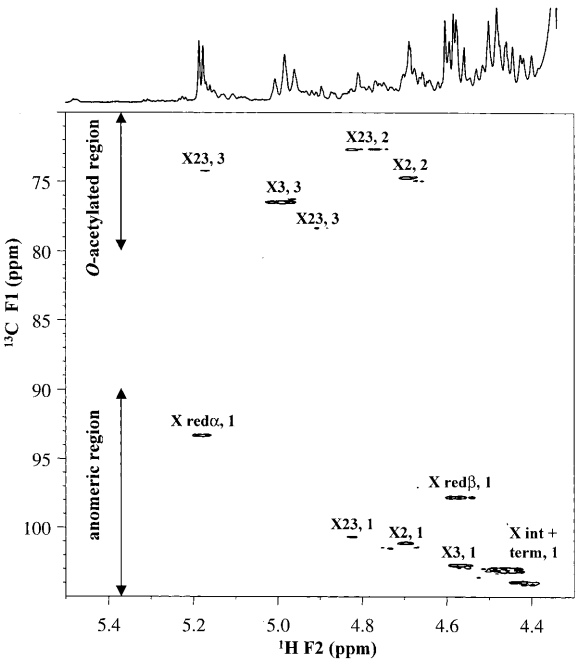


Fig. 4. The anomeric and O-acetylated xylose regions of the HSQC spectrum obtained from Fraction **10**. See footnote [a] in Table 1 for an explanation of the peak designations. The spectrum lined on the top is the 1D $^{1}H$ NMR spectrum of Fraction **10**.

2-O-acetylated xylose residues and the H-3 signals of 3-O-acetylated xylose residues in the fingerprint region (Table 4) and the degree of acetyl substitution (DS) on xylose residues subsequently calculated (Table 4). Similar values for the DS were obtained by integration of the signals assigned to acetyl groups at 2.2 ppm and of all carbohydrate signals. The degrees of acetylation for the xylans in Fractions **2** and **3** were greater than for Fraction **10**. These values for Fractions **2** and **3** agreed with an earlier reported value of 16% acetylation (DS = 0.67) for acetyl-4-*O*-methylglucuronoxylan isolated from aspen wood [13]. The acetylated xylan in Fraction **10** appeared to be a deacetylated fragment of an acetylated 4-*O*-methylglucuronoxylan. Some acetate was detected in the proton NMR spectra (Fig. 2) and it was unclear whether this originated from the liberation of *O*-acetyl groups.

The relative amounts of substituted and unsubstituted xylose residues are documented in Table 5. Splitting of signals indicated the occurrence of various possible combinations of the structural elements. Some of these combinations were identified in the case of the acetylated 4-*O*-methylglucuronoxylan in Fraction **3** (Table 3). The relative intensities of the H-1 and H-3 signals originating from 3-O-acetylated xylose residues and of the H-1 and H-2 signals originating from 2-O-acetylated xylose residues indicated that approximately one-third and two-thirds of the *O*-acetyl groups are linked to positions 2 and 3, respectively, of β-D-xylopyranosyl residues (Tables 4 and 5). The corresponding ratio for the thermodynamically controlled distribution of *O*-acetyl groups has been reported earlier to be approximately the same [16]. Thus, due to *O*-acetyl migration, it is unclear whether such equilibrium conditions are present in the wood itself or are established during the isolation procedure [5,8].

*Approximate degree of polymerization.*—In the proton NMR spectrum of Fraction **10**, intense resonances originating from reducing end and terminal end residues were seen. The many groups of anomeric protons exhibited unequal intensities in the fingerprint region indicating that this sample contains a mixture of several molecules. Integration results in an approximate value of 5 for the degree of polymerization. In the case of Fractions **2** and **3**,

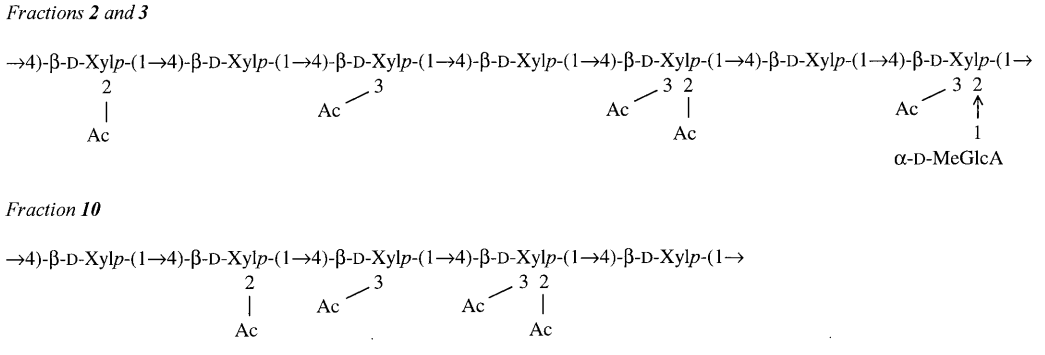


Fig. 5. Abbreviated structural formulae for the acetylated (4-*O*-methylglucurono)xylans isolated from aspen wood.

Table 3
Cross-peaks observed at the chemical shifts of 2.0–2.3, 3.46 and 4.3–5.4 ppm in the NOESY spectrum of Fraction **3**

| Proton [a] | $^1$H chemical shift (ppm) [b] | Structural element | NOESY cross-peak |
|---|---|---|---|
| X red α H-1 | 5.18 | -X red α | n.d. [c] |
| X red β H-1 | 4.58 | -X red β | X red β H-3 |
| X int H-1 | 4.48 | -X-X- | X H-3,5ax,5eq; X H-4 |
| X int H-1 | 4.46 | | n.s. [d] |
| X int H-1 | 4.42 | -X-XG3- | X H-3,5ax,5eq; XG3 H-4 |
| X int H-1 | 4.40 | | n.s. |
| X2 H-1,2 | 4.67, 4.68 | -X2-X- | X2 H-3,5ax; X int H-4 |
| X2 H-1 | 4.63 | | n.d. |
| X23 H-1,2 | 4.79, 4.81 | -X23-X- | X23 H-3,5ax,5eq; X int H-4 |
| X23 H-3 | 5.14 | -X23-X- | X23 H-1 |
| X3 H-1 | 4.58 | -X3-X- | X3 H-3, 5ax,5eq; X int H-4 |
| X3 H-3 | 4.98 | -X3-X- | X3 H-1,5ax |
| X3 H-1 | 4.53 | -X3-X3-, -X3-XG3- | X3 H-4 |
| XG3 H-1 | 4.73 | -XG3- | n.d. [e] |
| XG3 H-3 | 5.06 | -XG3- | XG3 H-1 |
| MeGlcA H-1 | 5.26 | -XG3- | MeGlcA H-2; XG3 H-2 |
| MeGlcA $OCH_3$ | 3.46 | -XG3- | MeGlcA H-4 |
| 2-*O*-Acetyl | 2.11 | -X2-, -X23- | n.d. |
| 3-*O*-Acetyl $CH_3$ | 2.17, 2.16 | -X3-, -X23- | n.d. |
| 3-*O*-Acetyl $CH_3$ | 2.22 | -XG3- | MeGlcA H-2,3 |

[a] See footnote [a] in Table 1 for an explanation of the designations used.
[b] See Table 1.
[c] n.d., not detected.
[d] n.s., not well separated from larger adjacent signals.
[e] Not detected due to overlap from the water resonance at 27 °C.

minor signals originating from reducing ends could also be detected and an approximate degree of polymerization of 35. The fact that the polysaccharides present in Fractions **2** and **3** are larger than the oligosaccharides in Fraction **10** agrees well with their order of elution in connection with size-exclusion chromatography. Moreover, the line width of the signals from the polysaccharides in Fractions **2** and **3** was generally broader than for the signals from the oligosaccharides in Fraction **10** (Fig. 3), indicating lower mobility of the molecules in the first two fractions.

## 3. Conclusions

The relative sugar and acetyl composition of the polysaccharides isolated here from aspen wood was the same as for a typical hardwood xylan [1,2]. It thus appeared that few acetyl and MeGlcA groups were removed by the microwave oven treatment and subsequent fractionation by SEC. However, the degree of polymerization obtained in this study was lower than for a natural hardwood xylan [1,2]. The structural element →4)[4-*O*-Me-α-D-

Table 4
Relative amounts [a] of acetyl groups and monosaccharide residues in xylans extracted from aspen wood

| Fraction | Structural element (mol%) | | | Degree of substitution |
|---|---|---|---|---|
| | Xyl | MeGlcA | Acetyl | |
| **2** | 59 | 6 | 35 [b] | 0.60 |
| **3** | 60 | 6 | 35 [b] | 0.58 |
| **10** | ~76 [c] | [d] | ~24 [b,c] | 0.32 |

[a] Determined by integration of quantitative 1D NMR spectra.
[b] In xylose residues substituted with *O*-acetyl groups, approximately one-third and two-thirds of these groups are at the C-2 and C-3 positions, respectively.
[c] Approximate value, due to several small resonances from acetylated mannan which overlap with the xylan resonances.
[d] Small resonance.

Table 5
Relative amounts of substituted and unsubstituted xylose residues in xylans extracted from aspen wood

| -(1→4)-β-D-Xyl*p* residue | Mol% [a] in Fraction | | |
|---|---|---|---|
| | **2** | **3** | **10** |
| Unsubstituted | 51 | 49 | 72 |
| 2-O-Acetylated | 13 | 14 | 10 |
| 3-O-Acetylated | 20 | 22 | 15 |
| 2,3-di-O-Acetylated | 5 | 6 | 3 |
| α-D-MeGlcA-(1→2), 3-O-acetylated | 11 | 9 | [b] |

[a] Approximate values.
[b] Small amount.

Glc*p*A-(1→2)][*O*-Ac-(1→3)]-β-D-Xyl*p*-(1→ was shown to be present. No xylose residues with a MeGlcA substituent and without 3-O-acetylation were detected. The oligosaccharide fraction (Fraction **10**) contained acetylated *xylo*-oligosaccharides that might be hydrolysis products of acetylated 4-*O*-methylglucuronoxylan.

## 4. Experimental

*Isolation of acetylated (4-O-methylglucurono)xylan.*—A total of 9.1 g (dry weight) of milled aspen (*Populus tremula*) chips in 100 mL water were extracted in a microwave oven at 180 °C for 10 min, resulting in a solution with a final pH of 3.5 [14]. The insoluble material (lignocellulose) was removed by filtration (Acrodisc® Syringer Filter, 0.2 μm, Gelman Laboratory) and the water-soluble oligomers and polymers further fractionated by size-exclusion chromatography (SEC). The filtrate (1.1 mg of material (according to sugar analysis after acid hydrolysis) in 0.5 mL) was loaded onto a fast protein liquid chromatography (FPLC) system from Pharmacia Biotech with three columns in series: (i) a pre-column of Superdex 75 (1 mL); (ii) a column containing Superdex 75 (HR 10/30); and (iii) a final column of Superdex 200 (HR 10/30) (Pharmacia Biotech). Elution was performed with Milli Q water at a flow rate of 0.5 mL/min and the refractive index (RI) and UV absorption (280 nm) of the eluant were monitored. Three fractions collected at the following elution times were used for the structural analysis: Fraction **2** at 34–39 min; Fraction **3** at 39–44 min; and Fraction **10** at 74–79 min. These same fractions from six independent separations were pooled and lyophilized.

*NMR spectroscopy.*—For NMR analysis, a portion of each of the dried samples (1.8–2.7 mg) was dissolved in 0.35 mL $D_2O$ (99.9 atom%D, Cambridge Isotope Laboratories). The pD of these solutions, which were clear and colorless, was measured and found to be 7. Shigemi NMR tubes (BMS-005B) were employed to obtain the $^1H$ and $^{13}C$ NMR spectra at 400.13 and 100.61 MHz, respectively, using a Bruker DPX 400 MHz spectrometer. 1D $^1H$ NMR spectra were recorded using an 85° pulse of 7 μs, a spectral width of 4000 Hz and a repetition time of 15 s. All spectra were acquired at probe temperatures of 27 or 70 °C. The chemical shifts are reported relative to an internal acetone standard at 2.225 and 31.55 ppm for $^1H$ and $^{13}C$ NMR spectra, respectively.

Standard pulse sequences and phase cyclings were employed to perform 2D phase-sensitive $^1H$,$^1H$-correlated spectroscopy (COSY) [29], total correlation spectroscopy (TOCSY) ($\tau_{mix} = 0.14$ s) [30,31] and nuclear Overhauser effect spectroscopy (NOESY) ($\tau_m = 0.5$ s) [32]. A spectral width of 2000 Hz was employed in both dimensions and the relaxation delay was 2.5 s. For each FID, 8 (or 16 in the case of TOCSY and 24 for NOESY) transients were acquired; the data size was 1024 (or 512 for TOCSY) in $t_1 \times 2048$ (or 1024 for TOCSY) in $t_2$. The final size of the data matrix after Fourier transformation was $1024 \times 2048$. The proton-detected heteronuclear single quantum (HSQC) spectra [33] were acquired over a $t_1$ spectral width of 11000 Hz and a $t_2$ width of 1800 Hz, with a $2048 \times 1024$ matrix (zero-filled to 2048 in $t_2$) and 12 transients per increment. The delay between transients was 2.5 s and the delay for polarization transfer was set to correspond to an estimated average $^1H$–$^{13}C$ coupling constant of 150 Hz. Data processing was performed using standard Bruker XWIN-NMR software.

## Acknowledgements

Financial support from NUTEK (the PROFYT program) is gratefully acknowledged. We wish to thank the Division of Wood Chemistry at the Royal Institute of Technology, Stockholm, Sweden, for allowing us to use their NMR spectrometer. We also thank Linda Junel for performing the microwave oven treatment.

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