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Oligosaccharides obtained by enzymatic hydrolysis of birch kraft pulp xylan: Analysis by capillary zone electrophoresis and mass spectrometry

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Abstract

Neutral and acidic oligosaccharides were obtained from an unbleached birch kraft pulp by treatment with a *Trichoderma reesei* endoxylanase pI 9 and subsequently characterized using capillary zone electrophoresis (CZE) and matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS). The borate complexes of unsaturated acidic oligosaccharides having a 4-deoxy-β-L-threo-hex-4-enopyranosyluronic acid (4ΔUA) residue linked to a β-D-(1 → 4)-xylooligosaccharide backbone were separated by CZE and detected by their UV absorption at 232 nm without prior derivatization. Pre-column derivatization with the chromophore 6-aminoquinoline (6-AQ) followed by CZE in alkaline borate buffer using detection based on absorption at 245 nm was used in the case of neutral xylosaccharides. Furthermore, MALDI-TOF-MS was employed to determine the molecular masses of both unsaturated and saturated acidic oligosaccharides. The acidic oligosaccharides released upon endoxylanase treatment of the birch kraft pulp were a (4ΔUA)-β-D-xylotetraose, a (4ΔUA)-β-D-xylopentaose, a (4-O-methyl-α-D-glucurono)-β-D-xylotetraose and a (4-O-methyl-α-D-glucurono)-β-D-xylopentaose. Analysis after enzymatic hydrolysis with β-xylosidase and α-glucuronidase from *Trichoderma reesei* strongly indicated that the uronic acid residue in these acidic oligosaccharides was linked to the D-xylose unit adjacent to the non-reducing D-xylose unit. The neutral xylosaccharides obtained after endoxylanase treatment of the pulp sample were D-xylose, β-(1–4)-D-xylobiose and β-(1–4)-D-xylotriose. © 1997 Elsevier Science Ltd.

Keywords: Aldouronic acids; Birch; Capillary zone electrophoresis; 4-Deoxy-L-threo-hex-4-enopyranosyluronic acid; *Trichoderma reesei* enzymes; Kraft pulp; Mass spectrometry; 4-O-Methyl-D-glucopyranosyluronic acid; NMR; Oligosaccharides; Xylan; D-Xylose

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1. Introduction

In hardwood species, such as birch, the major constituent of the hemicellulose is an O-acetyl-2-O-(4-O-methyl-α-D-glucurono)-β-(1–4)-D-xylan [1–3]. During kraft pulping, the structure of this xylan hemicellulose is extensively modified [2,4]. Upon subjecting birch wood xylan and model disaccharide xylans to strongly alkaline conditions, which simulate kraft cooking, 4-O-methylglucuronic acid residues (4-O-Me-D-GlcA *p*) in the xylan can be converted to 4-deoxy-β-L-threo-hex-4-enopyranosyluronic acid units (4ΔUA units) [5–7]. More recent studies have also demonstrated that 4ΔUA units are present as structural elements in xylans from unbleached softwood and hardwood kraft pulps [8–11], as well as in xylans dissolved during alkaline hydrogen peroxide bleaching of softwood and hardwood kraft pulps [12–14].

Teleman et al. [8] employed ^{1}H and ^{13}C NMR spectroscopy to identify 4ΔUA units attached to β-D-(1 → 4)-xylooligosaccharides in a xylanase hydrolysate of a pine kraft pulp. Furthermore, in a subsequent study Teleman et al. [15] utilized two-dimensional ^{1}H and ^{13}C NMR spectroscopy to determine the primary structures of certain acidic 4ΔUA-containing xylooligosaccharides. The acidic oligosaccharides were obtained by using a mixture of *Trichoderma reesei* enzymes to hydrolyze a commercially available 4-O-methyl-D-glucuronoxylan, which had been pretreated with strong alkali at a high temperature. In the latter study, the authors used high-performance anion-exchange chromatography involving pulsed amperometric detection to quantitate the relative amounts of different 4ΔUA-containing xylooligosaccharides in the hydrolysate.

Selective enzymatic hydrolysis is an established approach in determining the structure of polysaccharides [16]. Selective enzymatic hydrolysis combined with liquid chromatography of the products obtained has been used to characterize xylans originating from different kinds of wood [17–22], as well as for the characterization of kraft pulp hemicelluloses [9,23,24]. We have previously described the use of capillary zone electrophoresis for the analysis of the products obtained by enzymatic hydrolysis of xylans isolated from mechanical pulp [25,26] and kraft pulps [26,27].

This paper describes the use of capillary zone electrophoresis (CZE) and matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS) to characterize mixtures of oligosaccharides obtained from birch kraft pulp xylan with respect to distribution, molecular weight and composition. The mixtures of acidic and neutral oligosaccharides were obtained by treatment of an unbleached birch kraft pulp with *Trichoderma reesei* endoxylanase. Special attention has been focussed on the development of a rapid and simple technique, based on CZE with detection at 232 nm, for the selective analysis of acidic xylooligosaccharides containing 4-deoxy-β-L-threo-hex-4-enopyranosyluronic acid units as substituents on the backbone.

2. Experimental

Materials.—The xylan oligosaccharide sample, designated as HM-1, was obtained by treatment of an unbleached birch kraft pulp (Kappa No. 18.2) with a *Trichoderma reesei* endoxylanase pI 9 (5000 nkat/g) for 24 h at 5% consistency and a temperature of 45 °C. A more detailed description of this pulp sample and the enzymatic treatment is presented elsewhere [28].

The pure enzymes endoxylanase, β-xylosidase and α-glucuronidase from *Trichoderma reesei* [29–32] were generous gifts from M. Tenkanen at VTT Biotechnology and Food Research (Espoo, Finland). 4-O-Methyl-D-glucuronic acid (4-O-Me-D-GlcA) was a generous gift from Christian Krog-Jensen at Stockholm University, Sweden. D-Xylose and β-(1–4)-D-xylobiose were obtained from Sigma Chemical Co. (St Louis, MO, USA). β-(1–4)-D-Xylotriose, 4-O-Me-GlcA-β-D-xylobiose (aldotriuronic acids) and 4-O-Me-α-D-GlcA *p*-(1–2)-β-(1–4)-D-xylotrioses (aldotetrauronic acids) were procured from Megazyme (Sydney, Australia). The aldotriuronic and aldotetrauronic acids from Megazyme were mixtures of several isomers. Sodium cyanoborohydride, 6-aminoquinoline and acetic acid were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA).

Preparation of 6-aminoquinoline (6-AQ) derivatives.—The saccharides were derivatized under slightly acidic conditions using reductive amination in the presence of 6-AQ and sodium cyanoborohydride. To a 400 μL aqueous solution of the saccharide sample (approx. conc. 1 mg/mL), 140 μL reagent solution (0.5 M 6-AQ in 5 M aqueous acetic acid) was added. To this solution 40 μl 0.5 M aqueous sodium cyanoborohydride, followed by 400 μL aqueous 50% methanol were then added. This reaction mixture was maintained at 40 °C for 2 h. After cooling to room temperature, the mixture was filtered and analyzed by CZE.

Enzymatic hydrolysis.—To the HM-1 sample (containing 1 mg saccharides in 1 mL 50 mM sodium acetate buffer, pH 5), approximately 1 μL β-xylosidase (3 nkat/μL) was added. This mixture was maintained at 40 °C for 24 h to obtain a mixture of hydrolysis products, designated as HM-2. The third sample (HM-3) was obtained by treatment of HM-1 as above with 1 μL β-xylosidase (3 nkat/μL) for 24 h and thereafter with 1 μL α-glucuronidase (0.12 nkat/μL) for 48 h, both at 40 °C.

CZE of 4ΔUA xylooligomers.—These analyses were performed using a Dionex Capillary Electrophoresis System (Sunnyvale, CA, USA) equipped with a variable wavelength UV/VIS detector. Detection was performed at 232 nm, with the detector placed at the cathode 5 cm from the end of the capillary. An uncoated, fused silica capillary column (total length 43 cm and a 30 μm nominal I.D.; obtained from Skandinaviska GeneTec AB, Stockholm, Sweden) was employed.

Injections were performed in the hydrodynamic mode (gravity injection). The sample vial was elevated 75 mm during the injection period of 40 seconds. The instrument was run at a constant power level of 3000 mW, which corresponded to an applied voltage of approximately 28 kV. The running electrolyte was an alkaline (pH 10. 3) borate buffer (containing 438 mM H_3BO_3 and 300 mM NaOH).

CZE of 6-AQ oligosaccharides.—The 6-AQ derivatives were analyzed utilizing the same procedure as for the 4ΔUA xylooligomers except that the detection was performed at 245 nm. The sample vial was elevated 75 mm during the injection period of 10 seconds. The instrument was run at a constant power level of 1200 mW (corresponding to an applied voltage of approximately 21 kV) and the running electrolyte was an alkaline (pH 9) borate buffer (containing 420 mM H_3BO_3 and 220 mM NaOH).

Characterization of enzymatic hydrolyzates by MALDI-TOF-MS.—Matrix-assisted laser desorption ionization (MALDI) time of flight (TOF) mass spectrometry (MS) was run in the positive mode with a 8–10 μJ laser beam on an LDX-1700XS time of flight instrument using 2,5-dihydroxybenzoic acid as the matrix.

NMR spectroscopy.—A Bruker AM-X 300 NMR spectrometer was employed to obtain ^1H and ^{13}C NMR spectra of the oligosaccharide sample HM-1 (in which the acidic oligosaccharides were in their protonated form). ^1H NMR spectra were recorded at 300.13 MHz in a D_2O solution (sample concentration 20 mg/ml; temperature 50 °C; 90° pulses; pulse repetition time 5 s) and chemical shifts (ppm) are reported relative to the internal standard sodium 3-(trimethylsilyl)-propionate, giving a signal at 0 ppm. ^{13}C NMR spectra were recorded at 75.47 MHz in a DMSO-d_6 solution (sample concentration approx. 50 mg/ml; temperature 50 °C; 30° pulses; pulse repetition time 1 s) and chemical shifts are reported relative to the central signal of the solvent DMSO-d_6 at 39.6 ppm. Small amounts of an iron(III)-DPTA complex were added as a relaxation reagent.

3. Results and discussion

In a separate study, the mixture of neutral and acidic oligosaccharides, obtained by treatment of an unbleached birch kraft pulp (Kappa No. 18.2) with Trichoderma *reesei* endoxylanase p*I* 9 and designated as HM-1, was initially characterized using NMR spectroscopy. This previous study was designed primarily to verify the presence of acidic β-D-(1 → 4)-xylooligosaccharides containing 4-deoxy-L-threo-hex-4-enopyranosyluronic acid units linked to the xylosaccharide backbone. In addition to signals from 4-O-Me-α-D-GlcA *p* units present in other acidic β-D-(1 → 4)-xylooligosaccharides and to signals from β-D-(1 → 4)D-Xyl *p* units, important diagnostic signals from the 4ΔUA units were readily detected in the ^1H and the ^{13}C NMR spectra.

These signals appeared at 5.80 ppm (H-4) and 5.32 ppm (H-1) in the ^1H NMR spectra and at 162.9 ppm (C-6), 140.5 ppm (C-5), 112.4 ppm (C-4) and 98.1 ppm (C-1) in the ^{13}C NMR spectra. The assignment of these signals was based on previously reported ^1H NMR [6,8,12,15] and ^{13}C NMR [8,12,15,33] data for 4ΔUA units in β-D-(1 → 4)-xylooligosaccharides. From the ^1H and ^{13}C NMR spectra of the HM-1 sample, the ratio of 4-O-Me-D-GlcA *p* and 4ΔUA units linked to β-D-(1 → 4)-xylooligosaccharides in the mixture was found to be about 1:1.8. This determination was performed by comparing the areas of the H-1 (5.27 ppm) and C-6 (171.1 ppm) signals from the 4-O-Me-D-GlcA *p* residues with the areas of the corresponding signals from the 4ΔUA units.

In order to obtain information concerning the distribution of neutral and acidic saccharides in HM-1, this mixture was analysed using two different CZE procedures and MALDI-TOF-MS. HM-1 was also subjected to selective enzymatic hydrolysis using *Trichoderma reesei* β-xylosidase and α-glucuronidase. This hydrolysis was chosen, because the primary structures of three acidic 4ΔUA-xylooligosaccharides

Table 1
Structures and electrophoretic mobilities of the 4ΔUA-containing oligosaccharides separated by CZE as shown in Fig. 1

Peak No.	Structure [a]	Electrophoretic mobility $\mu \times 10^4$ ($cm^2\ V^{-1}\ s^{-1}$)
2	β-D-Xyl*p*-(1 → 4)-[β-L-4ΔUA *p*-(1 → 2)]-β-D-Xyl *p*-(1 → 4)-β-D-Xyl *p*-(1 → 4)-β-D-Xyl *p*-(1 → 4)-D-Xyl (**I**)	−1.36
3	β-D-Xyl*p*-(1 → 4)-[β-L-4ΔUA *p*-(1 → 2)]-β-D-Xyl *p*-(1 → 4)-β-D-Xyl *p*-(1 → 4)-D-Xyl (**II**)	−1.49
4	β-L-4ΔUA *p*-(1 → 2)-β-D-Xyl *p*-(1 → 4)-β-D-Xyl *p*-(1 → 4)-β-D-Xyl *p*-(1 → 4)-D-Xyl (**III**)	−1.52
5	β-L-4ΔUA *p*-(1 → 2)-β-D-Xyl *p*-(1 → 4)-β-D-Xyl *p*-(1 → 4)-D-Xyl (**IV**)	−1.71
6	β-L-4ΔUA *p*-(1 → 2)-β-D-Xyl *p*-(1 → 4)-D-Xyl (**V**)	−1.96

[a] L-4ΔUA = 4-deoxy-L-threo-hex-4-enopyranosyluronic acid; D-Xyl = D-xylose.

(obtained by treatment of an alkali-pretreated glucuronoxylan with a combination of *Trichoderma reesei* xylanase, β-xylosidase and α-glucuronidase) had been reported previously [15]. Thus, in order to elucidate the primary structures of the 4ΔUA-xylooligosaccharides in HM-1, we also analyzed the mixtures from HM-1 obtained by β-xylosidase treatment (designated as HM-2) and by combined treatment with β-xylosidase and α-glucuronidase (designated as HM-3) utilizing CZE and MALDI-TOF-MS. The results obtained were then compared with earlier studies in which other glucuronoxylans had been treated with the same enzymes [8,15,34].

Separation of kraft pulp-derived 4ΔUA xylooligomers by CZE.—Selective analytical separation was required in order to study the distribution of the acidic 4ΔUA-β-D-(1 → 4)-xylooligosaccharides, without interference from neutral xylooligosaccharides and acidic 4-O-Me-α-D-GlcA *p*-β-D-(1 → 4)-xylooligosaccharides which were also present in the samples investigated. In attempt to obtain such an analytical method, we employed CZE in an alkaline borate buffer with detection at 232 nm. The α,β-unsaturated uronic acid 4ΔUA linked to D-xylitol has been reported to exhibit a strong absorbance maximum at 230 nm ($\varepsilon = 5910$) [6]. More recently, acidic disaccharides such as 4ΔUA(1–3)-β-D-GlcNAc and 4ΔUA(1-3)-β-D-GalNAc, as well as other related 4ΔUA-oligosaccharides have been separated using CZE in an alkaline borate [35–38] or acidic phosphate buffer [38–40] with detection at 232 nm.

The electropherograms from CZE analysis of the hydrolysis mixtures HM-1, HM-2, and HM-3 are shown in Fig. 1. The first peak detected in these three electropherograms, with a migration time of about 3 minutes, corresponds to the electroosmotic flow (EOF) peak (peak 1). The borate complexes of the oligosaccharides containing 4ΔUA units, which are negatively charged under the conditions employed, migrate against the electroosmotic flow and are therefore detected after the EOF peak. These 4ΔUA-containing oligosaccharides migrate according to their charge-to-size ratio. This means in our case that the shortest 4ΔUA-containing xylooligomer in the sample will be detected last in the electropherogram.

CZE analysis of the hydrolysis mixture HM-1 revealed two major peaks, peaks 2 and 3 (Fig. 1A), in an approx. intensity ratio of 1:2. By comparison with

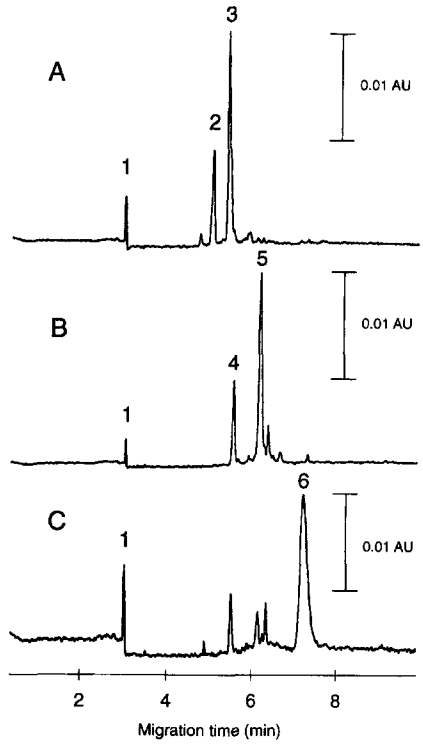

Fig. 1. Electropherogram of underivatized HM-1 (A), HM-2 (B) and HM-3 (C). The sample concentration was 10 mg/ml. Buffer: 438 mM alkaline borate, pH 10.3; capillary column: uncoated fused silica, $L = 43$ cm, $l = 38$ cm, I.D. = 30 μm; detection: Absorption at 232 nm; injection: 75 mm, 40 s. For the identifications of the peaks labelled 2–6, see Table 1 and the text.

the results obtained with MALDI-TOF-MS and enzymatic hydrolysis (see below), these peaks were identified as a 4ΔUA-β-D-(1 → 4)-xylopentaose (**I**) and a 4ΔUA-β-D-(1 → 4)-xylotetraose (**II**). In Table 1, the electrophoretic mobilities and the structures of the two acidic oligosaccharides **I** and **II** are presented.

CZE analyses of the 4ΔUA-containing oligosaccharides obtained by selective enzymatic hydrolysis of sample HM-1 by β-xylosidase (mixture HM-2) and by a combination of β-xylosidase and α-glucuronidase (mixture HM-3) are shown in Fig. 1B and C, respectively. The electropherogram of the HM-2 sample showed two new peaks, peaks 4 and 5 (Fig. 1B), in an approx. ratio of 1:2; whereas the corresponding electropherogram of the HM-3 sample showed only one major new peak (peak 6 in Fig. 1C). On the basis of their electrophoretic mobilities and by comparison with the MALDI-TOF-MS analyses (see below), peaks 4, 5 and 6 were identified as a 4ΔUA-β-D-(1 → 4)-xylotetraose (**III**), a 4ΔUA-β-D-(1 → 4)-xylotriose (**IV**), and a 4ΔUA-β-D-(1 → 4)-xylobiose (**V**). As mentioned above, the three 4ΔUA-β-D-(1 → 4)-xylooligosaccharides (compounds **III-V** in Table 1) have been isolated and identified earlier by Teleman et al. [15] from a 4ΔUA-xylan which had been subjected to treatment with the same enzymes used in the present study. Furthermore, the acidic oligosaccharides **IV** and **V** have also recently been isolated in a hydrolyzate after extensive treatment of an unbleached birch kraft pulp using cellulolytic and hemicellulolytic enzymes [41].

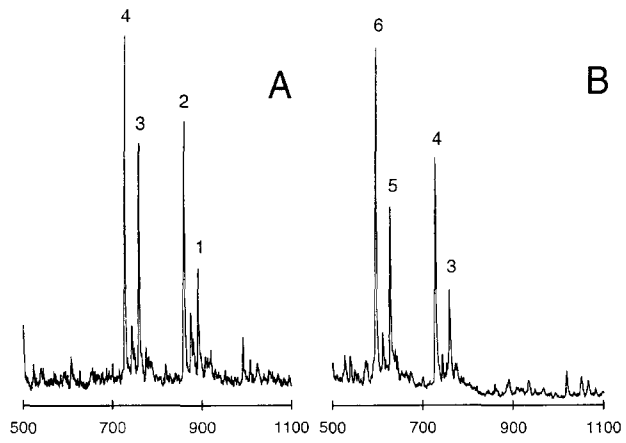

Fig. 2. MALDI-TOF-MS spectra of HM-1 (A) and HM-2 (B). Peak 1 = 4-O-Me-α-D-GlcA *p*-D-xylopentaoses, 2 = 4ΔUA-D-xylopentaoses, 3 = 4-O-Me-α-D-GlcA *p*-D-xylotetraoses, 4 = 4ΔUA-D-xylotetraoses, 5 = 4-O-Me-α-D-GlcA *p*-D-xylotrioses and 6 = 4ΔUA-D-xylotrioses (see also Table 2).

Characterization of birch pulp-derived oligosaccharides by MALDI-TOF-MS.—MALDI-TOF-MS is a powerful technique which has been used successfully in several studies on underivatized oligosaccharides [42–47]. The positive ion mass spectra of underivatized oligosaccharides consist essentially of the sodium adduct ions $(M + Na)^+$, from which the molecular weight of the saccharide can be obtained [43–45]. In the present study, the samples HM-1, HM-2 and HM-3 were analyzed by MALDI-TOF-MS in order to determine the molecular weights of the oligosaccharides present. The mono- and disaccharides in the samples were not detected by this technique.

Fig. 2A and 2B depict the MALDI-TOF-MS spectra of HM-1 and HM-2, respectively. These figures demonstrate peaks in the 500–1100 Da mass range. In this mass region, there were four peaks from the HM-1 sample (Fig. 2A). These four peaks were identified as the $(M + Na)^+$ ions of the following acidic oligosaccharides: 4-O-Me-α-D-GlcA *p*-β-D-xylopentaoses (peak 1), 4ΔUA-β-D-xylopentaoses (peak 2), 4-O-Me-α-D-GlcA *p*-β-D-xylotetraoses (peak 3) and 4ΔUA-β-D-xylotetraoses (peak 4).

In a similar manner, the following acidic oligosaccharides were identified in the HM-2 sample: 4-O-Me-α-D-GlcA *p*-β-D-xylotetraoses (peak 3), 4ΔUA-β-D-xylotetraoses (peak 4), 4-O-Me-α-D-GlcA *p*-β-D-xylotrioses (peak 5) and 4ΔUA-β-D-xylotrioses (peak 6). Thus, the analyses strongly indicate that one xylose unit was removed from the 4ΔUA- and 4-O-Me-α-D-GlcA *p*-containing acidic β-(1-4)-D-xylotetraoses and β-(1-4)-D-xylopentaoses in HM-1 upon β-xylosidase treatment. Since β-xylosidase cleaves an oligosaccharide chain from its non-reducing end and stops at a xylose residue carrying a branching unit [31], the uronic acid units in the acidic oligosaccharides in HM-1 must have been linked to the xylose unit adjacent to the terminal non-reducing xylose.

On the basis of these findings, the structures of the two unsaturated acidic xylooligosaccharides in HM-1 were concluded to be **I** and **II**. The two saturated acidic xylooligosaccharides in HM-1 were identified as β-D-Xyl *p*-(1 → 4)-[4-O-Me-α-D-GlcA *p*-(1 → 2)]-β-D-Xyl *p*-(1 → 4)-β-D-Xyl *p*-(1 → 4)-β-D-Xyl *p*-(1 → 4)-D-Xyl (**VI**) and β-D-Xyl *p*-(1 → 4)-[4-O-Me-α-D-GlcA *p*-(1 → 2)]-β-D-Xyl *p*-(1 → 4)-β-D-Xyl *p*-(1 → 4)-D-Xyl (**VII**). It is worth noting that Biely et al. [34] identified the pentasaccharide **VII** as the acidic oligosaccharide obtained by treatment of a 4-O-Me-α-D-GlcA *p*-xylan with Trichoderma *reesei* endoxyl-

Table 2
Identification of saccharides in the hydrolysis mixtures HM-1, -2 and -3 by MALDI-TOF-MS [a]

Saccharide	Mass No. $(M + Na)^+$	HM-1	HM-2	HM-3
β-L-4ΔUA-D-xylobioses	463			+ + +
β-L-4ΔUA-D-xylotrioses	595		+ +	
β-L-4ΔUA-D-xylotetraoses	727	+ +	+ +	+
β-L-4ΔUA-D-xylopentaoses	859	+ +		
β-L-4ΔUA-D-xylohexaoses	991	(+) [b]		
4-O-Me-α-D-GlcA *p*-D-xylotrioses	627		+ +	
4-O-Me-α-D-GlcA *p*-D-xylotetraoses	759	+ +	+ +	
4-O-Me-α-D-GlcA *p*-D-xylopentaoses	891	+ +		
4-O-Me-α-D-GlcA *p*-D-xylohexaoses	1023	(+) [b]		
β-(1-4)-D-xylotriose	437	+		

[a] + + +, + + and + denote major, medium and minor peaks in the spectra.
[b] Weak signal.

anase pI 9. Furthermore, these authors also reported that upon treatment with β-xylosidase, the acidic pentasaccharide **VII** was converted into an aldotetrauronic acid (with the uronic acid at the non-reducing end) by loss of one xylose unit. Table 2 summarizes the MALDI-TOF-MS findings for the samples investigated.

In the sample HM-3, which had been treated with β-xylosidase and α-glucuronidase, 4ΔUA-β-D-xylobiose **V** was found to be the dominant acidic oligosaccharide (see Tables 1 and 2) and only traces of 4-O-Me-α-D-GlcA *p*-β-D-xylobiose could be detected. These results indicated that the combined enzyme treatment removed the 4-O-Me-α-D-GlcA, but not the 4ΔUA unit from the acidic xylooligosaccharides investigated. These findings agree well with the earlier identification by Teleman et al. [15,41] of 4ΔUA-β-D-xylobiose **V** in similar enzymatic hydrolysis mixtures. The α-glucuronidase employed in our study has been reported to be active against 4-O-Me-α-D-GlcA *p*-β-D-xylotrioses and 4-O-Me-α-D-GlcA *p*-β-D-xylotetraoses, from which it removes the terminal uronic acid unit [32].

Separation of 6-AQ derivatives of xylan-derived saccharides by CZE.—By using a CZE-procedure developed recently in our laboratory [25], useful information concerning the distribution of neutral xylosaccharides in HM-1 was obtained. By labelling with the UV-chromophore 6-aminoquinoline, carbohydrates with a reducing end group can be detected with high sensitivity on basis of absorption at 245 nm. Table 3 documents the CZE electrophoretic mobilities of the 6-AQ derivatives of a number of commercially available neutral β-(1-4)-D-xylooligosaccharides and 4-O-Me-α-D-GlcA-containing aldouronic acids, used as reference compounds. It can also be seen from Table 3 whether HM-1, -2 and/or -3 contained components demonstrating electrophoretic mobilities identical to those of the standards. In addition to qualitative information, this

Table 3
Electrophoretic mobilities of the 6-AQ derivatives of commercially available saccharide standards. Identification of these saccharides in the hydrolysis mixtures HM-1, -2 and -3 [a]

Saccharide	Electrophoretic mobility $\mu \times 10^4$ ($cm^2/V^{-1}\,s^{-1}$)	HM-1	Presence in HM-2	HM-3
4-O-Me-D-GlcA	−2.22			+
4-O-Me-α-D-GlcA *p*-D-xylotriose [b]	−1.65		+	
4-O-Me-α-D-GlcA *p*-D-xylotriose [b]	−1.61		(+) [c]	
D-xylose	−1.51	+ +	+ + +	+ + +
β-(1-4)-D-xylobiose	−1.17	+ +		
β-(1-4)-D-xylotriose	−1.05	+		

[a] + + +, + + and + denote major, medium and minor constituents.
[b] 4-O-Me-α-D-GlcA *p*-D-xylotriose occurs in two isomeric forms in the standard, (see the text).
[c] Weak signal.

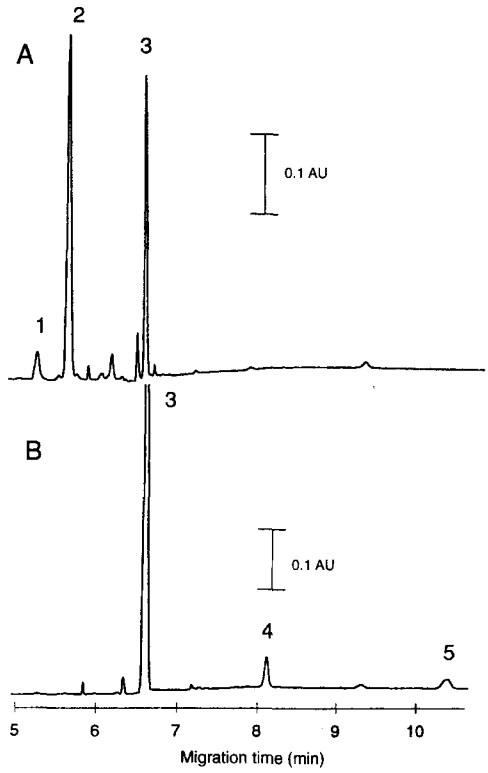

Fig. 3. Electropherogram of the 6-AQ derivatives of saccharides in HM-1 (A) and HM-3 (B). Buffer: 420 mM alkaline borate, pH 9.0; capillary column: uncoated fused silica, $L = 43$ cm, $l = 38$ cm, I.D. = 30 μm; detection: absorption at 245 nm; injection: 75 mm, 10 s; Peak 1 = β-(1-4)-D-xylotriose, 2 = β-(1-4)-D-xylobiose, 3 = D-xylose, 4 = 4ΔUA-D-xylobiose, 5 = 4-O-Me-D-glucuronic acid.

approach also provided a quantitative estimation of the relative abundance of the different saccharides (Table 3).

The electropherograms in Fig. 3 depict separation of the 6-AQ derivatives of saccharides in samples HM-1 (Fig. 3A) and HM-3 (Fig. 3B) by CZE. Two large peaks, originating from β-(1–4)-D-xylobiose and D-xylose, and a smaller peak from β-(1–4)-D-xylotriose are present in the electropherogram from HM-1. Some additional minor peaks are also seen and most probably originate from the acidic hexa- and pentasaccharides discussed above. The identities of these minor peaks in Fig. 3A were not investigated in detail. However, it is clear from this electropherogram that the neutral xylosaccharides were the major carbohydrate components of HM-1, whereas acidic saccharides were only minor constituents.

Information concerning the relative abundance of structural elements of the D-xylose, 4ΔUA and 4-O-Me-α-D-GlcA types present in HM-3 can be obtained from the CZE analysis shown in Fig. 3B. This sample contained D-xylose, 4-O-Me-D-GlcA and 4ΔUA-β-D-xylobiose **V** (the peak corresponding to 4ΔUA-β-D-xylobiose **V** appeared with a slightly longer migration time than that of the reference sample 4-O-Me-GlcA-β-D-xylobiose). By using the response factors obtained for the authentic reference compounds (D-xylose and 4-O-Me-D-GlcA) and by assuming the same molar response factor for 4ΔUA-β-D-xylobiose as for xylose, the relative amounts of the D-xylose, 4ΔUA and 4-O-Me-D-GlcA structural elements could be calculated to be 26.6:1.6:1. This result agrees reasonably well with the relative amounts of 4ΔUA and 4-O-Me-D-GlcA units revealed by NMR spectroscopy of the HM-1 sample (see above).

4. Conclusions

Capillary zone electrophoresis with UV-detection at 232 nm is a rapid and efficient analytical procedure for the characterization of birch kraft pulp xylan-derived oligosaccharides containing 4-deoxy-L-threo-hex-4-enopyranosyluronic acid units.

As illustrated by the examples presented here, the three analytical techniques employed (i.e. CZE of underivatized and derivatized saccharides and MALDI-TOF-MS) provide data that are in many respects complementary.

Selective enzymatic hydrolysis followed by analysis using CZE and MALDI-TOF-MS provided detailed information about the structures of the acidic oligosaccharides obtained form the birch pulp sample.

Finally, the relative amounts of D-xylose, 4ΔUA and 4-O-Me-D-GlcA units in the mixture obtained by endoxylanase treatment of the birch pulp sample could be estimated from the data provided by the analytical approach developed here.

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