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Carbohydrate Research 293 (1996) 1–13

CARBOHYDRATE RESEARCH

4-*O*-Methyl-β-L-idopyranosyluronic acid linked to xylan from kraft pulp: isolation procedure and characterisation by NMR spectroscopy

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Received 22 March 1996; accepted 13 June 1996

Abstract

The tetrasaccharide 2″-*O*-(4-*O*-methyl-β-L-idopyranosyluronic acid)xylotriose was isolated from enzymatically hydrolysed, unbleached, birch kraft pulp by anion-exchange chromatography in two steps. The primary structure of the tetrasaccharide was determined by ^{1}H and ^{13}C NMR spectroscopy, using homonuclear and heteronuclear two-dimensional techniques. NOE data and $^{3}J_{H,H}$ coupling constants show that the 4-*O*-methyl-β-L-idopyranosyluronic acid in the tetrasaccharide is predominantly in the $^{1}C_{4}$ chair conformation. The pK_a value (3.17) for 4-*O*-methyliduronic acid attached β-(1 → 2) to xylose was determined from the pH-dependent chemical shift of H-5. The amount of 4-*O*-methyliduronic acid (0.1–0.5 mol%) in surface xylan of unbleached birch and pine kraft pulps was determined by extensive xylanase treatment and further analysis by NMR spectroscopy and high-performance anion-exchange chromatography. © 1996 Elsevier Science Ltd.

Keywords: 4-*O*-Methyliduronic acid; Kraft pulp; Xylan; NMR spectroscopy; HPAED-PAD; Xylanase

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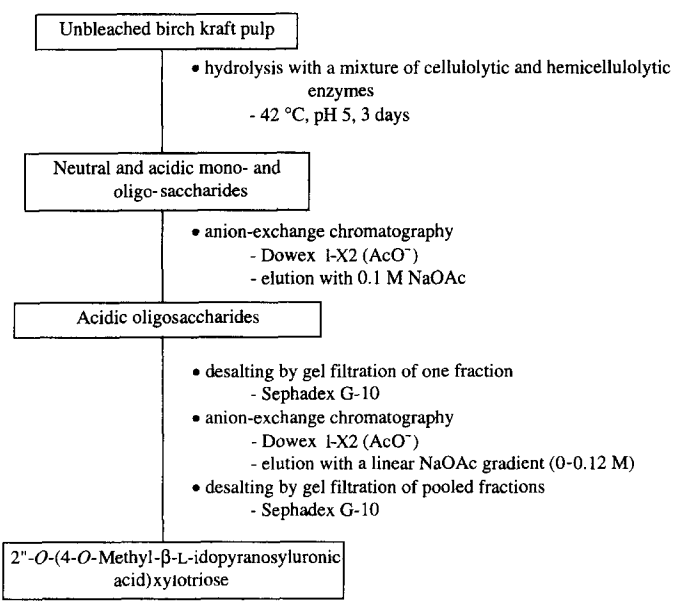
PII S0008-6215(96)00170-X

1. Introduction

The most abundant hemicellulose in hardwood is an *O*-acetyl-(4-*O*-methylglucurono)xylan, whereas an arabino(4-*O*-methylglucurono)xylan forms a part of softwood hemicellulose. On average, one-tenth and one-fifth to one-sixth of the (1 → 4)-linked xylosyl residues carry a 4-*O*-methylglucuronic acid (MeGlcA) group in hardwood and softwood xylan, respectively [1,2]. During kraft pulping the structure of xylan is modified. Some of the arabinose side-groups are degraded and the MeGlcA side-groups are converted into 4-deoxy-β-L-*threo*-hex-4-enopyranosyluronic acid (hexenuronic acid, HexA) side-groups [3,4]. The kinetics of the formation of HexA from MeGlcA have been investigated using 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylitol as model in 1 M NaOH at 150 °C [5]. The experiment showed that an epimerisation at C-5 of 4-*O*-methyl-α-D-glucopyranosyluronic acid occurs to give 4-*O*-methyl-β-L-idopyranosyluronic acid (MeIdoA) which is then degraded to hexenuronic acid [5]. Both MeIdoA [6] and hexenuronic acid [3] have been found in kraft pulps. However, only approximate amounts of MeIdoA have been reported [7]. Moreover, the β-(1 → 2) linkage of MeIdoA to a xylosyl residue in xylan has not been confirmed in samples from pulp.

The sugar residue L-iduronic acid is found in nature in heparin and heparin derivatives [8], in iduronic acid-rich proteoglycan [9] in connective tissues, as well as in chondroitin sulfates [10]. The inversion of configuration at C-5 of β-D-glucuronic acid



Scheme 1. A procedure for isolation of 2″-*O*-(4-*O*-methyl-β-L-idopyranosyluronic acid)xylotriose from unbleached birch kraft pulp.

to give α-L-iduronic acid is a vital step in the biosynthesis of heparin and its derivatives [8].

In this paper we present a procedure for the isolation of 4-*O*-methyl-L-iduronic acid linked to xylotriose from kraft pulp. We have also determined the primary structure of the isolated tetrasaccharide and the amount of MeIdoA in surface xylan of unbleached kraft pulps.

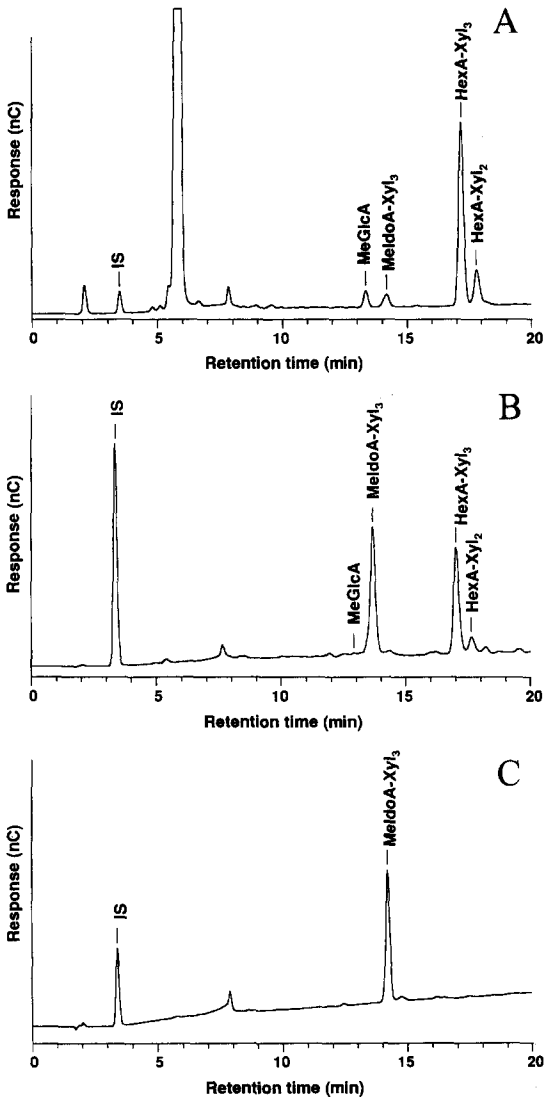


Fig. 1. HPAEC-PAD chromatograms from the isolation of MeIdoA-Xyl_3 from unbleached birch kraft pulp; IS = internal standard. (A) Hydrolysate after treatment with cellulolytic and hemicellulolytic enzymes, (B) acidic oligosaccharides in fraction 7 after the first anion-exchange chromatography, and (C) MeIdoA-Xyl_3.

2. Results and discussion

During the analysis of the carbohydrate composition of kraft pulps by anion-exchange chromatography [11,12] a minor unknown peak was observed in the HPAEC-PAD chromatograms. This unknown acidic oligosaccharide was isolated by the procedure outlined in Scheme 1 and Fig. 1. Analysis by HPAEC-PAD and NMR spectroscopy revealed that the isolated oligosaccharide consisted of one component, a tetrasaccharide (Figs. 1C and 2A). The structure of this acidic tetrasaccharide has been determined based on ^{1}H and ^{13}C NMR data alone, as described below.

Structure of 2″-O-(4-O-methyl-β-L-idopyranosyluronic acid)xylotriose.—The monosaccharide residues in 2″-*O*-(4-*O*-methyl-β-L-idopyranosyluronic acid)xylotriose (MeIdoA-Xyl_3) are designated **a**–**d** starting from the reducing end (Table 1). In the 1D ^{1}H NMR spectrum of MeIdoA-Xyl_3, four sets of resonances could be observed in the anomeric region (Fig. 2A). The relative intensity of the signals indicated a tetrasaccharide.

The assignment of proton NMR resonances for residues **a**–**d** was based on phase-sensitive COSY, relay COSY, and TOCSY experiments starting from the anomeric protons

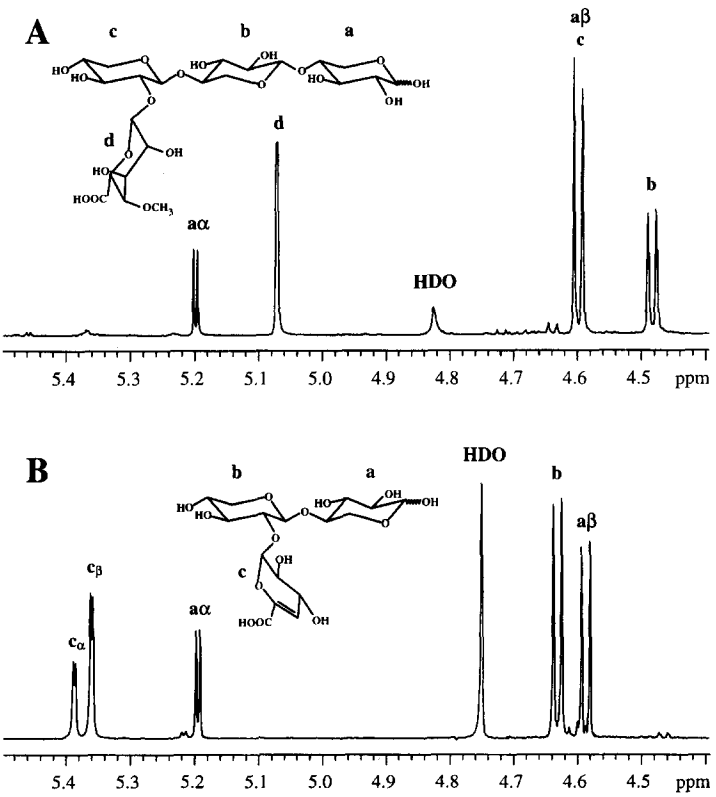


Fig. 2. Anomeric proton region of ^{1}H NMR spectra and structures of (A) MeIdoA-Xyl_3 (36 mg/0.74 mL D_2O, pH 7.6, 20 °C) and (B) HexA-Xyl_2 (6 mg/0.74 mL D_2O, pH 7.0, 27 °C).

(Table 2). The signals for the residues **a**β and **c** were distinguished by their relative abundances. The chemical shifts for H-2, H-3, and H-5 of **d** are in reasonably good agreement with published values of H-2, H-3, and H-5 of methyl β-D-idopyranosiduronic acid [13]. However, the chemical shifts for H-1 and H-4 of **d** and of methyl β-D-idopyranosiduronic acid [13] differ because of the differences in structure.

The ^{13}C chemical shifts of the five carbons (δ 99.26, 70.07, 67.57, 79.36, and 75.85, Table 3) to which the five ring protons of **d** are bound were identified from the HMQC spectrum. The non-protonated carbon atom (δ 176.66) was identified from the heteronuclear multiple-bond correlation (HMBC) spectrum. The assignment of ^{13}C resonances for residues **a**–**c** was based on heteronuclear correlation spectroscopy from the assigned proton signals (Table 3).

The point of attachment of the OCH_3 to residue **d** was confirmed by rotating-frame NOE spectroscopy (ROESY) and HMBC experiments. In the ROESY experiment cross-peaks were observed at the OCH_3 proton frequency for both H-3 and H-4 of residue **d** (Table 4). Unambiguous assignment of the point of attachment of the OCH_3 was obtained by the HMBC experiment. The data show that the OCH_3 proton signal at 3.43 has a cross-peak with C-4 of residue **d** at δ 79.36 and that the H-4 signal of residue **d** at δ 3.62 has a cross-peak with the OCH_3 ^{13}C signal at δ 59.29. A comparison of the ^{13}C chemical shifts of C-2 to C-5 of **d** shows that, among those, the C-4 signal has the highest chemical shift in agreement with an expected α-substituent effect. The same effect has been reported for 4-*O*-methyl-α-D-glucopyranosyluronic acid [16].

Table 1
Structures of the oligosaccharides MeIdoA-Xyl_3, HexA-Xyl_2, and HexA-Xyl_3

Code [a]	Structure
MeIdoA-Xyl_3 	**c** **b** **a** β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→4)-D-Xyl*p* β-L-MeIdo*p*A-(1→2) **d**
HexA-Xyl_2 	**b** **a** β-D-Xyl*p*-(1→4)-D-Xyl*p* β-L-Hex*p*A-(1→2) **c**
HexA-Xyl_3	β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→4)-D-Xyl*p* β-L-Hex*p*A-(1→2)

[a] Compounds are represented by short-hand notation: ●, Xyl; ▽, β-MeIdoA; □, β-HexA; –, linkage.

The HMBC and ROESY experiments confirmed the intra-residue interactions and assignments. The cross-peaks observed at the H-1 frequencies in the HMBC and ROESY spectra furthermore established the sequence of MeIdoA-Xyl_3 (Table 5). The presence of a β-(1 → 2)-linked MeIdoA on Xyl-**c** causes a shift to higher frequency of all the proton resonances in comparison to the signals of non-reducing Xyl in xylotriose [14]. The effect is especially large for H-2 (0.32 ppm) and H-1 (0.14 ppm). Likewise, the C-2 ^{13}C signal of **c** is at 6.42 ppm higher chemical shift than the corresponding signal of non-reducing xylose [3] in agreement with an expected α-substituent effect.

Conformational aspects.—For residue **d** all $^3J_{H,H}$ coupling constants are < 3 Hz indicating a gauche orientation of all the vicinal protons. The heteronuclear coupling constant $^1J_{C,H}$ (164 Hz) of the anomeric carbon of **d** is characteristic of an axial arrangement of H-1 [15]. The ROESY experiment (Table 4) and the $^3J_{H,H}$ coupling constants showed that **d** is predominantly in the $^1C_4$ chair conformation. The same observation has been reported for methyl β-D-idopyranosiduronic acid [13]. Thus, all the NMR data (chemical shifts, coupling constants, and proton–proton distances) for **d** are consistent with 4-*O*-methyl-β-L-idopyranosyluronic acid.

pK_a value.—A pH-titration showed that the largest chemical shift change (0.35 ppm) was obtained for H-5, as expected because of the proximity to the COOH group (Fig. 3). A non-linear regression fit (Fig. 3) gave a pK_a value of 3.17. We have earlier reported pK_a values for hexenuronic acid (3.03) and 4-*O*-methylglucuronic acid (3.14) attached (1 → 2) to xylooligomers [3], which are almost identical to the pK_a value of

Table 2
^1H NMR data for MeIdoA-Xyl_3 and HexA-Xyl_2

Compound [a]	Residue [a]	Chemical shift [b] (coupling constant [c])						
		H-1	H-2	H-3	H-4	H-5ax	H-5eq	OCH_3
	α-Xyl*p*-**a**	5.20	3.56	3.76	3.76	3.76 [d]	3.83 [d]	– [e]
	β-Xyl*p*-**a**	4.60	3.26	3.56	3.78	3.39	4.06	–
	β-Xyl*p*-**b**α	4.482	3.30	3.57	3.79	3.35	4.13	–
	β-Xyl*p*-**b**β	4.484	3.30	3.57	3.79	3.35	4.13	–
	β-Xyl*p*-**c**	4.60	3.58	3.55	3.69	3.33	3.98	–
	β-MeIdo*p*A-**d**	5.08 (~ 1)	3.69 (2.9)	4.28 (2.9)	3.62 (1.9)	4.33	–	3.43
	α-Xyl*p*-**a**	5.20	3.55	3.78	3.80 [f]	3.88 [f]	3.88 [f]	–
	β-Xyl*p*-**a**	4.59	3.25	3.57	3.80	3.43	4.13	–
	β-Xyl*p*-**b**	4.63	3.65	3.49	3.69	3.32	4.00	–
	β-Hex*p*A-**c**α	5.39	3.81	4.32	5.82	–	–	–
	β-Hex*p*A-**c**β	5.36	3.79	4.32	5.82	–	–	–

[a] See Table 1 for the key.
[b] In ppm relative to TSP at 0 ppm [D_2O, 20 °C (27 °C), and pD 7.6 (pD 7.0) for MeIdoA-Xyl_3 (HexA-Xyl_2)], acquired at 600 MHz.
[c] Observed first-order coupling in Hz.
[d,f] Assignment might have to be interchanged.
[e] – = Not relevant.

Table 3
^{13}C NMR data for MeIdoA-Xyl_3

Compound [a]	Residue [a]	Chemical shift [b] (coupling constant [c])						
		C-1	C-2	C-3	C-4	C-5	COOH	OCH_3
	α-Xyl *p*-**a**	92.88 (167)	72.23 (143)	71.79 (146)	77.43 (147)	59.66 (156)	– [d]	–
	β-Xyl *p*-**a**	97.37 (163)	74.85 (145)	74.74 (145)	77.25 (146)	63.82 (146)	–	–
	β-Xyl *p*-**b**	102.55 (162)	73.55 (145)	74.57 (145)	77.46 (147)	63.89 (146)	–	–
	β-Xyl *p*-**c**	102.52 (162)	80.02 (148)	75.12 (145)	69.95 (144)	65.98 (147)	–	–
	β-MeIdoA-**d**	99.26 (164)	70.07 (149)	67.57 (150)	79.36 (147)	75.85 (142)	176.66 (–)	59.28 (143)

[a] See Table 1 for the key.
[b] In ppm relative to external 1,4-dioxane at 67.4 ppm (D_2O, 20 °C, and pD 7.0), acquired at 150 MHz.
[c] Observed first-order $^1H–^{13}C$ coupling in Hz.
[d] – = Not relevant.

Table 4
Cross-peaks observed at the MeIdoA-**d** hydrogen frequencies in the ROESY spectrum of MeIdoA-Xyl_3

Compound [a]	Residue [a]	**d** H-1	**d** H-2	**d** H-3	**d** H-4	**d** H-5	**d** OCH_3
	MeIdoA-**d** H-1		X			X	
	MeIdoA-**d** H-2	X		X			
	MeIdoA-**d** H-3		X		X		X
	MeIdoA-**d** H-4			X		X	X
	MeIdoA-**d** H-5	X			X		
	MeIdoA-**d** OCH_3			X	X		

[a] See Table 1 for the key.

MeIdoA. However, the value is lower than apparent pK_a values (3.25, 3.95, and 4.2–4.5) reported for different forms of α-L-iduronic acid in heparin and heparin derivatives [8].

Relative amount of uronic acids in surface xylan of kraft pulp.—The accessible xylan of unbleached birch and pine kraft pulp was solubilised by extensive xylanase treatment. The carbohydrate composition was determined by NMR spectroscopy and HPAEC-PAD. In the ^1H NMR spectra of xylooligosaccharides, the anomeric proton resonances are well separated from the resonances of the other protons in the sugar ring and can thus be used as structural reporter signals and for obtaining quantitative data provided that the repetition time is long enough to avoid saturation. The anomeric proton resonances of MeGlcA, HexA, MeIdoA, Ara, and Xyl in xylan are well separated from each other and can thus be used for determination of the carbohydrate composition [3] (Table 2). In the HPAEC-PAD analysis xylose, arabinose, methylglucuronic acid, and HexA- and MeIdoA-xylooligomers were used as standards to obtain quantitative data. The structure of HexA-Xyl_2 (Fig. 2B, Tables 1, 2 and 5) and HexA-Xyl_3 [17] used as standards were determined by NMR spectroscopy. The assignment of the ^1H NMR data for HexA-Xyl_2

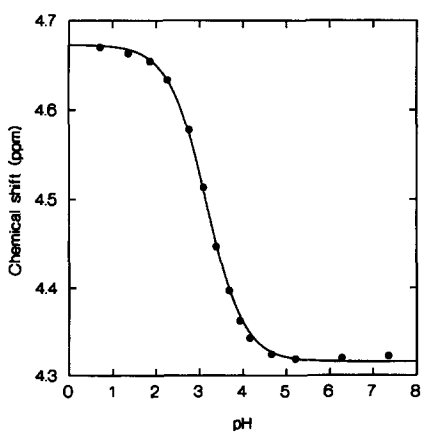


Fig. 3. pH-Dependence of the chemical shift of the proton (H-5) adjacent to the carboxyl group of 4-*O*-methyliduronic acid. The line is a non-linear-regression fit to the experimental points.

Table 5
Cross-peaks observed at the H-1 frequency in the HMBC spectrum of MeIdoA-Xyl_3 and ROESY spectra of MeIdoA-Xyl_3 and HexA-Xyl_2

Compound [a]	Residue [a]	HMBC cross-peak	ROESY cross-peak
	Xyl-**a**α H-1	Xyl-**a**α C-3,5	Xyl-**a**α H-2
	Xyl-**a**β H-1	Xyl-**a**β C-3	Xyl-**a**β H-3,5ax
	Xyl-**b** H-1	Xyl-**a**α C-4; Xyl-**a**β C-4; Xyl-**b**β C-3,5	Xyl-**b** H-3,5ax; Xyl-**a**β H-4,5eq; Xyl-**a**α H-4
	Xyl-**c** H-1	Xyl-**c** C-3; Xyl-**b** C-4	Xyl-**c** H-3,5ax; Xyl-**b** H-4,5eq
	MeIdoA-**d** H-1	MeIdoA-**d** C-2,5; Xyl-**c** C-2	MeIdoA-**d** H-2,5; Xyl-**c** H-2,3
	Xyl-**a**α H-1		Xyl-**a**α H-2
	Xyl-**a**β H-1		Xyl-**a**β H-2, 3, 5ax
	Xyl-**b** H-1		Xyl-**b** H-2, 3, 5ax; Xyl-**a**α H-4,5; Xyl-**a**β H-4, 5eq
	HexA-**c** H-1		HexA-**c** H-2, Xyl-**b** H-2

[a] See Table 1 for the key.

was based on phase-sensitive COSY, relay COSY, and TOCSY experiments starting from the anomeric protons (Table 2). The cross-peaks observed at the H-1 frequencies in the ROESY spectrum establish the sequence of HexA-Xyl_2 (Table 5). The H-1 signal of **c** was doubled because of the α and β forms of **a** (Fig. 2B). No such effect was observed on the H-1 signal of **b**, although that proton is closer in space to the reducing end according to the observed ROESY cross-peaks (Table 5). The same effect has been observed for the trisaccharide 2′-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)xylobiose [16].

The amount of MeIdoA was about one-fifth and one-tenth of the amount of MeGlcA for the surface xylan of birch and pine kraft pulp, respectively (Table 6). These values agree quite well with the earlier observed ratio for MeGlcA and MeIdoA in a model experiment with 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylitol in 1 M NaOH at 150 °C [5]. The amount of MeGlcA and HexA correlates well with earlier

Table 6
Relative amounts of sugar residues in the surface xylan of unbleached kraft pulps

Sample	Method	Carbohydrate content (mol%)					
		MeGlcA	MeIdoA	HexA	Ara	Xyl	Other
Birch kraft pulp	NMR [a]	1.8	0.3	3.7	–	94.1	0.2
	HPLC	2.2	0.5	4.9	–	92.4	nd [b]
Pine kraft pulp	NMR [a]	1.1	0.1	4.9	7.1	86.7	0.2
	HPLC	0.9	0.1	5.6	7.9	85.4	nd

[a] Determined by integration of anomeric protons in 1D ^{1}H spectra.
[b] nd = Not determined.

reported values for a pine kraft pulp [4]. NMR spectroscopy and HPAEC-PAD analysis resulted in very similar amounts for the different uronic acids (Table 6).

3. Conclusions

The 4-*O*-methyl-L-iduronic acid group in kraft pulp xylan is linked β-(1 → 2) to a xylopyranosyl residue. This side-group predominantly occupies the $^1C_4$ chair conformation. Quantitative analysis by HPAEC-PAD and NMR spectroscopy shows that 4-*O*-methyl-L-iduronic acid is found in low amounts in xylan of unbleached kraft pulp. About 6 and 2 mol% of the uronic acids were MeIdoA in the accessible xylan of birch and pine kraft pulps, respectively.

The pK_a values for 4-*O*-methylglucuronic acid, hexenuronic acid, and 4-*O*-methyliduronic acid linked to xylooligomers are almost identical. Hence, it can be concluded that the contribution of the uronic acid groups linked to xylan to the surface charge of cellulosic fibres in the pH range 2–5 can be treated as the sum of the uronic acids.

4. Experimental

Isolation of 2"-O-(4-O-methyl-β-L-idopyranosyluronic acid)xylotriose.—Conventionally cooked birch kraft pulp (kappa number 17) obtained from a Finnish pulp mill was hydrolysed by a mixture of cellulase (Econase CE, Primalco, Finland) and xylanase (Ecopulp X-200, Primalco, Finland) preparations. Water (40 L) was added to 22 kg of pulp (final concentration ~ 6 w/v%) and the pH was adjusted to 5 by sulfuric acid. Econase (2.2 L) and Ecopulp (220 mL) were added and the hydrolysis was carried out for 3 days at 42 °C after which the hydrolysis was terminated by boiling for 15 min. The hydrolysate was clarified by filtration after which the acidic mono- and oligo-saccharides were separated from the neutral saccharides by anion-exchange chromatography. A column (BP-252, 25.2 × 11 cm, Pharmacia, Sweden) of Dowex 1-X2 (Cl^-) 100–200 mesh (Fluka, Switzerland) was washed with 1 M NaOAc (10 column volumes) and equilibrated with water after which the hydrolysate was applied to the column. All acidic saccharides were bound and eluted in two steps, first with 0.1 M NaOAc and then with 1 M NaOAc. Fractions of 0.9 L were collected and analysed by HPAEC-PAD. The acidic oligosaccharides in fraction 7 (eluted with 0.1 M NaOAc) were further separated. After removal of NaOAc by gel filtration (Sephadex G-10 packed in 5 × 55 cm column, Pharmacia) the acidic oligosaccharides from fraction 7 were bound again to a Dowex 1-X2 column (1.6 × 10 cm) after which they were separated by elution with a linear NaOAc gradient from 0 to 0.12 M. The fractions containing an unknown acidic oligosaccharide were pooled, concentrated by evaporation, and desalted by gel filtration (Sephadex G-10 packed in 1.6 × 26 cm column, Pharmacia) after which the sample was dried by lyophilisation (yield, 36 mg).

Hydrolysis of pulps.—The amount of 4-*O*-methyliduronic acid in surface xylan of conventionally cooked birch and pine kraft pulp was determined using enzymatic peeling with xylanase. The production and properties of unbleached laboratory-cooked

birch and pine kraft pulps, with kappa numbers of 19.9 and 25.8, have been described previously [3]. The pulps were hydrolysed for 24 h with xylanase pI 9.0 of *Trichoderma reesei* [18] as described by Buchert et al. [4]. The amount of neutral and acidic sugars in the hydrolysates was quantified by NMR after freeze-drying. The hydrolysates were also analysed by HPAEC-PAD after a secondary enzymatic hydrolysis [19].

HPAEC-PAD analysis.—Analysis by high-performance anion-exchange chromatography on a DIONEX 4500i series Chromatograph with pulsed amperometric detection was performed using the gradient for acidic mono- and oligo-saccharides described earlier [3,11]. The xylose and arabinose used as standards were purchased from Fluka (Switzerland). The acidic mono- and oligo-saccharides used as standards were purified from different enzymatic hydrolysates. The 4-*O*-methylglucuronic acid (MeGlcA) was prepared from commercial birchwood xylan (Roth, Karlsruhe, Germany) by hydrolysis (24 h, 40 °C, 50 mM NaOAc buffer, pH 5) with xylanase pI 9 (10000 nkat/g) [18], β-xylosidase (5000 nkat/g) [20], and α-glucuronidase (1000 nkat/g) [21] of *T. reesei*. The free MeGlcA was purified using anion exchange (Dowex 1-X1) and gel filtration (Sephadex G-10) as described previously [3]. The hexenuronic acid–xylobiose (HexA-Xyl_2) was purified from the same hydrolysate as MeIdoA-Xyl_3. Fractions eluted from the Dowex column by 1 M NaOAc were combined and freeze-dried. The freeze-dried material was dissolved in water and desalted by Sephadex G-10. Fractions containing HexA-Xyl_2 were collected, concentrated by evaporation, and further purified by Bio-Gel P-2 (Bio-Rad) using 10 mM NaOAc buffer, pH 4.0 as eluent. The fractions containing HexA-Xyl_2 were pooled and desalted by Sephadex G-10. The hexenuronic acid xylotriose (HexA-Xyl_3) was prepared by hydrolysing birch kraft pulp (48 h, 40 °C, pH adjusted to 5 by H_2SO_4) with xylanase pI 6.9 of *Aspergillus oryzae* (2000 nkat/g) [22]. The HexA-Xyl_3 formed was purified by anion-exchange chromatography (Dowex 1-X2), using a linear NaOAc gradient (0–0.12 M) for elution, after which the pooled fraction was desalted by Sephadex G-10. The purification of MeIdoA-Xyl_3 was explained above.

Determination of the pK_a *value.*—For the pH-titrations, the pH (not corrected for isotopic effects) was adjusted by additions of NaOD or DCl.

NMR spectroscopy.—For NMR analysis the dried samples were redissolved in D_2O (99.8 atom %, Fluka) and the pH was adjusted to 7. The ^1H and ^{13}C NMR spectra were obtained at 599.86 and 150.85 MHz, respectively, on a Varian UNITY 600 MHz spectrometer. Typical acquisition parameters for 1D ^1H NMR (1D ^{13}C NMR) were a 90° pulse of 13 μs (12 μs), a spectral width of 8000 Hz (40000 Hz), and a repetition time of 21 s (10 s). Spectra were obtained at 20 °C (27 °C for HexA-Xyl_2). The chemical shifts are reported relative to sodium 3,3,2,2-tetradeuterio-4,4-dimethyl-4-silapentanoate (TSP) at 0 ppm and dioxane at 67.40 ppm for ^1H and ^{13}C NMR spectra, respectively.

Standard pulse sequences and phase cycling were utilised to obtain phase-sensitive COSY [23], R-COSY [24] (τ = 100 ms), TOCSY [25,26] (τ_{mix} = 0.14 s), and ROESY [27] (τ_m = 0.5 s) 2D spectra. A spectral width of 3000 Hz (2200 Hz for HexA-Xyl_2) was employed in both dimensions and the relaxation delay was 3.0 s. For each FID, 8 (4 for COSY and 16 for ROESY) transients were acquired; the data size was 350 (1024 for COSY and 512 for ROESY) in $t_1 \times 4096$ in t_2. The phase-sensitive ^1H-detected HMQC [28] spectrum was acquired over a t_1 spectral width of 10000 Hz and a t_2 width of 3000

Hz with a 512×4096 matrix (zero-filled to 1024 in t_1) and 8 transients per increment. The delay between transients was 3.0 s and the delay for polarisation transfer was set for $^1J_{CH}$ of 155 Hz. The multiple-bond ^1H–^{13}C shift correlation [29] (HMBC) spectrum resulted from 700×4096 data matrix size, with 16 scans per t_1 value, a delay time between scans of 3.0 s, and $\Delta_2 = 80$ ms. The spectral width was 19000 and 3000 Hz for t_1 and t_2, respectively. The final data size after Fourier transformation was 1024×4096.

Acknowledgements

The authors thank Professor Torbjörn Drakenberg for valuable discussions, Veera Ailasmaa for her help with NMR analysis of HexA-Xyl_2, and Tapani Vuorinen for drawing Fig. 3 and purification of MeGlcA. The skillful technical assistance of Riitta Isoniemi, Taina Simoinen, Pia Keinänen, Ulla Lahtinen, and Päivi Matikainen is gratefully acknowledged. Financial support from the Carbohydrate Research Programme of TEKES (Technology Development Centre, Finland) is gratefully acknowledged.

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