ELSEVIER

Carbohydrate Research 280 (1996) 197–208

CARBOHYDRATE RESEARCH

Identification of the acidic degradation products of hexenuronic acid and characterisation of hexenuronic acid-substituted xylooligosaccharides by NMR spectroscopy

Anita Teleman [a,*], Tiina Hausalo [b], Maija Tenkanen [c], Tapani Vuorinen [d]

[a] *Technical Research Centre of Finland, Chemical Technology, P.O. Box 1401, FIN-02044 VTT, Finland*
[b] *The Finnish Pulp and Paper Research Institute (KCL), P.O. Box 70, FIN-02151 Espoo, Finland*
[c] *Technical Research Centre of Finland, Biotechnology and Food Research, P.O. Box 1501, FIN-02044 VTT, Finland*
[d] *Laboratory of Forest Products Chemistry, Helsinki University of Technology, FIN-02150 Espoo, Finland*

Received 28 April 1995; accepted 16 August 1995

Abstract

A 4-*O*-methylglucuronoxylan was converted into a hexenuronoxylan at high temperature and alkalinity similar to the conditions used during kraft pulping. The hexenuronoxylan was hydrolysed with enzymes, and acidic xylooligosaccharides were separated from the hydrolysate by anion-exchange and size-exclusion chromatography. The primary structure of the two main hexenuronic acid-substituted xylooligosaccharides (a tetramer and a pentamer) was determined by two-dimensional ^{1}H and ^{13}C NMR spectroscopy. The 4-deoxy-hexenuronic acid is not stable under the acid hydrolysis step of conventional carbohydrate analysis. Here, we have identified the acidic degradation products of 4-deoxy-hexenuronic acid by NMR spectroscopy. Two degradation pathways were observed, both resulting in a furan derivative.

Keywords: Hexenuronic acid; Kraft pulp; NMR spectroscopy; Xylan; 2-Furoic acid; 5-Formyl-2-furoic acid

* Corresponding author.

SSDI 0008-6215(95)00309-6

1. Introduction

Acetyl-4-*O*-methylglucuronoxylan and arabino-4-*O*-methylglucuronoxylan are important constituents of hardwoods and softwoods, respectively [1]. During kraft pulping the structure of the hemicelluloses is extensively modified as a result of partial degradation of side-groups in the high alkalinity and temperature of the cooking liquor [2].

Over 30 years ago Clayton [3] proposed that the removal of 4-*O*-methyl-D-glucuronic acid (MeGlcA) during kraft pulping begins by β-elimination of methanol. Johansson and Samuelson [4] later verified that this reaction can occur in a model compound, 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylitol from which a new acidic substituent, 4-deoxy-β-L-*threo*-hex-4-enopyranosyluronic acid (hexenuronic acid, HexA) was formed. By ^{13}C NMR spectroscopy Šimkovic et al. [5] identified HexA in the xylan part of alkaline-degraded holocellulose and cellulose samples from beech. Also from NMR spectroscopic data we recently identified HexA in polymer xylan samples from kraft pulping liquor as well as in oligosaccharides from kraft pulp xylan obtained by enzymatic hydrolysis with xylanase [6]. The presence of HexA in kraft pulps has never been verified with conventional pulp analysis because this analysis includes an acid hydrolysis step, known to degrade HexA [4,7]. Here, we report the identification of the acidic degradation products of the 4-deoxy-hexenuronic acid by NMR spectroscopy.

2. Results and discussion

HexA-substituted xylooligosaccharides (**HXO**, Table 1) were obtained from 4-*O*-methylglucuronoxylan by the procedure outlined in Scheme 1. The modified hardwood xylan contained 4.1 mol% hexenuronic acid groups together with ~0.5 mol% residual 4-*O*-D-methylglucuronic acid groups. After enzymatic hydrolysis with endoxylanase, β-xylosidase, and α-glucuronidase, a mixture of monosaccharides and HexA-substituted xylooligosaccharides was obtained. From this mixture the **HXO** fraction was separated by using anion-exchange and size-exclusion chromatography. Analysis by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) revealed that the **HXO** fraction consisted of ~75 weight% of **HX-4**, ~20 weight% of **HX-5**, ~3 weight% of **HX-3** (Table 1), and traces of smaller oligosaccharides, Fig. 1.

Structure of hexenuronic acid-substituted xylooligosaccharides.—In the 1D ^1H NMR spectrum of the **HXO** fraction, five sets of resonances were seen in the anomeric region (Fig. 2A). The relative intensities of the signals indicated [6], in agreement with the HPAEC-PAD analysis, a mixture of a tetrasaccharide (**HX-4**), a pentasaccharide (**HX-5**), and a trisaccharide (**HX-3**). The structures of the two major components were determined by NMR spectroscopy. The monosaccharide units in **HX-4** and **HX-5** are designated **a**–**d** starting from the reducing end. The ^1H NMR data for **HX-4** and **HX-5** (D_2O, 27 °C) are given in Table 2. The assignments were based on COSY, relay COSY, and TOCSY experiments starting from the anomeric protons. In the TOCSY spectrum the resonances of **HX-5 b′** could clearly be separated from the resonances of **b** at the H-5eq frequencies (Fig. 3). The chemical shifts of **HX-5 a**, **b**, **c**, and **d** protons could not be distinguished from the chemical shifts of **HX-4 a**, **b**, **c**, and **d** protons.

Table 1
Structures of oligosaccharides **HXO**, **HX-3**, **HX-4**, and **HX-5**

Code	Structure
HXO	β-D-Xyl*p*-(1→4)-R β-L-Hex*p*A-(1→2) R= D-Xyl*p* or β-D-Xyl*p*-(1→4)-
HX-3	β-D-Xyl*p*-(1→4)-D-Xyl*p* β-L-Hex*p*A-(1→2)
HX-4	**c** **b** **a** β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→4)-D-Xyl*p* β-L-Hex*p*A-(1→2) **d**
HX-5	**c** **b** **b'** **a** β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→4)-D-Xyl*p* β-L-Hex*p*A-(1→2) **d**

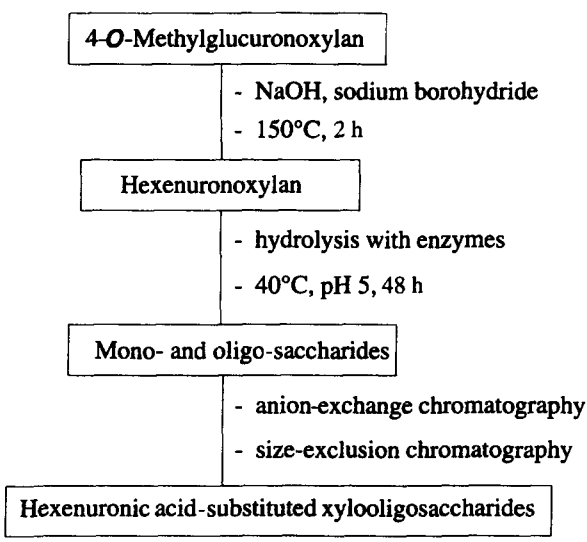
Similarly, the ^{13}C NMR data (150 MHz) for **HX-4** are given in Table 3. The assignment was based on heteronuclear correlation spectroscopy from the assigned proton signals. In the ^{13}C NMR spectrum of **HXO** a few minor signals, probably due to carbons of the pentasaccharide **HX-5 b'**, were also observed but assignment was not attempted.

Heteronuclear multiple-bond correlation (HMBC) and rotating-frame NOE spectroscopy (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 establish the sequence of **HX-4** (Table 4). The chemical shifts for HexA agree with earlier reported values [6]. The proton signals of **c** were shifted upfield compared with the corresponding signals of an internal xylose with HexA as side-group [6]. The same differences for internal and terminal units are observed for xylose without a side-group [8]. Similarly, the C-4 ^{13}C signal of **c** is shifted upfield (7.27 ppm) compared with the corresponding signal of internal xylose with HexA as a side-group [6] in agreement with an expected α-substituent effect. The presence of a β-(1 → 2)-linked HexA on Xyl-**c** causes a shift of all the proton resonances to higher frequency in comparison to the signals of non-reducing Xyl in xylotriose [8]. The effect is especially large for H-2 (0.39 ppm) and H-1 (0.18 ppm).

Table 2
^{1}H NMR data for hexenuronic acid-substituted xylooligosaccharides (**HX-4** and **HX-5**) and acidic degradation products of hexenuronic acid (**HD-1** and **HD-2**)

Compound [a]	Residue [b]	Chemical shift (ppm) [c]						
		H-1	H-2	H-3	H-4	H-5ax/H-5	H-5eq	CHO
	α-Xyl*p*-**a**	5.20	3.56	3.76	3.76	3.76 [d]	3.83 [d]	– [e]
HX-4	β-Xyl*p*-**a**	4.59	3.26	3.56	3.79	3.39	4.07	–
	β-Xyl*p*-$\mathbf{b}_\alpha$	4.476						–
	β-Xyl*p*-$\mathbf{b}_\beta$	4.480	3.302	3.574	3.813	3.42	4.17	–
	β-Xyl*p*-**c**	4.64	3.65	3.49	3.69	3.32	4.00	–
	β-HexA-**d**	5.36	3.80	4.32	5.82	–	–	–
	β-Xyl*p*-**b'** [f]	4.482	3.309	3.566	3.802	3.39	4.12	–
HX-5								
HD-1		–	–	7.01	6.56	7.60	–	–
HD-2		–	–	7.13	7.53	–	–	9.59

[a] Compounds are represented by short-hand notation: ●, Xyl; □, β-HexA; –, linkage.
[b] The Xyl*p* residue in the reducing position is denoted **a**, etc.
[c] Measured at 600 MHz on solutions in D_2O at 27 °C and pD 7.0 relative to internal TSP.
[d] Assignment might have to be interchanged.
[e] – = Not relevant.
[f] The chemical shifts of **HX-5 a**, **b**, **c**, and **d** protons could not be distinguished from the chemical shifts of **HX-4 a**, **b**, **c**, and **d** protons.



Scheme 1. A procedure for obtaining hexenuronic acid-substituted xylooligosaccharides from 4-*O*-methylglucuronoxylan.

Table 3
^{13}C NMR data for the hexenuronic acid-substituted xylooligosaccharide (**HX-4**) and degradation products of hexenuronic acid (**HD-1** and **HD-2**)

Compound [a]	Residue [b]	Chemical shift [c] (coupling constant [d])						
		C-1	C-2	C-3	C-4	C-5	COOH	CHO
	α-Xyl*p*-**a**	92.85 (171)	72.20 (144)	71.77 (144)	77.40 (150)	59.66 (150)	– [e]	–
HX-4	β-Xyl*p*-**a**	97.34 (163)	74.82 (144)	74.74 (144)	77.24 (147)	63.80 (148)	–	–
	β-Xyl*p*-**b**	102.55 (162)	73.49 (146)	74.59 (144)	77.15 (147)	63.80 (148)	–	–
	β-Xyl*p*-**c**	102.34 (162)	78.89 (147)	75.00 (142)	69.93 (145)	65.88 (147)	–	–
	β-HexA-**d**	99.01 (174)	70.84 (145)	66.74 (149)	107.82 (168)	146.29 (–)	170.11 (–)	–
HD-1		–	150.18 (–)	115.78 (177)	112.46 (177)	145.89 (204)	167.52 (–)	–
HD-2		–	154.93 (–)	116.83 (177)	125.91 (179)	152.68 (–)	165.97 (–)	182.60 (185)

[a,b] See Table 2 for the key.
[c] In ppm relative to internal 1,4-dioxane at 67.4 ppm (D_2O, 27 °C, and pD 7.0), acquired at 150 MHz.
[d] Observed first-order $^1H-^{13}C$ coupling in Hz.
[e] – = Not relevant.

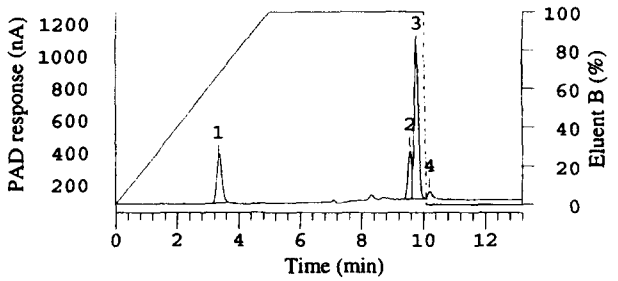


Fig. 1. HPLC chromatogram of the **HXO** fraction. Peaks: 1 = fucose (internal standard); 2 = **HX-5**; 3 = **HX-4**; 4 = **HX-3**.

Acidic degradation products of hexenuronic acid.—The **HXO** fraction was hydrolysed under acidic conditions (1 M trifluoroacetic acid) and the hydrolysate was analysed by ^{1}H NMR spectroscopy (Fig. 2B) without separation of the degradation products. In the anomeric proton region (4.4–5.5 ppm) only two resonances were observed. These were the resonances from the H-1 protons of α- and β-xylose. Thus all the glycosidic linkages had been hydrolysed and hexenuronic acid had been degraded as well. New resonances were observed between 6.5 and 9.8 ppm (Fig. 2B). Some of the resonances were related to degradation of xylose. An acid hydrolysis of xylotriose showed that a minor amount of 2-furaldehyde was formed (^{1}H chemical shifts in 1 M trifluoroacetic acid: 6.75, 7.57, 7.90, and 9.48 ppm [9]). This confirmed that one of the sets of minor

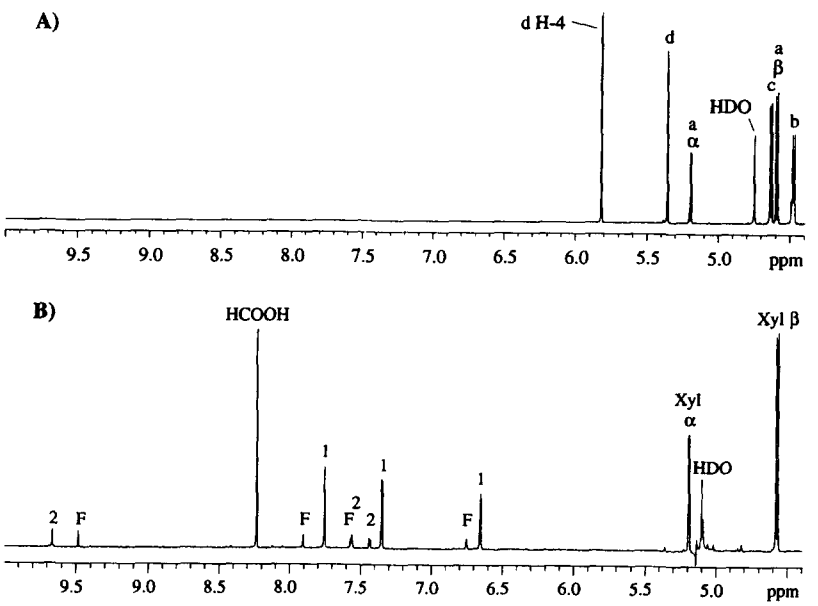


Fig. 2. ^{1}H NMR spectra of the **HXO** fraction: (A) 26 mg/0.74 mL D_2O, pH 7.0, and (B) 2 mg/mL after acid hydrolysis in 1 M trifluoroacetic acid. 1 = **HD-1**; 2 = **HD-2**; F = 2-furaldehyde.

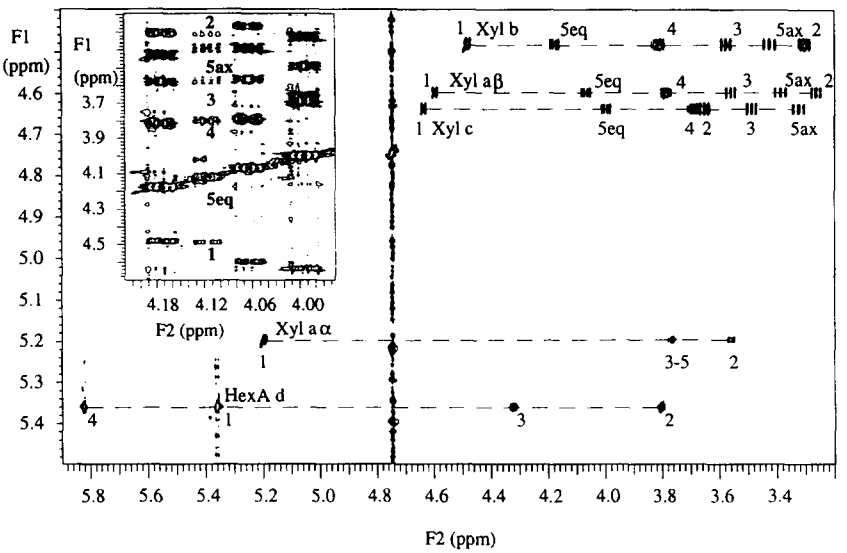


Fig. 3. TOCSY spectrum of the **HXO** fraction. Diagonal peaks of the anomeric protons are indicated. The numbers near cross-peaks refer to the protons of the scalar-coupling network belonging to a diagonal peak. In the inset, the cross-peaks at the β-Xyl b′ H-5eq frequencies are indicated.

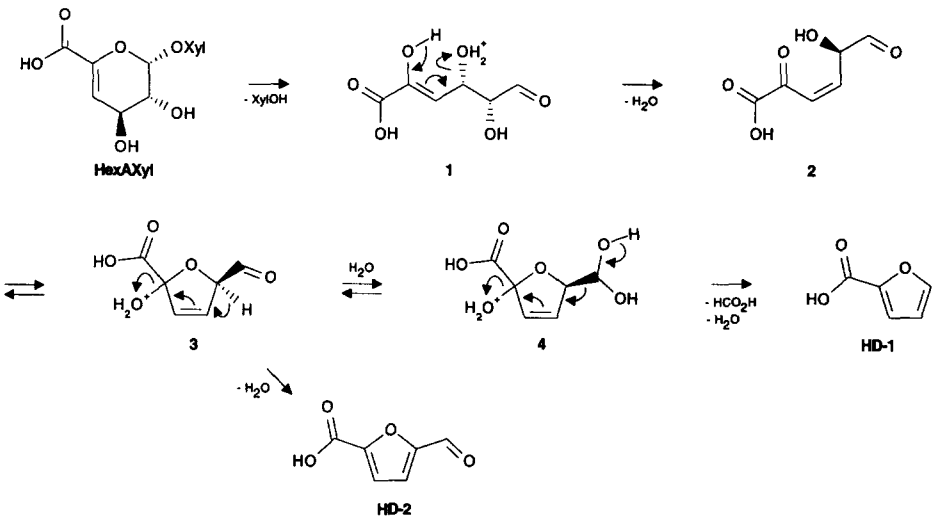
resonances in Fig. 2B is due to 2-furaldehyde. At the high temperature and acidic conditions used, ca. 4% of the xylose was converted into 2-furaldehyde. The structures of the acidic degradation products of hexenuronic acid were identified by ^{1}H (Table 2) and ^{13}C NMR (Table 3) spectroscopy. Two sets of signals, with an intensity ratio of 7:1, are observed for the degradation products (Fig. 2B). The main set was identified as 2-furoic acid (**HD-1**) and formic acid (Scheme 2). A minor amount of 5-formyl-2-furoic acid (**HD-2**) was also obtained. 4-Deoxy-L-*threo*-hex-4-enuronic acid (or its non-enolic form 4-deoxy-L-*threo*-hex-5-ulosuronic acid, see **1**, Scheme 2) that is liberated in the hydrolysis of HexA easily undergoes β-elimination of HO-3 (Scheme 2). The cyclised product (**3**) is stabilised through eliminations of water and formic acid or water alone

Table 4
Cross-peaks observed at the H-1 frequency in the HMBC and ROESY spectra of the **HXO** fraction

Compound [a]	Residue [b]	HMBC cross-peakROESY cross-peak
●●● □	Xyl-**a**α H-1	Xyl-**a**α C-3,5Xyl-**a**α H-2 (s [c])
HX-4	Xyl-**a**β H-1	Xyl-**a**β C-3,5Xyl-**a**β H-3(m),5ax(s)
	Xyl-**b** H-1	Xyl-**a**α C-4; Xyl-**a**β C-4Xyl-**b** H-3(s),5ax(s); Xyl-**a**β H-4(s),5eq(s)
	Xyl-**c** H-1	Xyl-**c** C-3,5; Xyl-**b** C-4Xyl-**c** H-2(w),3(s),5ax(s); Xyl-**b** H-4(s),5eq(s)
	HexA-**d** H-1	HexA-**d** C-2,3,5; Xyl-**c** C-2HexA-**d** H-2(s); Xyl-**c** H-2(s)

[a,b] See Table 2 for the key.

[c] Relative intensity of the cross-peaks in the ROESY spectrum determined by integration. The integrated volume has been divided by the relative abundance of the residue: w, weak; m, medium; s, strong.



Scheme 2. Acid degradation of hexenuronic acid.

depending on whether the aldehyde function is hydrated (**4**) or not (**3**). In principle the reaction steps can occur in a different order but ultimately they yield 2-furoic and 5-formyl-2-furoic acids. As deuterated acid and D_2O were used for the hydrolysis, in principle some deuterium could be incorporated in the products, at least to C-4 through an enol–keto equilibrium. However, according to the relative integrals of the proton NMR resonances, there is no incorporation of deuterium, which fits the mechanism proposed in Scheme 2. The resonances of **HD-1** and **HD-2** were clearly distinguished because of the lower amount of **HD-2**. For the ^{13}C NMR study, **HD-1** and **HD-2** were extracted from an acid hydrolysate of pulp.

The assignment of the NMR resonances of **HD-1** began at H-5, which has the largest $^{1}H-^{13}C$ direct coupling (204 Hz) [10]. The H-3 and H-4 resonances were assigned with the $J_{H,H}$ coupling constants ($J_{3,4} = 3.4$, $J_{3,5} = 0.8$, and $J_{4,5} = 1.8$ Hz). A pH-titration showed that the largest chemical shift change (0.35 ppm) was obtained for H-3 as expected because of the proximity to the COOH group (Fig. 4). The assignment of the ^{13}C spectrum was obtained from selective decoupling of the $^{1}H-^{13}C$ satellites in the ^{1}H NMR spectrum. The C-2 resonance was identified in the undecoupled ^{13}C NMR spectrum by the absence of direct coupling. The ^{1}H and ^{13}C chemical shifts for **HD-1** are in reasonably good agreement with earlier published values in another solvent [9,10].

The assignment of the NMR resonances of **HD-2** began at the aldehyde proton resonance. The C-3 and C-4 signals were identified in the undecoupled ^{13}C NMR spectrum by both having one direct $^{1}H-^{13}C$ coupling. The C-4 resonance was recognised by having two long-range $^{1}H-^{13}C$ couplings, whereas the C-3 signal has only one. The assignment of the H-3 and H-4 resonances was obtained from selective decoupling of the $^{1}H-^{13}C$ satellites in the ^{1}H NMR spectrum. A pH-titration showed that the largest chemical shift change (0.33 ppm) was obtained for H-3 as expected because of the proximity to the COOH group (Fig. 4). The C-2 and C-5 resonances were identified in

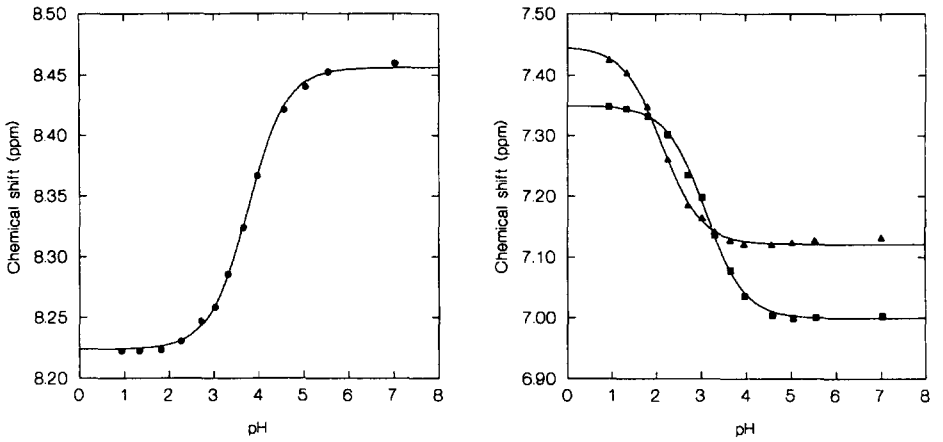


Fig. 4. pH Dependence of the chemical shift of protons adjacent to the carboxylic acid: ● denotes HCOOH; ■, H-3 of **HD-1**; and ▲, H-3 of **HD-2**. The lines are non-linear-regression fits to the experimental points.

the undecoupled ^{13}C NMR spectrum by the absence of direct coupling. They were distinguished by the number of long-range $^{1}H-^{13}C$ couplings, two and three for C-2 and C-5, respectively.

Non-linear regression fits (Fig. 4) gave pK_a values of 3.11 and 3.77 for **HD-1** and formic acid, respectively, which are in good agreement with reported values of 3.164 and 3.751, respectively [11]. Similarly a pK_a value of 2.15 was determined for **HD-2**.

Recently, Gellerstedt et al. [12] have identified the same furan derivatives in the lipophilic fraction obtained after acid hydrolysis of unbleached industrial pulp. These furan derivatives, as demonstrated here, originate from HexA formed during pulping and subsequently degraded during the acid hydrolysis.

3. Conclusions

A procedure for obtaining hexenuronic acid-containing xylooligosaccharides from 4-*O*-methylglucuronoxylan is presented. The glycosidic bond of β-HexA-(1 → 2)-D-Xyl is acid labile in contrast to the glycosidic bond of 4-OMe-α-D-Glc*p*A-(1 → 2)-D-Xyl [13]. Furthermore, 4-deoxy-hexenuronic acid itself is not stable under acid conditions. In consequence the acid hydrolysis step of conventional pulp carbohydrate analysis has hitherto prevented the identification of HexA. Two degradation pathways of HexA were observed, both resulting in a furan derivative.

4. Experimental

Preparation of hexenuronic acid-substituted oligosaccharides.—4-*O*-Methylglucuronoxylan (Roth, Karlsruhe, Germany, 15 g) and $NaBH_4$ (0.2 g) were dissolved in 1 M NaOH (300 mL) and kept overnight under N_2. The solution was heated to 150 °C

for 2 h. After cooling, glycerol (15 mL) was added to break xylan–borate complexes and the solution was neutralised (pH 7) with formic acid. The xylan was precipitated by slow addition of MeOH (300 mL). The precipitate was separated by centrifugation, washed with MeOH–water (1:1, 300 mL) and MeOH (300 mL), and dried under vacuum at room temperature (yield 12.6 g).

The modified xylan (10 g) was hydrolysed for 48 h at 40 °C and pH 5 (50 mM sodium acetate buffer) with an enzyme mixture containing purified endoxylanase (5000 nkat/g), β-xylosidase (1000 nkat/g), and α-glucuronidase (1000 nkat/g). The endoxylanase p*I* 9, β-xylosidase, and α-glucuronidase used were isolated from the *Trichoderma reesei* culture filtrate according to the methods described by Tenkanen et al. [14], Poutanen and Puls [15], and Siika-aho et al. [16], respectively.

The concentrated hydrolysate (100 mL) was desalted and prefractionated with a column (2.6 × 100 cm) of Sephadex G-10 (Pharmacia) using water as eluent. The oligosaccharide fraction was eluted through a column (1 × 20 cm) of Dowex 1-X1 anion-exchange resin in the acetate form and the elution continued with water (100 mL). The acidic oligosaccharides were eluted from the column with 0.5 M NaOAc (100 mL). The eluate was concentrated and fractionated with a column (2.6 × 100 cm) of Sephadex G-15 (Pharmacia) using water as eluent. The major acidic oligosaccharide fraction (dp 4-5) was recovered and freeze-dried (yield 0.45 g).

Acid hydrolysis.—The hexenuronic acid-substituted oligosaccharide fraction (2.2 mg/mL) and xylotriose (2.1 mg/mL) were hydrolysed in 1 M CF_3CO_2D at 120 °C for 90 min (D_2O) [17].

Preparation of acidic degradation products of hexenuronic acid.—An acid hydrolysate from oxygen and peroxide-bleached hardwood pulp (100 g) was concentrated to a volume of 100 mL and extracted with diethyl ether (100 mL). The ether layer was washed with water (50 mL) and evaporated to dryness. The solid residue (yield 0.39 g) contained mainly furan derivates.

HPAEC-PAD analysis.—Analysis by high-performance anion-exchange chromatography on a DIONEX 4500i series Chromatograph with pulsed amperometric detection was performed as described earlier [6], except that the elution was carried out with a linear gradient from 100% eluent A (100 mM NaOH) to 100% eluent B (300 mM CH_3COONa–100 mM NaOH) during 5 min and thereafter 100% eluent B during 5 min. The column was washed with eluent C (300 mM NaOH) during 3 min.

Determination of the pK_a values.—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 7.0. 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 were for 1D ^1H NMR (1D ^{13}C NMR) a 90° pulse of 15 μs (13 μs), a spectral width of 8000 Hz (40 000 Hz), and a repetition time of 21 s (10 s) for the hexenuronic acid-substituted oligosaccharides and 154 s (17 s) for the furan derivatives. The repetition times were long enough to obtain quantitative data. Spectra were obtained at 27 °C. The chemical shifts are reported relative to internal sodium 2,2,3,3-tetradeuterio-4,4-dimethyl-4-silapentanoate (TSP) at 0 ppm and 1,4-dioxane at 67.40 ppm for ^1H and ^{13}C NMR spectra, respectively.

Assignment of the ^{13}C NMR resonances for the furan derivatives was performed by selective ^{13}C decoupling (continuous wave decoupling power, 15 dB) and observation of which $^{1}H-^{13}C$ satellite in the ^{1}H NMR spectrum disappears.

Standard pulse sequences and phase cycling were utilised to obtain phase-sensitive COSY [18], R-COSY [19] ($\tau = 70$ ms), TOCSY [20,21] ($\tau_{mix} = 0.14$ s), and ROESY [22] ($\tau_m = 0.5$ s) 2D spectra. A spectral width of 2000 Hz was employed in both dimensions and the relaxation delay was 2.5 s. For each FID, 8 (16 for TOCSY and ROESY) transients were acquired; the data size was 350 (700 for COSY) in $t_1 \times 4032$ in t_2. The phase-sensitive ^{1}H-detected HMQC [23] spectrum was acquired over a t_1 spectral window of 10000 Hz and a t_2 window of 2000 Hz with a 512×3584 matrix (zero-filled to 2048 and 4096 in t_1 and t_2, respectively) and 16 transients per increment. The delay between transients was 3.5 s and the delay for polarisation transfer was set for $^{1}J_{CH}$ of 155 Hz. The multiple-bond $^{1}H-^{13}C$ shift correlation [24] (HMBC) spectra resulted from 540×3584 data matrix sizes, with 32 scans per t_1 value, a delay time between scans of 3.5 s, and $\Delta_2 = 80$ ms. The spectral window was 19000 and 3500 Hz for t_1 and t_2, respectively. Full details of the 1D and 2D experiments have been reported [6].

Acknowledgements

The authors thank Professor T. Drakenberg for valuable discussions. This work was financed by TEKES (Technology Development Centre, Finland).

References

[1] K. Shimizu, in D.N.-S. Hon and N. Shiraishi (Eds.), *Wood and Cellulosic Chemistry*, Dekker, New York, 1991, pp 177–214.
[2] E. Sjöström, *Wood Chemistry: Fundamentals and Applications*, Academic, New York, 1993, pp 140–161.
[3] D.W. Clayton, *Sven. Papperstidn.*, 66 (1963) 115–124.
[4] M.H. Johansson and O. Samuelson, *Carbohydr. Res.*, 54 (1977) 295–299.
[5] I. Šimkovic, J. Alföldi, and M. Matulová, *Carbohydr. Res.*, 152 (1986) 137–141.
[6] A. Teleman, V. Harjunpää, M. Tenkanen, J. Buchert, T. Hausalo, T. Drakenberg, and T. Vuorinen, *Carbohydr. Res.*, 272 (1995) 55–71.
[7] K. Shimizu, *Carbohydr. Res.*, 92 (1981) 219–224.
[8] R.A. Hoffmann, B.R. Leeflang, M.M.J. de Barse, J.P. Kamerling, and J.F.G. Vliegenthart, *Carbohydr. Res.*, 221 (1991) 63–81.
[9] F. Fringuelli, S. Gronowitz, A.-B. Hörnfeldt, I. Johnson, and A. Taticchi, *Acta Chem. Scand., Ser. B*, 28 (1974) 175–184.
[10] S. Gronowitz, I. Johnson, and A.-B. Hörnfeldt, *Chem. Scr.*, 7 (1975) 211–222.
[11] J.A. Dean (Ed.), *Lange's Handbook of Chemistry*, 13th ed., McGraw-Hill, New York, 1985, pp 5–39.
[12] G. Gellerstedt, J. Pranda, and E-L. Lindfors, *J. Wood Chem. Technol.*, 14 (1994) 467–482.
[13] J. Jansson, *Faserforsch. Textiltech.*, 25 (1974) 375–382.
[14] M. Tenkanen, J. Puls, and K. Poutanen, *Enzyme Microb. Technol.*, 14 (1992) 566–574.
[15] K. Poutanen and J. Puls, *Appl. Microbiol. Biotechnol.*, 28 (1988) 425–432.

[16] M. Siika-aho, M. Tenkanen, J. Buchert, J. Puls, and L. Viikari, *Enzyme Microb. Technol.*, 16 (1994) 813–819.
[17] D. Fengel, M. Przyklenk, and G. Wegener, *Das Papier*, 30 (1976) 240–243.
[18] L. Braunschweiler, G. Bodenhausen, and R.R. Ernst, *Mol. Phys.*, 48 (1983) 535–560.
[19] G. Wagner, *J. Magn. Reson.*, 55 (1983) 151–156.
[20] L. Braunschweiler and R.R. Ernst, *J. Magn. Reson.*, 53 (1983) 521–528.
[21] A. Bax and D.G. Davis, *J. Magn. Reson.*, 65 (1985) 355–360.
[22] A. Bax and D.G. Davis, *J. Magn. Reson.*, 63 (1985) 207–213.
[23] M.F. Summers, L.G. Marzilli, and A. Bax, *J. Am. Chem. Soc.*, 108 (1986) 4285–4294.
[24] A. Bax and M.F. Summers, *J. Am. Chem. Soc.*, 108 (1986) 2093–2094.