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Carbohydrate Research 307 (1998) 107–111

Isolation, ^{1}H and ^{13}C NMR studies of (4-*O*-methyl-D-glucurono)-D-xylans from luffa fruit fibres, jute bast fibres and mucilage of quince tree seeds

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Received 26 September 1997; accepted 19 December 1997

Abstract

The (4-*O*-methyl-D-glucurono)-D-xylans isolated from luffa fruit fibres (*Luffa cylindrica*), jute bast fibres (*Corchorus capsularis*) and mucilage of the seeds of the quince tree (*Cydonia oblonga*) were studied by ^{1}H and ^{13}C NMR spectroscopy. Their NMR spectra were grossly similar, but the molar proportions of D-Xyl and 4-*O*-Me-D-GlcA varied and were found to be, respectively, 7.5:1, 5:1 and 2:1 for luffa fibres, jute bast fibres and quince tree seeds mucilage. The mucilage extracted from the seeds of the quince tree contained cellulose microfibrils strongly associated with a glucuronoxylan possessing a very high proportion of glucuronic acid residues. In addition to 4-*O*-methyl-α-D-glucopyranosyluronic residues the presence of α-D-glucopyranosyluronic residues was noticed, respectively, in the molar ratio 4-*O*-Me-D-GlcA and-D-GlcA 9:1. © 1998 Elsevier Science Ltd. All rights reserved

Keywords: Luffa xylan; Jute xylan; Quince xylan; Quince mucilage; Glucuronoxylan; ^{1}H and ^{13}C NMR spectroscopy

1. Introduction

During the last 20 years there have been numerous studies on plant cell wall polysaccharides, concerning their isolation and their characterisation, either by classical chemical methods or by the powerful modern techniques of ^{1}H and ^{13}C NMR spectroscopy. It is well known that hardwood xylans consist of a main chain possessing β1,4-linked xylose residues which are substituted every 8th to 20th unit at position O-2 by a 4-*O*-methyl-α-D-GlcA acid residue [1,2]. Jute xylan obtained from retted jute fibre bundles has already been studied and contained D-Xyl (89%) and 4-*O*-methyl GlcA (10%) glycosidically linked to the O-2 of Xyl [3,4]. Luffa constituents were reported to be cellulose, lignin, hemicellulose, and small amounts of mannan and galactan [5]. Alkali-soluble hemicellulose from luffa was identified as xylan by paper chromatography after it was hydrolyzed with sulfuric acid in the usual manner [6].

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PII S0008-6215(98)00002-0

Early studies by Smith and Montgomery [7] showed the mucilage of quince tree seeds to be a mixture of cellulose and water soluble polysaccharides. Lindberg *et al.* [8]. reported that the major water-soluble polysaccharide in the mucilage of the seeds of quince tree, is a partially-O-acetylated (4-*O*-methyl-D-glucurono)-D-xylan with an exceptionally high proportion of glycuronic acid residues.

In this paper we report on the extraction of glucuronoxylans from luffa cell-wall (*Luffa cylindrica*) and jute bast fibre (*Corchorus capsularis*), after removal of the pectic polysaccharides. We report also on the mucilage released during water extraction of quince tree seeds, consisting of a suspension of cellulose microfibrils solvated by a glucuronoxylan [9–12], and on their fractionation into acidic xylan and cellulose microfibrils. These three 4-*O*-methyl glucuronoxylans were purified and characterized principally by proton and ^{13}C NMR spectroscopy.

2. Experimental

General methods.—Uronic acid content was determined according to Blumenkrantz and Asboe-Hansen [13]. Neutral sugars were released by Saeman hydrolysis and analysed by GLC as their corresponding alditol acetates [14] using a Packard and Becker 417 instrument coupled to a Hewlett–Packard 3380 A integrator. Glass columns (3 mm×2 m) packed with 3% SP 2340 on Chromosorb W-AW DMCS (100–120 mesh), or 3% OV 17 on the same support, were used. The carboxyl groups of the 4-*O*-methyl-D-glucopyranosyluronic residue were reduced according to the method of Taylor and Conrad [15]; the carbodiimide reduction was performed twice.

Materials.—The luffa samples were supplied by Vela C^{ie} (Milano, Italy). The retted jute fibre bundles were supplied by the embassy of Bangladesh. The quince tree seeds were collected from local quince fruits.

*Isolation of (4-*O*-methyl-*D*-glucurono)-*D*-xylans.*—The luffa samples (30 g), after elimination of fats and waxes in a Soxhlet apparatus by refluxing 24 h with 19:31 toluene–EtOH, were extracted sequentially with boiling water (2×4 h), aq 0.5% ammonium oxalate (2×4 h at 100 °C), and aq 0.5% EDTA Na_2 (0.5%, 80 °C), in order to remove pectic polysaccharides. The luffa residue was then extracted twice at 80 °C with 0.5 M NaOH solution. After neutralisation with 0.1 M HCl solution, extensively dialysed against distilled water and freeze-dried, 2.4 g (8%) of luffa xylan was obtained. The retted jute fibre bundles (30 g) were fractionated according to the procedure described above for luffa samples, and 2.69 g (8.9%) of an alkali soluble jute xylan was obtained.

Extraction of the quince tree seeds mucilage. Quince tree seeds (200 g) were extracted with 2 L of cold water over 24 h. The mucilage solution was filtered through cheese cloth and the filtrate poured into three volumes of ethanol. The resulting precipitate was collected by centrifugation (3000 g, 30 min), resuspended in water, dialysed and then freeze-dried (2.5%).

Purification of the mucilage.—Two hundred mg of the crude mucilage was dispersed in water (150 mL), precipitated by a solution of 3% Cetavlon [16] (hexadecyltrimethylammoniumbromide, 100 mL), redissolved in 2 M sodium chloride (100 mL) and reprecipitated by ethanol (400 mL). This attempt to purify the acidic polymer gave 162 mg of polysaccharide. After hydrolysis, the polysaccharide precipitated with Cetavlon presented the same composition as the original mucilage. In order to remove the remaining cellulose, O-deacetylation of the glucuronoxylan (80 mg) was achieved with 10 ml of 0.1 M NaOH (overnight, N2, 4 °C). After neutralisation followed by dialysis against distilled water, the resulting polysaccharide was freeze-dried and fractionated by anionic exchange chromatography on a column (80×2 cm) of DEAE–Sepharose, equilibrated with 0.02 M acetate buffer (pH 4.5). The column was eluted with 300 ml of buffer and then with a gradient of buffer containing respectively, 0.125, 0.25, 0.5, and 1 M NaCl. The resulting fractions that contained the acidic polysaccharide were dialysed and freeze-dried, to give 42 mg of 4-*O*-methylglucuronoxylan.

NMR spectroscopy.—^1H NMR experiments were recorded on a Varian Unity plus 500 spectrometer operating at frequency of 500 MHz. Samples were studied as solutions in D_2O (5 mg in 0.75 mL of solvent) at 65 °C in 5 mm o.d. tubes (internal acetone ^1H (CH_3): 2.1 ppm relative to Me_4Si). ^1H spectra were recorded using 90° pulses, 3300 Hz spectral width, 12480 data points, 1.891 s acq. time and 32 scans were accumulated. 1D TOCSY experiments were recorded using a soft pulse sequence with an eburp1-256 shape, with selective power −5dB, pulse of 186 ms, arrayed selective

frequency, mixing time (10 to 80 ms), number of transients (64 to 288) and relaxation delay of 1 s.

^{13}C NMR experiments were obtained with an AC 300 Bruker spectrometer (operating frequency, 75.468 MHz). Samples were examined as solutions in D_2O (20 mg in 0.5 mL of solvent) at 65 °C in 5 mm o.d. tubes (internal acetone ^{13}C (CH_3): 31.5 ppm relative to Me_4Si).

3. Results and discussion

Luffa and jute xylans were composed of Ara, Xyl, Gal and Glc in the molar proportions 1:95:2:1 and 1:93:3:3, respectively. Uronic acid was estimated by colorimetric method in luffa and jute xylan to be 10 and 13%, respectively.

Crude quince seed mucilage was obtained by a simple extraction with water followed by precipitation with ethanol, giving a mucilage in a yield of about 2.5% of the dry weight of the seeds. An hydrolysate of the mucilage revealed that it was composed of Ara, Xyl, Gal and Glc in the proportions 8:54:4:34. Uronic acid content, determined by the colorimetric method, was estimated to be 20%. Carboxyl reduction followed by total hydrolysis and GC–MS analysis of the acetylated alditol derivatives, allowed the identification of the uronic acid as 4-*O*-methylglucuronic acid. The proportions of the neutral sugars were slightly modified in the carboxyl-reduced mucilage, where Ara, Xyl, Gal, 4-*O*-methyl-D-Glc and Glc were identified in the proportions 7:54:2:24:13. The effectivness of the reduction was confirmed by the measurement of residual uronic acid content, which was less than 0.5%.

The increase in Xyl suggested that this sugar was linked to 4-*O*-methyl GlcA as an aldobiuronic acid. This aldobiouronic acid was only partly hydrolyzed in the original mucilage. On the other hand, the decrease in glucose may be explained by the loss of cellulose during the carboxyl reduction step. Earlier studies [10,12] showed that the quince seed mucilage was composed of cellulose, Xyl, aldobiouronic acids and small amount of Ara. Lindberg et al. [8] showed that the major water-soluble polysaccharide in the mucilage of quince tree seeds is a partially O-acetylated (4-*O*-methyl-D-glucurono)-D-xylan with an exceptionally high proportion of glycuronic acid residues.

Examination of the ^1H and ^{13}C spectra of the crude quince mucilage shows the complexity of the structure exhibited by the numerous peaks present in the anomeric regions (between 4.4 and 5.5 ppm in proton and 98 and 107 ppm in carbon-13). The presence of a signal at δ 2.1 (^1H) or δ 22 ppm (^{13}C) unambiguously indicated the presence of O-acetyl groups, a structural feature often observed in hardwood xylans. The highly substituted xylan structure was confirmed by the multiplicity of the signals relative to the anomeric carbon of Xyl residues between 101 and 105 ppm. Carboxyl-reduced mucilage gave a slightly simplified spectrum due to the removal of O-acetyl groups during the reduction, and the consequent disappearance of the corresponding signals. Carboxyl reduction was accompanied by small changes in the chemical shifts, except that of the characteristic peak at 61.8 ppm assigned to C-6 carbon atoms of 4-*O*-methyl-α-D-Glc. No further information could be obtained on the constituents of the quince seed mucilage, without considering their separation and purification. For this reason we isolated and purified the 4-*O*-methyl-glucuronoxylan.

The significant percentage of uronic acid in the crude mucilage led us to attempt the selective isolation of the acidic polymer by Cetavlon, but this led to the isolation of a precipitate having the same composition as the original mucilage. It was concluded that very strong interactions existed between the cellulose microfibrils and the acidic glucuronoxylan, hence causing their co-precipitation. Therefore the acidic glucuronoxylan was isolated by DEAE–Sepharose chromatography. Carbohydrate analysis showed a great enrichment in Xyl residues, as with a composition Ara, Xyl and Glc 2:92:6. Uronic acid content, determined by the colorimetric method, was estimated to be 24%.

NMR studies were performed on the glucuronoxylans from the three different substrates. The complete assignment of the proton spectra was achieved by doing 2 D COSY and 1 D TOCSY experiments; the proton data are reported in Table 1. Examination of the proton spectrum of luffa or jute xylan (Fig. 1) shows the relative simplicity of the structure exhibited by: (i) major signals at δ 4.34 (H-1), 3.97 (H-5_{eq}), 3.65 (H-4), 3.43 (H-3), 3.26 (H-5_{ax}) and 3.17 ppm (H-2), corresponding to non-substituted β-D-Xyl residues; (ii) minor signals at δ 5.12 (H-1), 4.15 (H-5), 3.62 (H-3), 3.45 (H-2), 3.33 (OCH_3), 3.12 ppm (H-4), corresponding to 4-*O*-methyl-α-D-GlcA acid residues, and at δ 4.49 (H-1), 4.01 (H-5_{eq}), 3.67 (H-4), 3.51

Table 1
Chemical shift data (D_2O, 338 K) for glycosyl residues of luffa, jute, and quince (4-*O*-methyl-D-glucurono)-D-xylans

Glycosyl residues		Assignment				
		1	2	3	4	5
Luffa xylan						
(1→4)-β-D-Xyl*p*	^{1}H	4.35	3.17	3.44	3.66	3.97;3.26
	^{13}C	102.87	73.95	74.98	77.67	64.25
(1→4)-β-D-Xyl*p*-2-*O*-Glc*p*A	^{1}H	4.44	3.33	3.5	3.67	4.01;3.27
	^{13}C	102.43	77.97	73.46	77.38	64.05
4-OMe-α-D-Glc*p*A	^{1}H	5.15	3.45	3.61	3.10	4.15
	^{13}C	98.79	72.63	78.07	83.51	73.61 (60.82,OCH_3) (177.40,C-6)
Jute xylan						
(1→4)-β-D-Xyl*p*	^{1}H	4.34	3.17	3.43	3.65	3.97;3.26
	^{13}C	102.87	73.95	74.98	77.68	64.25
(1→)-β-D-Xyl*p*-2-*O*-Glc*p*A	^{1}H	4.49	3.33	3.51	3.67	4.01;3.30
	^{13}C	102.43	77.92	73.46	77.39	64.05
4-OMe-α-D-Glc*p*A	^{1}H	5.12	3.45	3.62	3.12	4.15
	^{13}H	98.80	72.63	78.07	83.51	73.66 (60.77,OCH_3) (177.40,C-6)
Quince xylan						
(1→4)-β-D-Xyl*p*	^{1}H	4.34	3.18	3.45	3.66	3.98;3.28
	^{13}C	103.02	73.95	75.03	77.78	64.05
(1→4)-β-D-Xyl*p*-2-*O*-Glc*p*A	^{1}H	4.47	3.31	3.49	3.64	3.99;3.25
	^{13}C	102.48	78.02	73.42	77.43	63.76
4-OMe-α-D-Glc*p*A	^{1}H	5.09	3.42	3.62	3.13	4.15
	^{13}C	98.90	72.58	78.07	83.37	73.71 (60.77,OCH_3) (177.38,C-6)

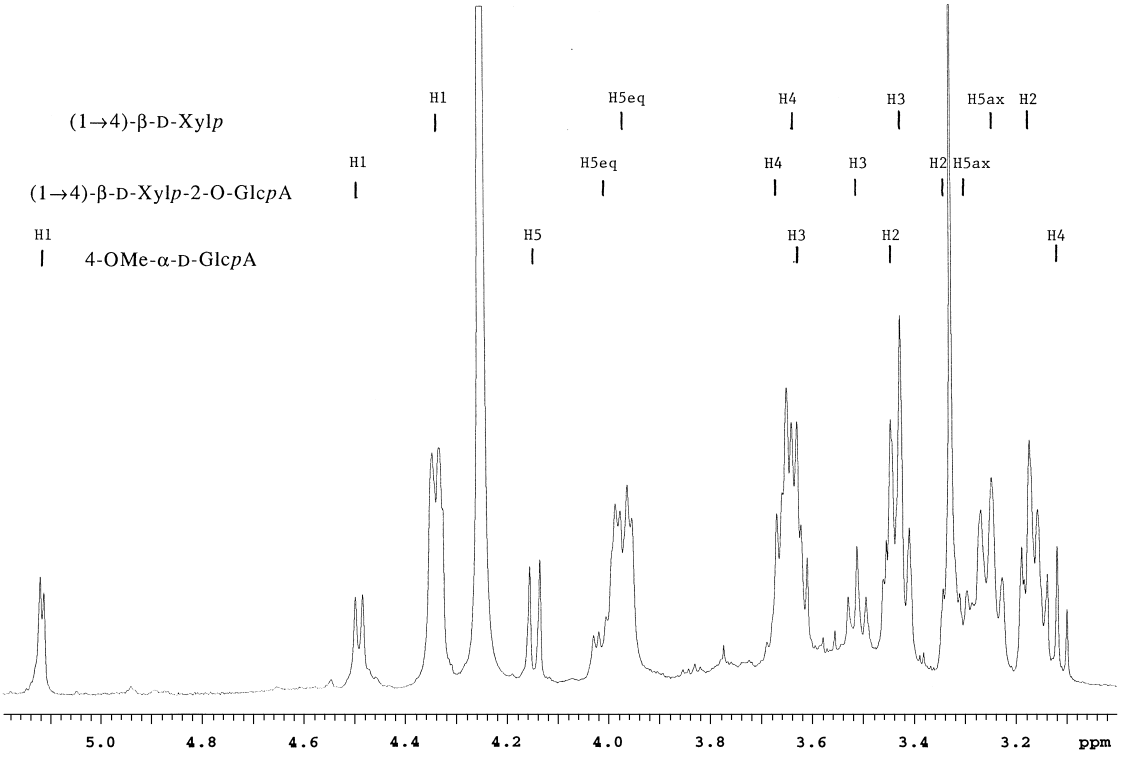

Fig. 1. 500 MHz 1D ^{1}H spectrum of jute (4-*O*-methyl-D-glucurono)-D-xylan in D_2O at 338 K.

(H-3), 3.33 (H-2) and 3.30 ppm (H-5_{ax}) assigned to β-D-Xyl units substituted with 4-*O*-methyl-α-D-GlcA.

By using $^{13}C/^{1}H$ shift correlated 2D experiments, the ^{13}C NMR spectra were completely assigned from the proton spectra, and the corresponding chemical shifts are reported in Table 1. The ^{13}C spectra of luffa and jute xylans contained, inter alia, signals for anomeric carbons at δ 98.80, 102.43 and 102.87 ppm, assigned to C-1 of 4-*O*-methyl-α-D-GlcA residues, β-D-Xyl units substituted with such residues, and β-D-Xyl residues, respectively. The signal of the methoxylated C-4 appeared at 83.51 ppm. The carbon signal for the methoxyl group was at 60.80 ppm. The NMR data were in agreement with those obtained either on acidic oligo- [17,18] or polysaccharides [8,19–23] related to 4-*O*-methyl glucuronoxylans.

The ^{1}H and ^{13}C NMR spectra of the purified quince xylan appeared much less complex than those of the crude quince mucilage, due to removal of acetyl groups and neutral polysaccharides. Nethertheless, they were more complex than the spectra of luffa and jute xylans, due to the presence of extra signals at δ 5.24, 4.40, 3.75–3.85 and 3.51 ppm in the proton spectrum corresponding to glucuronic acid residues.

The main differences in the spectra of the three glucuronoxylans are from the relative amounts of 4-*O*-methyl-α-D-GlcA residues. The molar proportions of D-Xyl and 4-*O*-methyl-α-D-GlcA were determined by integration of corresponding anomeric protons, and were found respectively to 7.5:1 for luffa, 5:1 for jute and 2:1 for quince tree seeds xylans. Bazus et al. [22] found a ratio of 8–9:1 on purified fractions extracted from sunflower hulls. The data obtained by proton NMR for quince tree seeds xylan, are in good agrement with the sugar composition obtained by G.C–M.S analysis of the carboxyl reduced mucilage, where a molar proportion of D-Xyl and 4-*O*-methyl-α-D-GlcA was found, respectively, to 2.2:1. Concerning the jute xylan we found by NMR an amount of 4-*O*-methyl-α-D-GlcA twice the quantities obtained by classical sugar analysis [3,4]; these discrepancies can be easily explained by the fact that aldouronic acids are difficult to hydrolyze. In addition to 4-*O*-methyl-α-D-glucopyranosyluronic residues the presence of α-D-glucopyranosyluronic residues was noticed, respectively, in the molar ratio 4-*O*-Me-D-GlcA and-D-GlcA 9:1.

Acknowledgements

We thank Mrs A. Lefèvre and M.-F. Marais for sugar analysis, Dr H. Chanzy and Professor J.-P. Joseleau for useful discussions.

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