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Structural features of the pectic polysaccharides isolated from retted hemp bast fibres

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Abstract

Pectic polysaccharides were solubilized from retted hemp bast fibre bundles, by sequential extraction with water and ammonium oxalate at 100 °C. The polysaccharides isolated from the extracts were de-esterified and fractionated by anion exchange chromatography, yielding five fractions from boiling water extract and five fractions from oxalate extract. Five of these fractions were characterized by chemicals methods, ^{1}H and ^{13}C NMR, as polysaccharides containing galacturonic acid, rhamnose and galactose units in variable ratios. Their chemical structure comprises a disaccharide repeating unit $\rightarrow$ 2)-α-L-Rha*p*-(1 $\rightarrow$ 4)-α-Gal*p*A-(1 $\rightarrow$ backbone, with short side chains attached to the rhamnosyl residues. The β-D-Galactose residues are attached to O-4 of the rhamnosyl residues, but the amount of L-rhamnosyl residues that are 2,4-linked can vary from 36 to 75%, from one fraction to another. A fraction corresponding to a mixture of oligosaccharides with an average dp of 13, with ten galacturonic acid, two rhamnose and one galactose units was also isolated. © 1996 Elsevier Science Ltd.

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1. Introduction

Fibrous bast plants such as flax, hemp, jute, etc, have been extensively used by mankind, especially when the navies of the world were powered by sail. With the prevalence of motorized boats and the appearance of more competitive synthetic fibres,

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their production decreased drastically. Flax fibres are still utilized in the textile industry and hemp fibres can be used in the pulp and paper industry to strengthen thin paper sheet (bank notes, religious books, cigarettes, etc). The flax and hemp bast fibres are embedded in pectic polysaccharides and arranged in bundles; a retting process is required to degrade the pectins and allow to separate the bast fibres from the rest of the plant. The ultrastructure and morphology of hemp stems have already been published [1–5], but the chemical composition of hemp cell-wall polysaccharides is not well established yet. Primary cell walls are characterized by a high content of pectic polysaccharides [6]. Pectins are homo- or hetero-polysaccharides composed mainly of galacturonic acid, rhamnose, galactose and arabinose [7].

In this paper, we report on the cell-wall polysaccharides isolated from retted hemp bast fibre. The pectic fractions extracted mainly from the middle lamellae of hemp bast fibres were characterized principally by ^{1}H and ^{13}C NMR spectroscopy. We found a rhamnose/galacturonic acid ratio particularly high for most of these polysaccharide fractions, which were very similar to the so-called 'hairy' regions isolated from primary cell walls pectic polysaccharides, but with very short neutral sugar side chain.

2. Experimental

General methods.—The uronic acid content was determined according to Blumenkrantz and Asboe-Hansen [8]. The neutral sugars were identified and quantified by GLC of the corresponding alditol acetates [9], 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 D-galactosyluronic acid of BWP3 and OXP2 polysaccharides (H^+ form), were reduced according to the method of Taylor and Conrad [10]; the carbodiimide reduction was performed twice.

Materials.—The retted hemp fibre bundles were supplied by "La Chanvrière de l'Aube", Bar sur Aube (France).

Isolation of pectic polysaccharides.—The retted hemp fibre bundles (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) and aq 0.5% ammonium oxalate (2 × 4 h at 100 °C), resulting in a polymer soluble in boiling water (BWP*, 1.4 g) and a polymer soluble in aq ammonium oxalate (OXP*, 2 g). The two fractions BWP* and OXP* were, respectively, saponified with 0.1 M NaOH (overnight, N_2, 4 °C), in order to hydrolyze the acetyl and methyl esters. The solutions were then acidified to pH 4–5 by addition of 0.1 M HCl solution and extensively dialyzed against distilled water and freeze-dried to yield deacetylated BWP and OXP samples in their H^+ form.

Fractionation of BWP and OXP.—The BWP sample (660 mg), was suspended in 100 mL of 0.05 M phosphate buffer (pH = 6.3) and the solution was passed through a column (2 × 15 cm) of DEAE-Trisacryl M (phosphate form) eluted at 40 mL/h flow rate. The fractions were eluted sequentially with 300 mL of buffer and successively by

300 mL of buffer containing, respectively, 0.125, 0.25, 0.5, and 1 M NaCl. The fractions were then desalted by ultrafiltration with a membrane having a molecular weight cut-off of 500 and freeze-dried. The amounts of sample recovered in each fraction were: buffer, 65.6 mg (9.9%, BWP1); 0.125 M NaCl, 60 mg (9%, BWP2); 0.25 M NaCl, 175.1 mg (26.5%, BWP3); 0.5 M NaCl, 88.8 mg (13.5%, BWP4); 1 M NaCl, 87.6 mg (13.3%, BWP5). Further elution with 0.5 M NaOH removed a significant amount of additional material, (110 mg, 16.6%). BWP1 which was not retained on the column, corresponded to a neutral polysaccharide constituted mainly of xylose residues, whereas BWP2, BWP3, BWP4, and BWP5 were acidic polysaccharides.

The OXP sample, in its H^+ form (1.170 g), was fractionated according to the procedure described for BWP. The different fractions were then desalted by ultrafiltration and freeze-dried. The amount of sample recovered in each fraction were: buffer, 69.7 mg (6%, OXP1); 0.125 M NaCl, 141 mg (12.1%, OXP2); 0.25 M NaCl, 421 mg (36%, OXP3); 0.5 M NaCl, 94.2 mg (8.1%, OXP4); 1 M NaCl, 39 mg (3.3%, OXP5). Further elution with 0.5 M NaOH removed significant amounts of additional material (0.37 g, 31%).

Methylation analysis.—The carboxyl-reduced BWP3 polysaccharide was methylated twice by the Hakomori procedure, as described by Jansson et al. [11]. The partially methylated carbohydrates were then converted into their alditol acetates by successive treatments with $NaBH_4$ and pyridine–Ac_2O and analyzed on a fused-silica widebore column (30 m × 0.53 mm) half-bonded with SP-2380. The conditions were as follows: 165 °C for 4 min, then 2.5 °C/min to 225 °C; injector temperature 260 °C; detector temperature 280 °C; nitrogen as carrier gas. Peak identification was based on retention times using partially methylated alditol acetate standards and confirmed by GLC by using a SP 2380 capillary column (0.32 mm) coupled to a Nermag R1010C mass spectrometer. The conditions were: 165 °C for 3 min, then 2 °C/min to 220 °C; injector temperature 250 °C; helium as carrier gas. Mass spectrometer conditions: electron impact; ionizing voltage 70 eV; ion current 0.150 mA; interface and source temperature 200 °C. Peak areas were corrected by using the molar response factors according to Sweet et al. [12].

NMR spectroscopy.—^1H Experiments were recorded on a Varian Unity Plus 500 spectrometer equipped with an ultrashim system (operating frequency: 499.836 MHz). Samples were studied as solutions in D_2O (5 mg in 0.75 mL of solvent) at 70 °C in 5 mm o.d. tubes without spinning (internal acetone ^1H (CH_3): 2.1 ppm relative to Me_4Si). ^1H spectra were recorded using 90° pulses, 3300 Hz spectral width, 12,480 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 −5 dB, pulse of 186 μs, arrayed selective frequency, mixing time (10 to 80 ms), number of transients (64–288), and relaxation delay of 1 s.

^{13}C Experiments were obtained with an AC 300 Bruker spectrometer (operating frequency: 75.468 MHz). Samples were examined as solutions in D_2O (30 mg in 0.35 mL of solvent) at 60 °C in 5 mm o.d. tubes (internal acetone ^{13}C (CH_3): 31.5 ppm relative to Me_4Si). Quantitative ^{13}C spectra were recorded using the Invgate Bruker sequence, with 90° pulse length (6.5 μs), 15,000 Hz spectral width, 8 K data points, 0.54 s acquisition time, relaxation delay of 1.2 s, and 100,000 scans.

3. Results and discussion

Chemical studies.—The hemp fibre bundles were first extracted with boiling water (BWP*), and then with ammonium oxalate (OXP*), which acts as a chelating agent of the 'bridging' Ca^{2+}. The ^{1}H NMR spectra showed that BWP* and OXP* were partially esterified. The amount of acetyl groups in BWP* was 5% and 2% in OXP*. The amount of methyl esters in BWP* and OXP* is less than 1%. The methyl and acetyl ester groups of BWP* and OXP* polysaccharides were saponified to yield BWP and OXP. The de-esterified BWP and OXP samples were fractionated by anion-exchange chromatography, giving 5 fractions. After removal of the salts, 72.2% of BWP fractions, and 65.5% of OXP fractions were recovered, the major fractions being BWP3 (26.5%) and OXP3 (36%).

The sugar composition of Fractions BWP2, BWP3, BWP4, BWP5, OXP2, and OXP3 are reported in Table 1. BWP2, BWP3, and BWP4 Fractions contained, respectively, 16.5, 24 and 25% of galacturonic acid. OXP2 and OXP3 contained respectively 22.5 and 63% of galacturonic acid. As expected during the DEAE-Trisacryl M fractionation, the galacturonic acid/neutral sugar ratio increased with the ionic strength. The neutral sugar data reported in Table 1 showed that BWP2, BWP3, BWP4, and OXP2 were very similar and were composed mainly of rhamnose, arabinose and galactose, in ratios 1:1:2 for BWP2, 2:1:3 for BWP3, 4:2:3 for BWP4, and 3:1:4 for OXP2. These data suggested that BWP2, BWP3, BWP4, BWP5, and OXP2 samples resembled the so-called 'hairy regions' as described by De Vries et al. [13]. Furthermore, the poor yields of total neutral sugars, due to incomplete hydrolysis of the GalA → Rha linkage, which induced an under-estimation of the rhamnose and galactose content, prompted us to undertake a general proton and ^{13}C NMR study in order to determine the sugar composition of all the pectin fractions.

All the carboxyl groups of the D-galacturonic acid residues of Fraction BWP3 were reduced ($NaBH_4$) after two carbodiimide treatments, as indicated on the ^{13}C NMR spectrum, by the disappearance of signals at 176.4 ppm (COOH) and 100.2 ppm (C-1)

Table 1
Sugar composition [a] of BWP2, BWP3, BWP4, OXP2, and OXP3

	BWP2	BWP3	BWP4	OXP2	OXP3
Galacturonic acid [b]	16.5	24	25	22.5	63
Total neutral sugars [c]	34	20	15.6	28	10.3
Total sugars	50.5	44	40.6	50.5	70.3
Glucose	1.5	2	1.2	1.5	1.3
Galactose	17	8.5	4.5	12	2.3
Rhamnose	8	6	6.3	10.4	3.6
Mannose	0.5	–	0.5	0.5	0.3
Xylose	–	0.5	0.3	0.4	1
Arabinose	7	3	2.8	3.2	1.8

[a] As % of dry matter.
[b] Acid content calculated by a colorimetric method according to Blumenkrantz and Asboe-Hansen [8].
[c] Neutral sugars determined by GLC of the alditol acetates.

Table 2
Partially methylated alditol acetates from BWP3 and OXP2

Methylated alditol acetates	$NaBH_4$ carboxyl-reduced BWP3 [a]	$NaBD_4$ carboxyl-reduced BWP3 [a]	$NaBD_4$ carboxyl-reduced OXP2 [a]
2,3,4,6-Me_4-Gal [b]	18	20	25
2,3,6-Me_3-Gal-6,6-d_2	–	35	36
2,3,6-Me_3-Gal	40	–	–
3-Me-Rha	18	21	23.5
3,4-Me_2-Rha	23.5	24	15.5

[a] Relative mole ratio.
[b] 2,3,4,6-Me_4-Gal = 1-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-galactitol, etc.

and the emergence of new signals at 101.1 and 63.2 ppm, corresponding respectively to C-1 and C-6 of α-galactose residues. The results of methylation analysis of Fraction BWP3 after carboxyl reduction are given in Table 2. These data showed that the rhamnose residues were 1 → 2 linked and partially branched at O-4, and that the proportion of 2 and 2,4-linked L-rhamnosyl residues was not exactly equal, being 56.5% and 43.5, respectively.

In another experiment, the carboxyl groups of BWP3 were reduced with $NaBD_4$ into the corresponding 6,6′-dideutero-D-galactosyl residues. The methylation analysis data are reported in Table 2. The galacturonic acid residues were converted into deuterium-labelled D-galactosyl residues, in order to differentiate the galactose arising from the reduction of the galacturonic acid residues and the galactosyl residues already in the side chain. The data showed that 2,3,6-tri-*O*-methyl galactitol from the $NaBH_4$ carboxyl reduction corresponded exclusively to the 1 → 4 linked galacturonic acid residues in the main chain. The proportion of 2,3,4,6-tetra-*O*-methyl galactitol was found to be equal to the proportion of 2,4-linked L-rhamnosyl residue (3-*O*-methyl rhamnitol), which shows that there is only one galactose unit as side chain. The amount of 4-linked D-galacturonic acid was approximately equal to the sum of the 3-*O*-methyl rhamnitol and 3,4-di-*O*-methyl rhamnitol, indicating that BWP3 consisted of a rhamnogalacturonan backbone with rhamnose units linked either in O-2 (53.4%) or both in O-2 and O-4 (46.6%), and with the same proportion of galacturonic acid and rhamnose units.

The carboxyl groups of the D-galacturonic acid residues of OXP2 were reduced with $NaBD_4$. The corresponding methylation analysis results are reported in Table 2. The amount of 2,3,6-tri-*O*-methyl galactitol 6,6′-d_2 was approximately equal to the sum of the 3-*O*-methyl rhamnitol and 3,4-di-*O*-methyl rhamnitol, indicating that there is one galacturonic acid per rhamnose residue. Regarding the rhamnosyl residues, 40% were linked only in O-2 and 60% were linked both in O-2 and O-4. The proportion of 2,3,4,6-tetra-*O*-methyl galactitol was found to be equal to the proportion of 2,4-linked L-rhamnosyl residue (3-*O*-methyl rhamnitol), which shows that there is only one galactose unit as side chain.

NMR studies.—Studies were focused only on BWP2, BWP3, BWP4, OXP2, and OXP3 fractions. These fractions contained mainly galacturonic acid, rhamnose, galactose, and arabinose moieties in variable amounts. The NMR spectra showed that all the

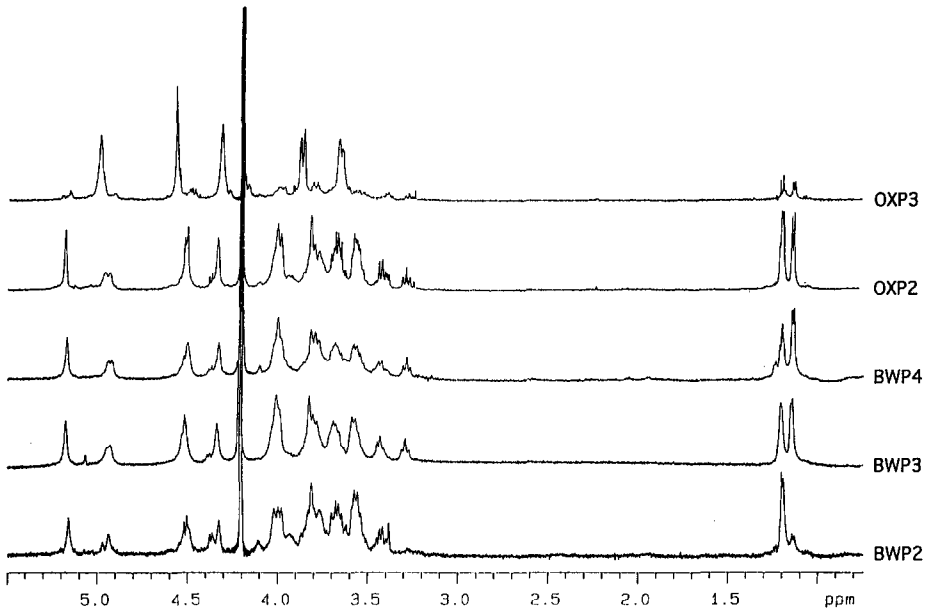

Fig. 1. Comparative 500 MHz 1D ^{1}H spectra of BWP2, BWP3, BWP4, OXP2, and OXP3 samples.

sugar residues were pyranoid, excepted for arabinose which was furanoid. The absolute configurations were assumed to be the same as those invariably found in plant cell wall polysaccharides [14], i.e., D-GalA, L-Rha, D-Gal, and L-Ara.

Both ^{1}H and ^{13}C NMR spectra were recorded for the five different fractions and are reported in Figs. 1 and 2. The more acidic fraction OXP3, which is easier to analyze because the major signals in the ^{1}H and ^{13}C NMR spectra correspond to data for α-(1 → 4) homogalacturonan already reported in the literature [15], was first characterized. In addition to the main signals, minor signals corresponding to rhamnopyranosyl and galactopyranosyl residues were also seen.

The ^{1}H and ^{13}C NMR spectra in Figs. 1 and 2, corresponding to BWP2, BWP3, BWP4 and OXP2, showed the same general features as already observed in the case of OXP3, but the signals corresponding to two rhamnosyl and one galactosyl residues were found in much larger amounts. The signals in the region 4.4–5.2 ppm corresponded to anomeric protons; the signals in the range 1.12–1.3 ppm were easily assigned to the H-6,6′,6″ of the rhamnose units. These methyl rhamnoses appeared generally as two doublets due to the presence of two different rhamnose residues. The two pairs of doublets, centred at $\delta = 1.13$ ppm and $\delta = 1.20$ ppm, were respectively assigned to the rhamnosyl residues linked only at O-2 and to the rhamnosyl residues linked both at O-2 and O-4, in agreement with Colquhoun et al. [16]. Due to partial overlapping of the proton resonances, the ^{1}H spectra were analyzed by TOCSY 1D experiments [17,18], resulting in the assignment of most of the protons.

TOCSY experiments, performed on the OXP2 fraction (Fig. 3), showed a selective

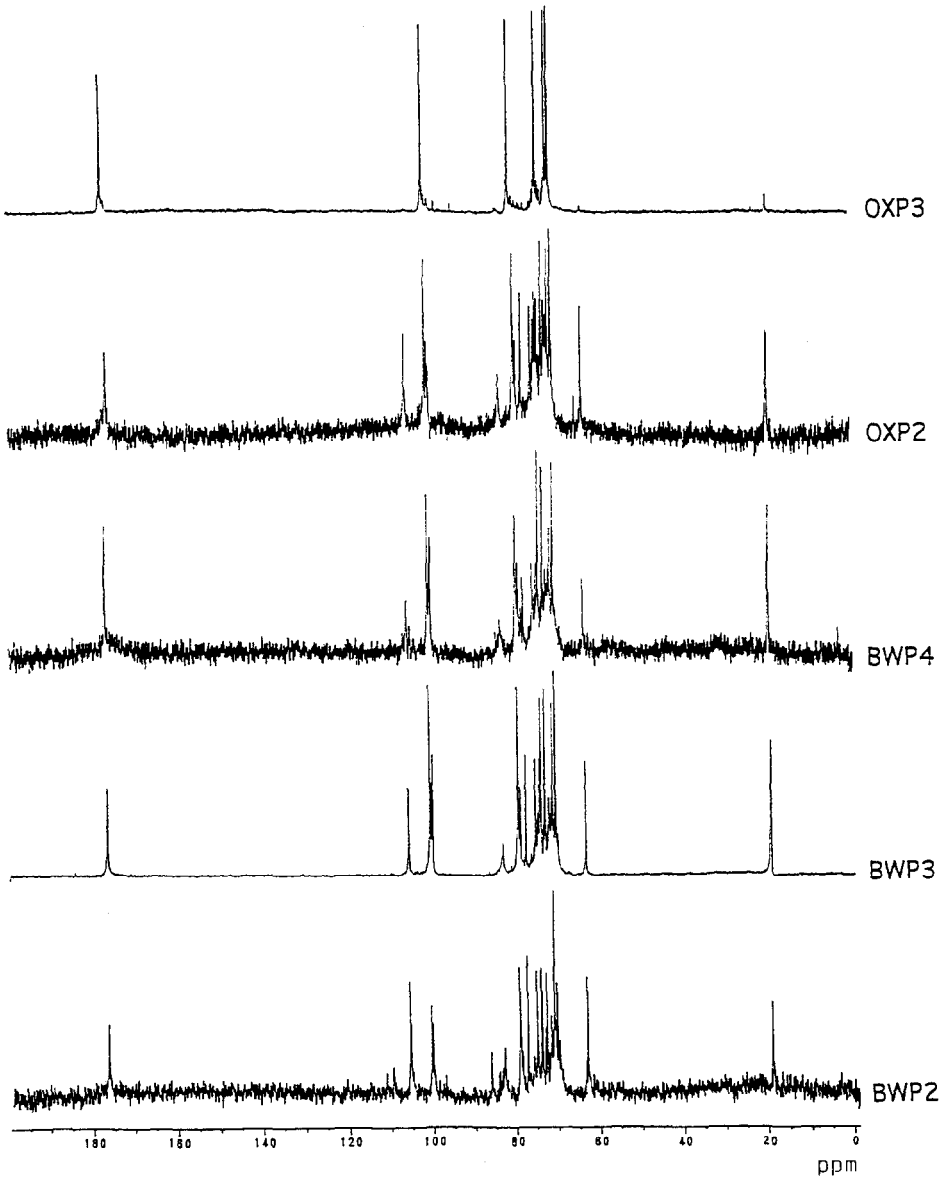

Fig. 2. Comparative 75.468 MHz 1D quantitative ^{13}C spectra of BWP2, BWP3, BWP4, OXP2, and OXP3 samples.

saturation of the methyl protons at higher field ((H-6,6′,6″), doublet at $\delta = 1.13$ ppm) which has been assigned to the rhamnosyl residues linked only at O-2. A contact time of 10 ms (experiment **b**), gave a specific response at H-5. A contact time of 20 ms (experiment **c**), induced responses at H-5 and H-4. Contact times of 30 and 40 ms

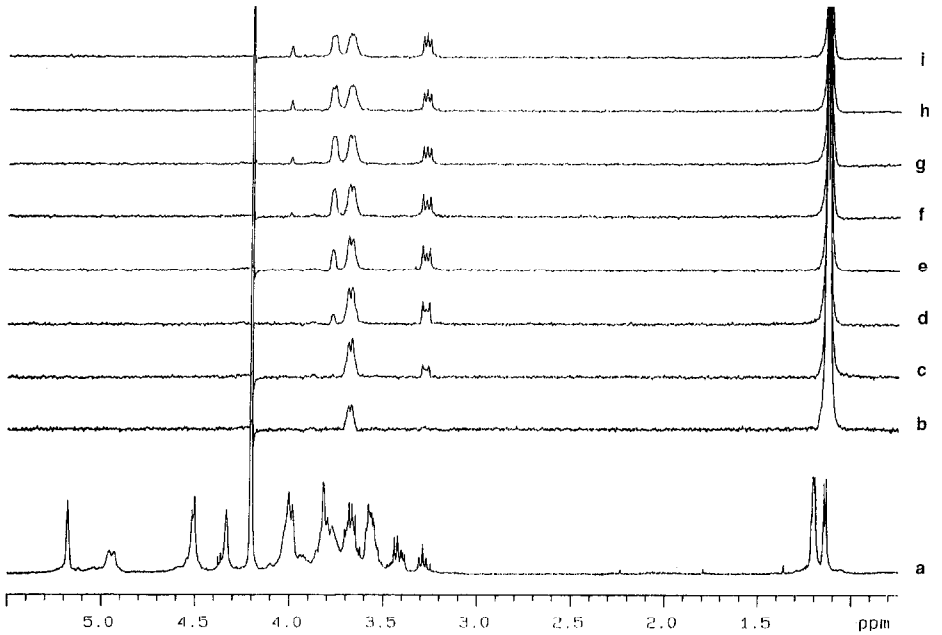

Fig. 3. 500 MHz 1D TOCSY proton spectrum of OXP2, with selective inversion of methyl protons of the linear rhamnosyl residues linked only in O-2; (a) 1-D spectrum; (b) 10 ms contact time; (c) 20 ms contact time; (d) 30 ms contact time; (e) 40 ms contact time; (f) 50 ms contact time; (g) 60 ms contact time; (h) 70 ms contact time; (i) 80 ms contact time.

(experiments **d** and **e**) gave responses at H-5, H-4, and H-3 and contact times of 50, 60 and 70 ms (experiments **f**, **g**, and **h**), showed responses at H-5, H-4, H-3, and H-2. Finally, a contact time of 80 ms (experiment **i**), gave responses at H-5, H-4, H-3, H-2, and H-1 (rhamnosyl residues linked only in O-2).

The methyl protons at lower field (H-6,6′,6″, doublet at δ 1.20 ppm), assigned to the rhamnosyl residues linked both at O-2 and O-4, gave by selective saturation, successive responses at H-5, H-4, H-3, and H-2. It should be noted that we could not obtain a response at H-1.

Selective saturation of H-2 of galactose residue at δ 3.42 ppm, with increasing contact time, gave successive responses at H-3 and H-1, H-3, H-1, and H-4, H-3, H-1, H-4, and H-5. Finally, we were able to assign the overall proton signals of the main sugars contained in sample OXP2 (galacturonic acid, O-2 linked rhamnopyranosyl, O-2 and O-4 linked rhamnopyranosyl, and galactopyranosyl residues). The chemical shifts of the corresponding protons are reported in Table 3.

The same TOCSY NMR experiments were performed on BWP2, BWP3, and BWP4 samples and assignments could be made for most of the proton signals corresponding to the main sugars contained in these pectic polysaccharides.

From the data reported in Table 3, each sugar residue was identified by at least one characteristic signal. (i) The signals at δ 4.98, 4.96 or 4.94 ppm are characteristics of

H-1 α of Gal*p*A, respectively in α-D-(1 → 4)-homogalacturonan, in → 4)-α-D-Gal*p*A-(1 → 2)-α-L-Rha*p*-(1 → or in

→ 4)-α-D-Gal*p*A-(1 → 2)-α-L-Rha*p*-(1 →
4
↑
1
β-D-Gal*p*

(ii) The signals at δ 4.51 and 3.42 ppm are characteristics, respectively of H-1 and H-2 of β-(1 → 4)-galactose residues. (iii) The signals at δ 1.13 and 3.38 ppm are characteristic, respectively of methyl H-6,6′,6″ and H-4 protons of the O-2 linked rhamnosyl residues. (iv) The signal at δ 1.2 ppm is characteristic of methyl H-6,6′,6″ protons of the rhamnosyl residues linked both at O-2 and O-4. The anomeric signals of H-1 α

Table 3
Chemical shift data (D_2O) for glycosyl residues of the acidic fractions (BWP2, BWP3, BWP4, OXP2, and OXP3)

Glycosyl residues		Assignment					
		1	2	3	4	5	6
α-Galacturonosyl residues							
→ 4)-α-D-Gal*p*-A(1 →	^{13}C	100.95	70.35	71.00	80.10	73.35	176.95
	^{1}H	4.98	3.65	3.86	4.30	4.56	–
→ 4)-α-D-Gal*p*A-(1 → 2)-α-L-Rha*p*-(1 →	^{13}C	100.20	69.90	70.60	78.90	72.30	176.40
	^{1}H	4.96	3.80	3.98	4.32	4.50	–
→ 4)-α-D-Gal*p*A-(1 → 2)-α-L-Rha*p*-(1 → 4 ↑ 1 β-D-Gal*p*	^{13}C	100.20	69.90	70.60	78.90	72.30	176.40
	^{1}H	4.94	3.80	3.98	4.32	4.50	–
α-Rhamnosyl residues							
→ 4)-α-Gal*p*-A(1 → 2)-α-L-Rha*p*-(1 →	^{13}C	99.90	77.45	70.75	73.35	70.50	18.50
	^{1}H	5.17	3.99	3.77	3.38	3.68	1.13
→ 4)-α-D-Gal*p*A-(1 → 2)-α-L-Rha*p*-(1 → 4 ↑ 1 β-D-Gal*p*	^{13}C	99.45	n.a. [a]	n.a.	n.a.	n.a.	18.70
	^{1}H	5.17	4.00	3.54	3.98	3.75	1.20
β-Galactosyl residues							
→ 4)-α-D-Gal*p*A-(1 → 2)-α-L-Rha*p*-(1 → 4 ↑ 1 β-D-Gal*p*	^{13}C	105.20	73.25	74.40	70.20	76.85	62.80
	^{1}H	4.51	3.42	3.56	3.81	3.58	3.64

[a] n.a.: Not assigned.

Table 4
Sugar composition of BWP2, BWP3, BWP4, OXP2, and OXP3 as calculated by proton NMR [a] spectroscopy

	BWP2	BWP3	BWP4	OXP2	OXP3
Gal*p*A	1	1	1	1	1
Rha*p* (2-linked)	0.3	0.6	0.7	0.45	0.1
Rha*p* (2,4-linked)	0.9	0.5	0.4	0.6	0.1
Gal*p*	1.15	0.55	0.45	0.65	0.1
Ara*f*	0.3	–	–	–	–

[a] Anomeric protons and methyl of rhamnose residues were selected and the corresponding peak areas were measured.

resonances of rhamnose residues are not affected by the O-4 linkage and resonate at δ 5.17 ppm.

The surface area of the proton signals characteristics of each individual sugar unit for BWP2, BWP3, BWP4, OXP2, and OXP3 was measured, allowing us to deduce the corresponding sugar composition reported in Table 4.

All the ^{13}C NMR spectra of BWP2, BWP3, BWP4, and OXP2 pectic polysaccharides (Fig. 2) exhibited one signal in the region 176–177 ppm (C-6 of the GalA units), four signals in the region 99–106 ppm corresponding to anomeric carbons (Gal, GalA, Rha units), one signal around 63 ppm (assigned to C-6 of Gal not O-6-linked), and mainly two peaks in the region 18–19 ppm (C-6 of the Rha units). Their relative intensities, however, varied, in particular the peaks at 99.9 and 99.45 (C-1) and at 18.5 and 18.7 ppm (C-6) which corresponded to the rhamnosyl residues differently linked (O-2 or O-2 and O-4). Some resonances of specific sugar units were also assigned. The anomeric signal at δ 99.9 ppm and the methyl signal at 18.5 ppm are characteristic of O-2 linked rhamnose residues, whereas the anomeric signal at δ 99.45 ppm and the methyl signal at 18.7 ppm are characteristic of the rhamnose residues linked both at O-2 and O-4. The resonances at δ 100.2 and 176.4 ppm are characteristic of C-1 and C-6 of galacturonic acid residues from hairy regions. A downfield shift is observed in the C-1 chemical shifts (100.95 ppm) of galacturonic acid residues from homogalacturonan blocks (Table 3). The signals at δ 105.2 and 62.8 ppm correspond to C-1 and C-6 of galactose units.

In the ^{13}C spectrum of BWP2, the presence of minor signals at 110.98, 109.35 and 85.8 ppm can be seen. These are ascribed to C-1 and C-4 of terminal or O-5 linked arabinofuranosyl residues [19].

Our data (Table 4) on the sugar composition, as calculated by proton NMR spectroscopy, are in good agreement with the carboxyl reduced methylation analysis of BWP3 and OXP2 given in Table 2, showing that ^{1}H NMR spectroscopy can give valuable information on the glycosyl and glycosyl-linkage composition of pectic polysaccharides.

BWP2, BWP3, BWP4, and OXP2 presented a rhamnose/galacturonic acid ratio close to 1, which indicated that their chemical structures were based on a disaccharide repeating unit backbone → 2)-α-L-Rha*p*-(1 → 4)-α-Gal*p*A-(1 → backbone, but with different degrees of branching. Indeed, BWP2 corresponded to a highly branched rhamno-

galacturonan backbone, as 75% of the L-rhamnosyl residues were linked both in O-2 and O-4. BWP3, BWP4, and OXP2 were less ramified, as they contained, respectively, 45, 36 and 57% of L-rhamnosyl residues linked both in O-2 and O-4.

The ammonium oxalate extracts were less acetylated (2%) than the hot water extracts (5%), and contained a higher ratio of galacturonic acid to rhamnose, in particular Fraction OXP3, which corresponded to a mixture of acidic oligosaccharides, with an average dp of 13 determined by quantitative ^{13}C NMR experiments (ten galacturonic acid, two rhamnose, and one galactose units).

Our results suggest that during the retting process, a wide spectrum of enzymes is secreted by microorganisms. We found that the side chains of BWP2, BWP3, BWP4, and OXP2 were very short, as they generally contained only one galactose unit. The presence of terminal galactose units, linked to O-4 of the rhamnosyl residues, indicated an endo-galactanase activity which removes the galactan and arabinogalactan side chains. The lack of arabinose could be explained, either by the preceding endo-galactanase, or by endo-arabinanase and arabinofuranosidase activities, which removes the arabinose of the side chain. Endo-1,4-α-polygalacturonase and pectin and pectate lyases are also produced, which cleave the α-(1 $\rightarrow$ 4)-Gal*p*A glycosidic bonds between contiguous galacturonic acid residues, which explains why a galacturonic rich oligosaccharides fraction with a relatively low molecular weight was found (OXP3). The low degree of methyl esterification of our extracts indicates involvement of pectin methylesterase in the retting process.

The objective of this study was to investigate the primary structure of hemp pectic polysaccharides. Five different pectic fractions, with increasing amounts of galacturonic acid when going from BWP2 to BWP4 and from OXP2 to OXP3 were characterized. We have been able to assign most of the proton signals corresponding to the main sugars present in these pectin polysaccharides and to determine their sugar composition by measuring the ^{1}H and ^{13}C surface area of specific resonances.

The BWP2, BWP3, BWP4, and OXP2 pectic polysaccharides can be compared to the modified hairy region of apple pectin already described by Colquhoun et al. [16], but with higher molecular weight because no signals characteristic of the terminal reducing end can be observed in their ^{1}H and ^{13}C spectra. Their chemical structures were very similar to those already described for rhamnogalacturonan I (RG-I)-like polysaccharides, which are solubilized during ethylenediamine tetraacetic acidic disodium salt extraction of retted flax fibres [20], or during water extraction of the walls of suspension-cultured sycamore cells [21,22], after digestion of the walls with a highly purified endo-α(1 $\rightarrow$ 4)-polygalacturonase. They presented an alternating sequence of rhamnose and galacturonic acid residues in the backbone, but with only one β-D-galactose residue attached at O-4 of the rhamnosyl residues.

The ratio of rhamnosyl residues linked either at O-2 or both at O-2 and O-4 are close to 1 in BWP3 and OXP3, but slightly different in BWP2 (1:3), OXP2 (2:3), and in BWP4 (2:1). Generally, a 1:1 average is found in the RG-I type polysaccharides already described in the literature, but our results show that locally, in hemp we can find rhamnogalacturonan blocks with different amounts of (1 $\rightarrow$ 2) and (1 $\rightarrow$ 2,4) linked rhamnopyranosyl residues.

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