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Isolation and structural characterisation of rhamnogalacturonan oligomers generated by controlled acid hydrolysis of sugar-beet pulp

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Abstract

Controlled acid hydrolysis was applied to a deesterified beet pulp and the resulting soluble fraction was fractionated on a Biorad AG 1X8 column eluted by ammonium acetate pH 6 from 0.05 to 2 M. Eight retained fractions were obtained, containing almost exclusively GalA and Rha. Three types of oligomers could be identified: homogalacturonans, of which mono-, di- and tri-GalA were isolated as individual components, and two series of rhamnogalacturonan (RG) oligomers. One RG oligomer, isolated after ion-exchange chromatography, was identified as α-D-GalA *p*-(1 → 2)-α-L-Rha*p*-(1 → 4)-α-D-GalA *p*-(1 → 2)-L-Rha*p*. The major peak contained oligomers of dp 6 to more than 20, of which dp 6 to 16 could be isolated on Bio-Gel P-6 + P-4. NMR of the oligomers of dp 6 to 10 showed the following structure: α-D-GalA *p*-(1[→ 2)-α-L-Rha*p*-(1 → 4)-α-D-GalA *p*-(1]$_n$ → 2)-L-Rha*p*. A second, quantitatively minor, series of RG oligomers eluted at higher ionic strength. These oligomers, which could be hydrolysed by RG-hydrolase and RG-lyase, were based on the alternating RG structure. Their non-reducing end was GalA, susceptible to hydrolysis by RG-galacturonohydrolase, and their reducing end might have more than one consecutive GalA. © 1998 Elsevier Science Ltd

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1. Introduction

The backbone of pectins, one of the main polysaccharides from the primary cell walls of plants, is composed of at least three distinct structural units: (1) homogalacturonans, (2) rhamnogalacturonans (RG) (backbone of GalA and Rha, with Rha carrying side-chains of mostly arabinans and arabinogalactans), and (3) xylogalacturonans, in which Xyl residues are carried by GalA [1]. Oligomers obtained by enzymic hydrolysis of the RG backbone have shown that this

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fraction is essentially composed of alternating Rha and GalA residues [2–7]. Oligomers that have been described were either products of RG-hydrolase (formerly known as rhamnogalacturonase), with GalA at the reducing end [2–4], or products of RG-lyase [7], with Rha at the reducing end and unsaturated GalA at the nonreducing end. They always contained residual Gal (on the C-4 of Rha) and represented minor or non-quantified amounts of the Rha initially present in the cell walls. One study, using acid hydrolysis, summarily characterised RG oligomers of degree of polymerisation (dp) up to 6 [5].

We have used controlled acid hydrolysis to study the length of homogalacturonan regions [8] and the distribution of Rha in commercial pectins [9]. At pH values lower than 2, the linkages between uronic acid residues are very stable, more so than the linkages between a uronic acid and a neutral sugar, i.e., the linkages $\rightarrow$4)-α-D-GalA*p*-(1$\rightarrow$2)-L-Rha*p*-(1$\rightarrow$ or $\rightarrow$2)-α-L-Rha*p*-(1$\rightarrow$4)-α-D-GalA*p*-(1$\rightarrow$, the latter themselves more stable than the linkages between neutral sugars. This leads to fast degradation of the side-chains to oligomers and monomers, while the backbone is more resistant, and is cleaved preferentially between Rha and GalA. After acid hydrolysis of commercial apple, beet and citrus pectins, RG oligomers devoid of Gal were observed [9]. They represented 15–27% of the Rha originally present in the pectins and formed a homologous series with dp of 6 to $\sim$20 [9]. The H-1 signals in the NMR spectrum of a mixture of these oligomers showed a repetitive structure with Rha at the reducing end. A preliminary study on controlled acid hydrolysis of sugar-beet pulp, a substrate particularly rich in Rha, showed the same main oligomer series, and indicated that oligomers of dp $\geq$ 12 could be digested by rhamnogalacturonase [10].

The work presented here describes further results on the acid hydrolysis of sugar-beet pulp, and the fractionation and fine structure of its RG oligomers. Our aims were to estimate the (minimum) length of strictly alternating RG fractions of the pectic backbone, to quantify the proportion of Rha actually present in such structures, and conversely to attempt to isolate 'aberrant' stuctures such as portions with successive Rha or GalA residues, which might be missed in enzymic degradation studies. Information on the length of the RG fractions is highly relevant to the functional properties of pectins, while the degree or absence of randomness in their backbone is a decisive indication for their mode of synthesis.

2. Materials and methods

Materials.—Sugar-beet pulp from Sofalia (Ennezat, France) was deesterified as described previously [11]. Rhamnogalacturonan (RG) degrading enzymes were isolated as described: RG-lyase [7], RG-hydrolase [12], RG-rhamnohydrolase [13] and RG-galacturonohydrolase [14].

Acid hydrolysis.—Approximately 8 g of deesterified sugar-beet pulp were hydrolysed for 72 h in 333 mL of 0.1 M HCl at 80 °C. After hydrolysis, the slurry was filtered on a G-4 sintered glass filter. The residue was washed with distilled water until the washing water was at pH $\sim$ 6, and dried by solvent exchange. The filtrate was brought to pH 6 by adding 1 M NaOH, then left overnight at 4 °C before centrifugation (20 min, 12,000 $\times$ *g*). The precipitate formed upon neutralisation was collected on a G-4 sintered glass filter and dried by solvent exchange. The filtrate was stored frozen.

Ion - exchange.—The filtrate (neutralised hydrolysate) was diluted with distilled water until its conductivity reached 3.2 mS. A 350 mL sample was injected onto a Biorad AG 1X8 column (22 $\times$ 3.5 cm) eluted with ammonium acetate pH 6: 400 mL of 0.05 M buffer (conductivity 3.2 mS), to 0.8 M over 1300 mL, to 1.2 M over 400 mL, maintained for 600 mL before rinsing with 0.05 M buffer. Fractions of 24 mL were collected and analysed for neutral sugars and uronic acid content (see Section 2.5). Peak-forming fractions were pooled, concentrated on a rotary evaporator, desalted on a Sephadex G-10 column (90 $\times$ 4.5 cm) (monitored by conductimetry), reconcentrated and frozen.

Gel - permeation chromatography.—Concentrated and desalted fractions from ion-exchange were injected on combined Bio-Gel P-4 and P-6 columns (90 $\times$ 2.5 cm) mounted in series, eluted with sodium acetate 0.1 M, pH 3.6 at 40 °C. Fractions (4 to 5 mL) were collected and analysed for neutral sugars and uronic acid content (see Section 2.5). The columns were calibrated using an oligogalacturonide mixture obtained as described by Gillet et al. [15].

Analytical.—Dry weight of solids was determined by drying at 120 °C for 2 h. Neutral sugars were measured as alditol acetates after hydrolysis of the samples. Deesterified beet pulp and the residue were pre-treated with 72% sulfuric acid (1 h and 3 h, respectively, at 20 °C) then hydrolysed in 1 M sulfuric acid (3 h, 100 °C) before conversion to alditol acetates [16]. The hydrolysate and chromatography

fractions were hydrolysed by TFA (2 M, 2 h, 120 °C). GalA and total neutral sugar (NS) concentrations were measured by automated *meta*-hydroxy-diphenyl [17] and orcinol assays [18] respectively. Corrections were made for interference of uronic acids in the orcinol assay. Proteins were determined by a semi-automated Kjeldahl assay (using N × 6.25). Ash was determined as the residue after incineration for 16 h at 550 °C and 1 h at 900 °C. HPAEC was carried out on a Carbopac PA1 column (4 × 250 mm) eluted at a flow rate of 1 mL/min with 0.1 M NaOH with a NaOAc gradient from 0.15 to 0.5 M over 50 min then to 0.6 M over 5 or 10 min, followed by 15 min reequilibration time, or (monitoring of enzyme hydrolyses) on a Carbopac PA-100 column (4 × 250 mm) eluted with 0.1 M NaOH with a NaOAc gradient from 0 to 0.45 M over 50 min then to 1 M over 5 min, followed by 15 min reequilibration time, with pulsed amperometric detection (Dionex, Sunnyvale, USA).

Nuclear magnetic resonance.—^{13}C and ^{1}H NMR spectra of oligosaccharide solutions (15–30 mg/mL) in deuterium oxide were recorded on a Bruker ARX 400 spectrometer. ^{13}C and ^{1}H chemical shifts were referenced to acetone assigned to 31.4 and 2.225 ppm, respectively. Oligosaccharides were adjusted to pH ~ 7 and deuterium-exchanged twice in 99.9% deuterium oxide before solubilisation in 0.5 mL 100% deuterium oxide with a trace of acetone as internal reference. Non-exchangeable proton assignments of oligosaccharides and sugar sequences were determined from two-dimensional COSY90 and TOCSY (HOHAHA) spectra. Carbon chemical shifts were determined from ^{1}H–^{13}C HMQC and HMQC–TOCSY experiments using the conventional pulse sequences provided by Bruker.

Enzyme digestions.—Oligomers (ca. 0.5 mg/mL) were incubated for 22 h at 40 °C in 0.05 M NaOAc pH 5.0 either with RG-rhamnohydrolase, RG-galacturonohydrolase, RG-hydrolase or RG-lyase (at respectively 7.05, 0.047, 0.044 and 0.21 μg/mL).

3. Results

Hydrolysis of sugar-beet pulp and isolation of the charged fractions.—The deesterified sugar-beet pulp was rich in Ara, Glc and GalA (Table 1), as reported earlier [9]. It contained 2.6% of Rha, implying RG represents up to 5% of sugar-beet pulp (assuming all the Rha was present in strictly alternating RG). After acid hydrolysis, a solid residue contained all the Glc, most of the GalA (homogalacturonans are insoluble in acidic conditions), > 90% of the Xyl, about 5% of the Rha, and virtually no Ara and Gal. The hydrolysate was rich in Ara, Gal and GalA, and contained most of the Rha (82%); some was lost, probably degraded during hydrolysis. A precipitate, formed upon neutralisation of the hydrolysate, contained almost no carbohydrates (Table 1).

Chromatography of the hydrolysate on Biorad AG 1X8 (Fig. 1) separated the neutral and acidic oligomers: 71.5% of the uronic acids but only 4.0% of the neutral sugars (estimated from the orcinol measurement) were recovered in the retained fractions (total recoveries: 82.5% for the uronic acids, 105% for the neutral sugars). Composition of the non-retained material was as reported earlier [10]. A flatter gradient allowed better separation of the retained material: eight fractions were collected (Fig. 1); fractions 2, 5, 7 and 8 were rich in GalA, while

Table 1
Composition of the deesterified beet pulp and its acid hydrolysis products

	Deesterified beet pulp	Residue	Hydrolysate	Precipitate
Rha	26	3 (5%)	339 (82%)	2
Fuc	2	0	39 (120%)	0
Ara	292	1	3392 (73%)	0
Xyl	20	25 (55%)	97 (30%)	0
Man	16	32 (87%)	0	0
Gal	71	2 (1%)	776 (68%)	0
Glc	294	664 (98%)	tr	4
GalA	201	324 (70%)	1040 (32%)	40
Proteins	110	36	nd	423
Ash	46	9	nd	nd

The composition data are in mg/g except for the hydrolysate (mg/mL). The values between parentheses are in % of the amount present in the deesterified beet pulp.
tr = Traces; nd = not determined.

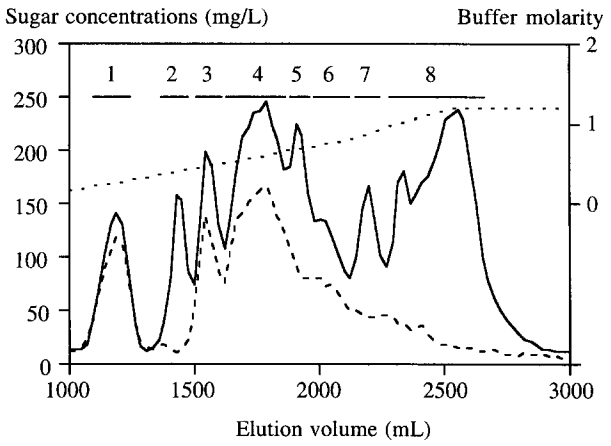

Fig. 1. Ion-exchange fractionation of the sugar-beet pulp hydrolysate on Biorad AG 1X8 resin eluted by ammonium acetate at pH 6. (————) GalA; (——) neutral sugars; (——) buffer molarity.

fractions 1, 3, 4 and 6 (Table 2) were also rich in Rha.

The main components of fractions 2, 5, 7 and 8 (Fig. 2, HPAEC (not shown)) were respectively mono- (fraction 2), di- (fraction 5), and tri-GalA (fraction 7), and a mixture of higher oligogalacturonides (fraction 8). Fraction 5 and to a lesser extent 7 also contained some higher RG oligomers.

Bio-Gel P-4 + P-6 chromatography of the RG fractions.—Bio-Gel P-4 + P-6 chromatography of fractions 1, 3, 4 and 6 (Fig. 2) gave fraction 1 as mostly di- or trimers, with Rha and Gal as the main neutral sugars. Its major peak, fraction 1d, contained at least 5 oligomers (Fig. 3A) and was not investigated further. Fraction 3 showed one major peak (fraction 3c), its K_{av} indicating a tetramer, composed of Rha and GalA in equimolar proportions, with residual contamination (Fig. 3A).

Fractions 4 and 6 were composed of homologous series of oligomers (Fig. 2). The pattern was particularly clear for fraction 4, with peaks separated by the equivalent of one Rha plus one GalA. All were composed of Rha and GalA in equimolar proportions except peak 4i. Fractions 4b to 4g showed one single peak on HPAEC (Fig. 3B); fractions 4e, 4f and 4g were chosen for further characterisation. Their K_{av}s indicated oligomers of dp 6 (4g), 8 (4f) and 10 (4e). The Bio-Gel P-6 + P-4 pattern of fraction 6 was less clear cut, with broader peaks, of K_{av}s intermediary between those of fraction 4. The peaks at the higher K_{av}s showed shoulders indicating two components. HPAEC of fractions 6b to 6d (Fig. 3B) showed one peak present in fraction 4 at the same K_{av} plus one new oligomer, more retained on the Carbopac column. Fraction 6i was di-GalA.

The Biorad AG1X8 chromatography pattern indicated a superposition of sharp peaks of oligogalacturonides, with a less well-resolved fractionation of RGs, some of which were collected with the oligogalacturonides. Oligomers were eluted as a function of the proportion of charged residues and the degree of polymerisation: a RG oligomer of dp 4 eluted between mono- and di-GalA. Dialysed samples [9] injected on the same column did not contain fractions 1, 2, and 3, and less of fractions 5 and 7 compared to fraction 8. Only GalA and RGs up to a dp of 4 were quantitatively eliminated by dialysis against water, which confirms the observations of Mort et al. [19]. For RGs, a shift of the 'average' molecular weight can be observed for the oligomers

Table 2
Composition (mol%) of charged fractions from the acid hydrolysate obtained on Biorad AG1X8 followed by Bio-Gel P-4 + P-6

	GalA	Rha	Fuc	Ara	Xyl	Gal	Glc
Hydrolysate	15	6	1	65	2	12	tr.
Fraction 1	54	28	2	1	3	11	1
1a	27	4	0	2	3	7	57
1b	40	8	0	1	3	45	3
1c	56	10	0	1	13	19	1
1d	50	33	3	0	2	11	1
Fraction 2	92	5	0	0	0	3	0
Fraction 3	54	45	0	0	0	1	0
3a	37	46	0	1	1	14	2
3b	40	34	0	6	11	7	1
3c	50	50	0	0	0	0	0
Fraction 4	48	50	0	0	0	2	0
4a	53	44	0	0	0	2	1
4b	55	42	0	0	0	2	1
4c	58	41	0	0	0	1	0
4d	54	44	0	0	0	1	0
4e	56	43	0	0	0	1	0
4f	51	48	0	0	0	1	0
4g	58	40	0	0	0	1	0
4h	54	42	0	0	0	3	1
4i	67	28	0	0	0	2	2
Fraction 5	73	26	0	0	0	1	0
Fraction 6	51	47	0	0	0	1	1
6c	55	44	0	0	0	0	0
6d	46	53	0	0	0	1	0
6e	41	58	0	0	0	1	0
6f	44	55	0	0	0	1	1
6g	48	50	0	0	0	1	1
6h	44	49	0	0	6	0	1
Fraction 7	74	23	0	0	1	2	0
Fraction 8	86	11	0	0	1	1	2

For coding see Figs. 1 and 2.

of the main series as the elution progresses from fraction 4 to fraction 6 (Fig. 2). At the same time a second series of oligomers appeared and overlaid the pattern of the oligomers of the first series: this series is still minor in fraction 5 but becomes the most important in fraction 6.

NMR characterisation of the RG oligomers.— Oligomers 3c and 4g to 4e, all composed of Rha and GalA in a 1:1 molar ratio, were investigated by ^{1}H NMR spectroscopy. All sugars were in the pyranose form. Absence of Gal resulted in clearer patterns than observed by Colquhoun et al. [2], Schols et al. [3] and Mutter et al. [7]. The spectra (Fig. 4) contained six signals in the anomeric region: three (at 5.00, 5.05 and 5.16 (Table 3)) with coupling constants of ~4 Hz, corresponding to H-1 of GalA, and two (at 5.22 and 4.93) with coupling constants <2 Hz (typical for H-1 of Rha [20]) and one not resolved (at 5.27).

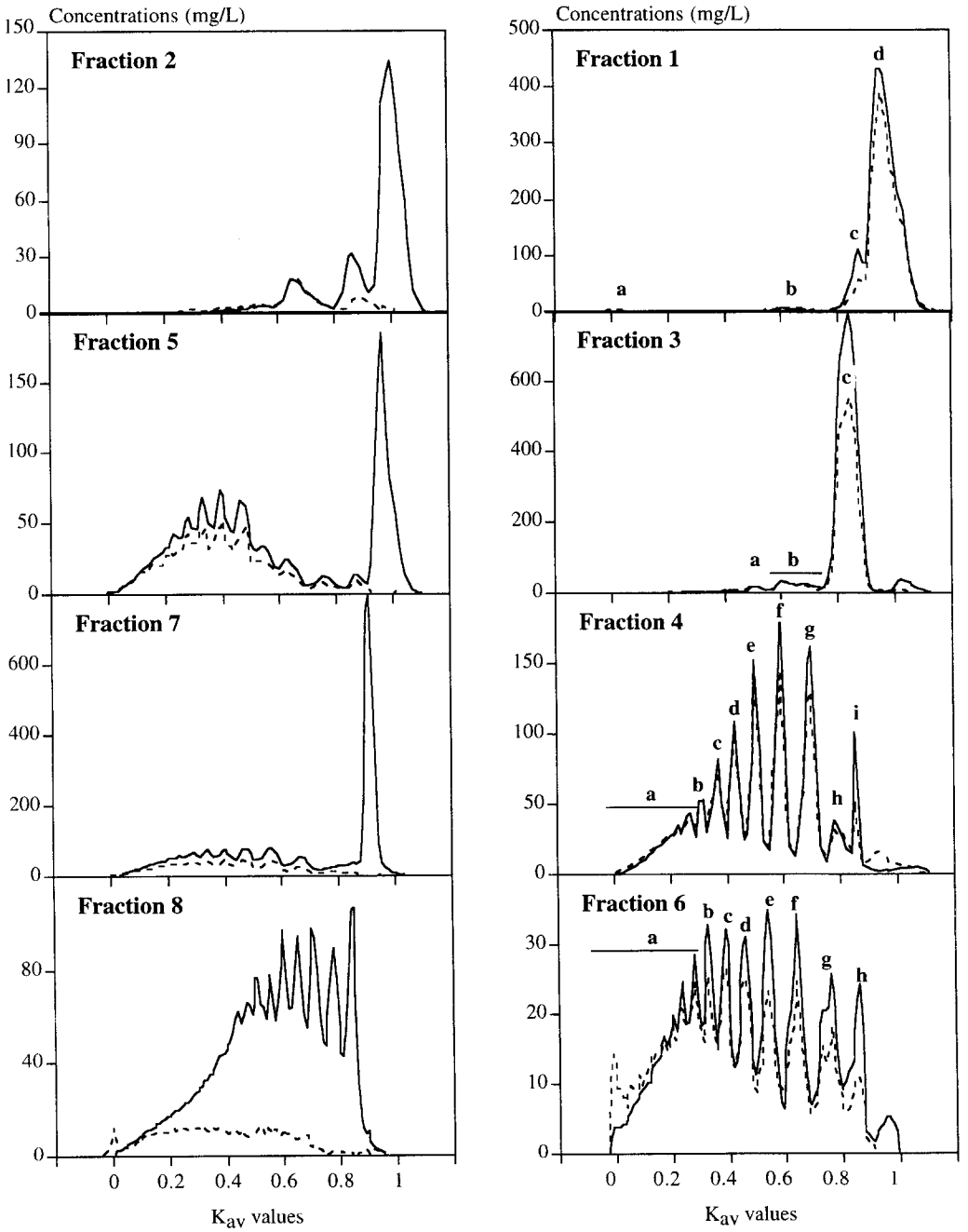

Fig. 2. Bio-Gel P-4 + P-6 chromatography of ion-exchange fractions of the sugar-beet-pulp hydrolysate (elution by sodium acetate/acetic acid 0.1 M, pH 3.6, 40 °C). For identification of the fractions see Fig. 1. (————) GalA; (– – –) neutral sugars.

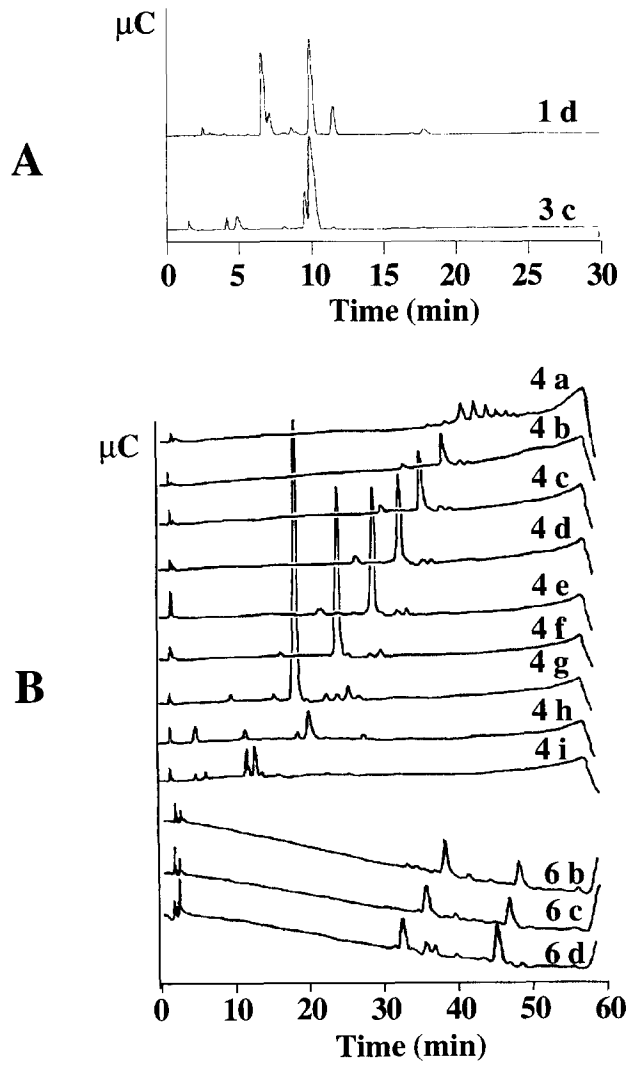

Fig. 3. HPAEC of selected fractions of the sugar-beet pulp hydrolysate after ion-exchange and gel-filtration. For identification of the fractions see Fig. 2.

Literature data [2,4,7] confirmed assignment of the two most intense signals, which increase with the dp of the oligomer, to 2-linked α-Rha*p* (5.27) and 4-linked GalA *p* (5.00). The signals at 5.22 and 4.93 were those of the α and β form, respectively, of reducing-end Rha [4]. The anomeric form of the reducing-end Rha affected the signals of the linked GalA, with peaks at 5.05 and 5.16 for GalA linked to the α and β-Rha, respectively. Integration of H-1 signals (ratio of integral of signals of reducing-end Rha at 5.22 and 4.93 to all other H-1 signals) confirmed the sizes deduced from the K_{av}s: dp 4 for fraction 3c, dp 6 for fraction 4g, dp 8 for fraction 4f and dp 10 for fraction 4e. Other signals that could be easily identified were those of the H-6 of Rha at circa 1.3 ppm, and those of the H-4 of Rha at 3.4 ppm. In the absence of Gal, this region contained three overlapping triplets. The main triplet, centered on 3.4 ppm, was attributed to the internal Rha residues, with indications in the spectra of the octa- and decamer of a splitting between the Rha linked to the non-reducing end GalA and internal Rhas. The α and β anomers of the reducing-end Rha gave signals at 3.47 and 3.34, respectively.

The hexamer (4g) was chosen for further experiments. The ^{1}H signals (Table 3) were assigned from COSY90. The coupling networks beginning with the H-1 resonance at 5.00 diverged from the resonance of the H-2, making it possible to distinguish the internal GalA (G_{int})(H-3 at 4.12 and H-4 at 4.41) from the non-reducing end (G_{nr}) (H-3 at 3.97 and H-4 at 4.29). These two networks converged again for the H-5 resonance. Resonances for the GalA linked to the reducing-end Rha (Gα and Gβ) were similar (except for the H-1) to those of the internal GalA, confirming that both were substituted on C-4, in contrast to the non-reducing end residue. As reported by Colquhoun et al. [2], the signal at 4.6 arose from H-5 of GalA. Tracing the coupling network for Rha residues was more complex in spite of presence of two starting points (H-1 and H-6), due to interferences between the coupling of the H-3 and the H-5, which had similar chemical shifts (especially for the α anomer of the reducing end residue, Rα), to the H-4. In addition, the H-5 signal of the Rβ fell within the zone of the H-4 signals. Signals for the H-5 were identified from their coupling to the H-6. R_{int} and R_{nr} had very similar spectra, the only visible difference being for the H-4 signals, as noted above. All assignments of internal residues were comparable to literature data obtained for unsubstituted residues of RG oligomers [2,7].

The ^{13}C NMR signals of this hexamer were assigned from HMQC and HMQC–TOCSY experiments (Table 3). The low resolution of the crowded proton signals between 3.75–4.00 ppm and the small chemical shift differences between carbon signals in the region between 68.5–71.0 ppm (Fig. 5) precluded the full unambiguous attribution of all the resonances of the carbons in the different sugars constituting this oligosaccharide. This was particularly the case for the carbons of the reducing-end β-Rha and those of the GalA linked to this residue. Small resonances at 71.6 and 69.8 remain unattributed and may correspond to β-Rha carbons. The three H-4 triplets observed for the different Rha residues in the proton spectrum of oligomer 4g (Fig. 4) were correlated with only one carbon resonance at 73.2 ppm. Two signals at 77.3

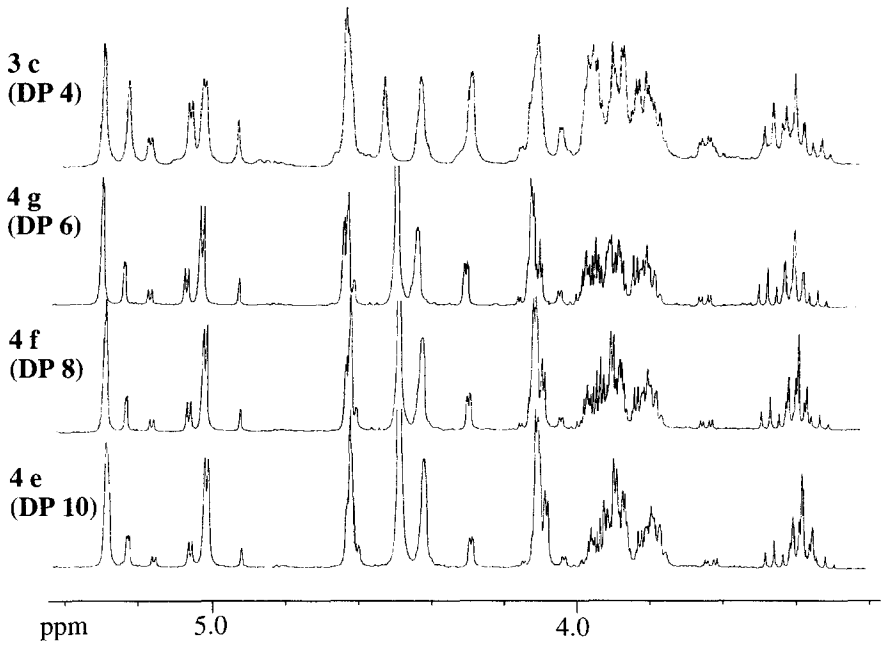

Fig. 4. ^{1}H NMR spectra of RG oligomers α-D-GalA *p*-(1[→ 2)-α-L-Rha*p*-(1 → 4)-α-D-GalA *p*-(1]$_n$ → 2)-L-Rha*p* of dp 4 (3c) to 10 (4e).

and 77.2 ppm were attributed to C-2 of the R_{int} and R_{nr} since these were correlated with the only proton resonance for the H-2 of these sugars on the HMQC spectrum. These chemical shift variations may reflect a small difference between the dihedral angles of the glycosidic linkage between the non-reducing end and the internal galacturonic to the Rha C-2. No other small chemical shift differences between the other carbons in these sugars were observed. The large chemical shift differences between the carbon signals of the internal GalA and Rha residues can be of use in future studies aimed at locating the site(s) of substitution(s) or branching of alternating RG regions in pectins.

Table 3
^{13}C and ^{1}H NMR chemical shifts assignments (coupling constants in Hz) of the hexamer 4g at 27 °C (G_{nr}-R_{nr}-G_{int}-R_{int}-Gα/β-Rα/β)

			1	2	3	4	5	6
R	α	^{1}H	5.22 (1.8)	3.97	3.88 (9.7)	3.47	3.88 (6.2)	1.29
		^{13}C	92.8	77.9	70.5a	73.2	69.8a	17.8
	β	^{1}H	4.93 (1.0)	4.04 (3.2)	3.66 (9.6)	3.33 (9.5)	3.42	1.30
		^{13}C	95.1	nd	nd	73.2	nd	17.9
	int/nr	^{1}H	5.27	4.11 (3.1)	3.90 (9.7)	3.40 (9.7)	3.80 (6.1)	1.25
		^{13}C	99.6	77.3/77.2	70.6	73.2	70.2	17.8
G	α	^{1}H	5.16 (4.4)	3.96 (10.6)	4.12	4.41	4.65	
		^{13}C	98.9	69.11b	nd	78.44c	72.57d	nd
	β	^{1}H	5.05 (3.9)	3.92 (10.1)	4.14	4.42	nd	
		^{13}C	102.2	nd	nd	nd	nd	nd
	int	^{1}H	5.00 (3.8)	3.92 (10.2)	4.12 (3.1)	4.41 (1.6)	4.65	
		^{13}C	98.7e	69.07b	71.5	78.38c	72.51d	176.1
	nr	^{1}H	5.00 (3.8)	3.82 (10.0)	3.97 (3.4)	4.29 (1.5)	4.66	
		^{13}C	98.6e	69.2	70.6	72.0	73.2	177.1

nd = Not determined; same letters means possible interchange.

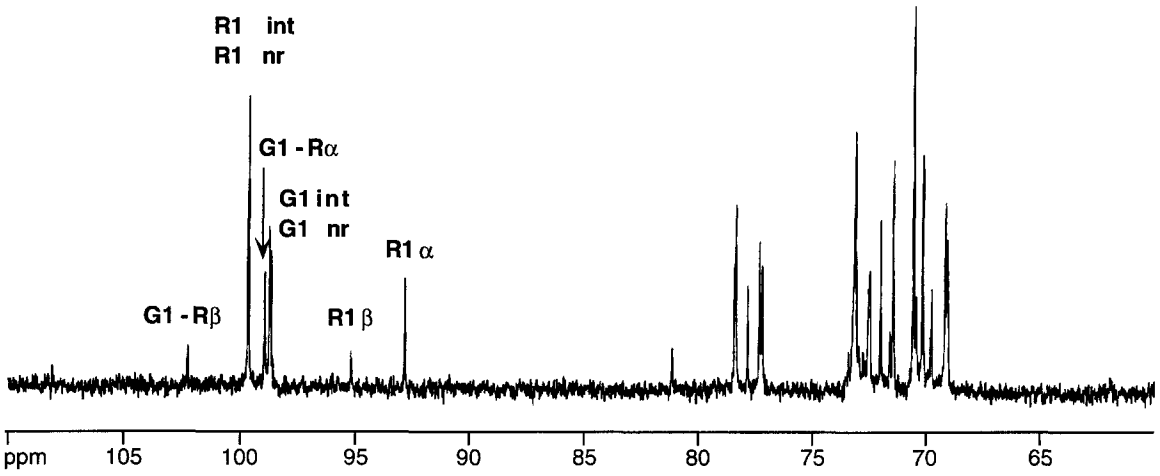

Fig. 5. ^{13}C NMR spectrum of the hexamer 4g (G_{nr}-R_{nr}-G_{int}-R_{int}-$G\alpha/\beta$-$R\alpha/\beta$).

HPAEC and action of RG-degrading enzymes on the rhamnogalacturonic oligomers of fraction 6.— Establishing the structure of the minor series of oligomers present in fraction 6 was hampered by their low abundance and incomplete separation from oligomers of the main series. Enzymic degradation was chosen as able to give structural information on minute amounts of substrate: degradation by the (highly specific) RG-degrading enzymes, would imply a strictly alternating structure with minor variations on the main series oligomers, such as presence of a different reducing or non-reducing end. RG oligomers with the strictly alternating structure and all four possible combinations of reducing/non-reducing end residues have been prepared and characterised (Mutter et al., unpublished results). The original oligomers of fraction 6 did not co-elute with any of these standards, and the curve for log k' versus dp (estimated from the Bio-Gel P-4 + P-6 fractionation) did not fit with that of any of the RG oligomers

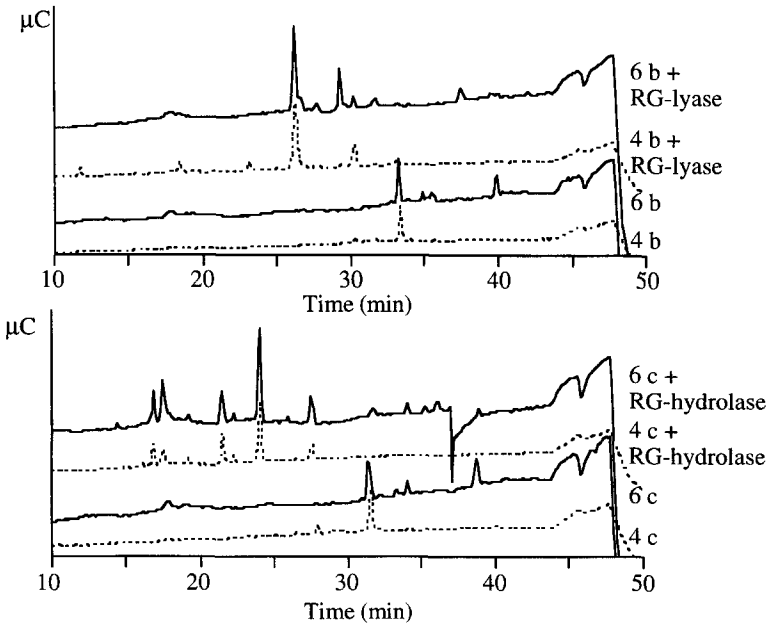

Fig. 6. Degradation of RG oligomers by RG-lyase and RG-hydrolase. HPAEC patterns of the fractions 6c and 6b (————) and of corresponding oligomers of fraction 4 (——).

series. The elution times were much higher than for any of the standard RG oligomer of similar dp, whatever their end groups.

These oligomers could be cleaved by RG-hydrolase in fractions 6d to 6b, and also by RG-lyase in fractions 6c and 6b (i.e., the largest oligomers), like the G-(R-G)$_n$-R oligomers. Similar minimal degrees of polymerisation for susceptibility to RG-hydrolase and RG-lyase applied and most reaction products were identical for both series (Fig. 6): this further implies a difference limited to ends of the oligomers. RG-rhamnohydrolase and RG-galacturonohydrolase are exo-acting enzymes able to remove a Rha or a GalA, respectively, from the non-reducing ends of RG chains. Oligomers 6e to 6b could be degraded by RG-galacturonohydrolase but not RG-rhamnohydrolase, identifying their non-reducing end as GalA on a RG backbone. The reaction products again had much higher elution times than corresponding products from the main series, suggesting the presence of additional GalA residues.

4. Conclusion

Two series of RG oligomers were present in the sugar-beet pulp hydrolysate, the main series (fraction 4) being RG oligomers with the strictly alternating structure: α-D-GalA *p*-(1[→ 2)-α-L-Rha*p*-(1 → 4)-α-D-GalA *p*-(1]$_n$ → 2)-L-Rha*p*, with $n = 1$ to > 10. NMR spectra of four oligomers of dp 4 to 10 clearly show the alternating RG structure. These are the first spectra reported for higher RG oligomers with Rha at the reducing end, GalA at the non-reducing end and devoid of Gal. The oligomers of the minor series appeared to present only a limited variation: as they could be cleaved by RG-hydrolase and RG-lyase, the body of the oligomers is likely to be strictly alternating RG, while susceptibility for RG-galacturonohydrolase indicates GalA (on a RG backbone) as the non-reducing end. Increased acidity compared to available RG standards suggests the presence of more than one GalA at the reducing end; the puzzling question then that remains is why a homogeneous series with a defined number of GalA and not a more or less random number of GalA. The second option could arise from the reducing end side of the RG region with a remnant of the HG backbone. The first implies the presence of sections of the RG with a defined number of consecutive GalA residues. Further work is necessary to isolate sufficient amounts of these oligomers and complete their characterisation.

Taking into account the losses during acid hydrolysis (13%) and the non-retained Rha (12%), 75% of the initial Rha was present in the retained fractions. Out of this, 12.5% was recovered in fraction 1, i.e., as di- or trimers, 8% in fraction 3 (strictly alternating tetramer), 36% in fraction 4 (strictly alternating oligomers of dp ≥ 6), 5% in fraction 5 and 10.5% in fraction 6. Most of the Rha present in sugar-beet pulp was thus found in strictly alternating Rha-GalA oligomers, though additional complications seem present.

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