Pergamon

PII: S0031-9422(98)00119-8

Phytochemistry, Vol. 49, No. 5, pp. 1195–1202, 1998

Printed in Great Britain
0031–9422/98/$—see front matter

OLIGOSACCHARIDES GENERATED BY PARTIAL HYDROLYSIS OF THE BORATE-RHAMNOGALACTURONAN II COMPLEX FROM SUGAR BEET†

TADASHI ISHII* and SATOSHI KANEKO

Forestry and Forest Products Research Institute, P. O. Box 16, Tsukuba Norin Kenkyu Danchi-nai, Ibaraki 305–8687, Japan

(*Received* 10 *November* 1997)

Key Word Index—*Beta vulgaris*; chemopodiaceae; borate-rhamnogalacturonan II; oligosaccharides; partial acid hydrolysis; sugar beet.

Abstract—The borate-rhamnogalacturonan II complex (B-RG-II), isolated from sugar beet (*Beta vulgaris*), was partially acid-hydrolyzed. The oligosaccharides generated were characterized by glycosyl-composition and glycosyl-linkage analyses, ES-mass, and NMR spectroscopy. Two disaccharides, α-L-Rha*p*-(1→5)-D-Kdo and α-L-Ara*f*-(1→5)-Dha, an aceric acid-containing oligosaccharide, and a 2-*O*-Me-Xyl-containing oligosaccharide, in addition to partially methyl-esterified α-(1→4)-oligogalacturonides were characterized. The data provide additional evidence that B-RG-II isolated from different plant species have identical structures.

INTRODUCTION

Rhamnogalacturonan II (RG-II) is a low M_r, structurally complex pectic polysaccharide [1]. It has been isolated from cell walls of dicots [2, 3], monocots [4] and gymnosperms [5], and is present in the commercial enzyme preparation, Pectinol AC [6], and in red wine [7].

RG-II contains at least 11 different monosaccharides, including the following seldom-observed sugars, apiose [Api; 3-*C*-(hydroxymethyl)-D-*glycerol*-aldotetraose], 2-*O*-Me-L-fucose, 2-*O*-Me-D-xylose, and aceric acid (AceA; 3-*C*-carboxy-5-deoxy-L-xylose). The monosaccharide constituents of RG-II are interconnected by at least 20 different glycosidic linkages. Chemical fragmentation of the RG-II isolated from the walls of suspension-cultured sycamore (*Acer pseudoplatanus*) cells led to the isolation and structural characterization of two apiose-containing oligosaccharides [6, 8, 9] and two 3-deoxy sugar containing disaccharides, that is, α-L-Rha*p*-(1→5)-D-Kdo [10] and β-L-Ara*f*-(1→5)-D-Dha*p* [11].

The functions of RG-II are not known [1]. However, it has been shown recently that RG-II exists in the wall as a dimer that is cross-linked by a borate diester [12–16]. These findings provide support for the hypothesis that cell wall-localized, borate-containing pectic polysaccharides are required for the normal growth and development of plants [17].

Identification of the borate-binding glycosyl residues in the borate RG-II complex (B-RG-II) is required to determine its structure and function in cell walls. To study the structure in detail, B-RG-II was subjected to mild acid hydroysis to cleave acid-labile glycosyl linkages and the generated oligosaccharides were characterized by glycosyl-residue and glycosyl-linkage analyses, mass and NMR spectroscopy. We now report the structural characterization of the oligosaccharides generated by partial acid hydrolysis of the B-RG-II from sugar beet. These studies confirm that B-RG-IIs isolated from different plant species have similar structures.

RESULTS AND DISCUSSION

Sugar beet B-RG-II (**1**) was methylated by a modified Hakomori procedure [18, 19], as described by Stevenson *et al.* [11]. The methylated B-RG-II (**2**) was reduced with $LiEt_3BD$ to give **3**, thus converting galacturonic residues in the native polysaccharide to 6,6-dideuteriogalactosyl residues. Glycosyl-linkage analysis of **3** showed that the B-RG-II contained the linkages typically found in RG-II from other species [1]. B-RG-II contained 2, 3, 3′-linked apiosyl residues, in addition to 3′-linked apiosyl residue (Table 1), indicating that 3′-linked apiosyl residues were involved in

* Author to whom correspondence should be addressed.

† This work is dedicated to Prof. emeritus Juzo Nakano, who passed away on 27 July, 1997.

Table 1. Glycosyl-linkage composition of B-RG-II from sugar beet

Glycosyl linkage	mol%	
	B-RG-II	Saponified B-RG-II*
T-Rha	5	8
2-Rha	4	n.d.+
3-Rha	7	6
2,3,4-Rha	8	8
T-(2-*O*-Me)-Fuc	6	6
3,4-Fuc	3	3
3′-Api	7	8
2,3,3′-Api	3	3
T-Ara*f*	5	5
T-Ara*p*	6	6
T-(2-*O*-Me)-Xyl	7	6
T-Gal	7	7
2,4-Gal	6	6
T-GalA	14	14
4-GalA	2	2
3,4-GalA	3	3
2,4-GalA	4	4
2-GlcA	5	5

*Saponified B-RG-II B-RG-II saponified and treated with Driselase. +n.d.: not detected.

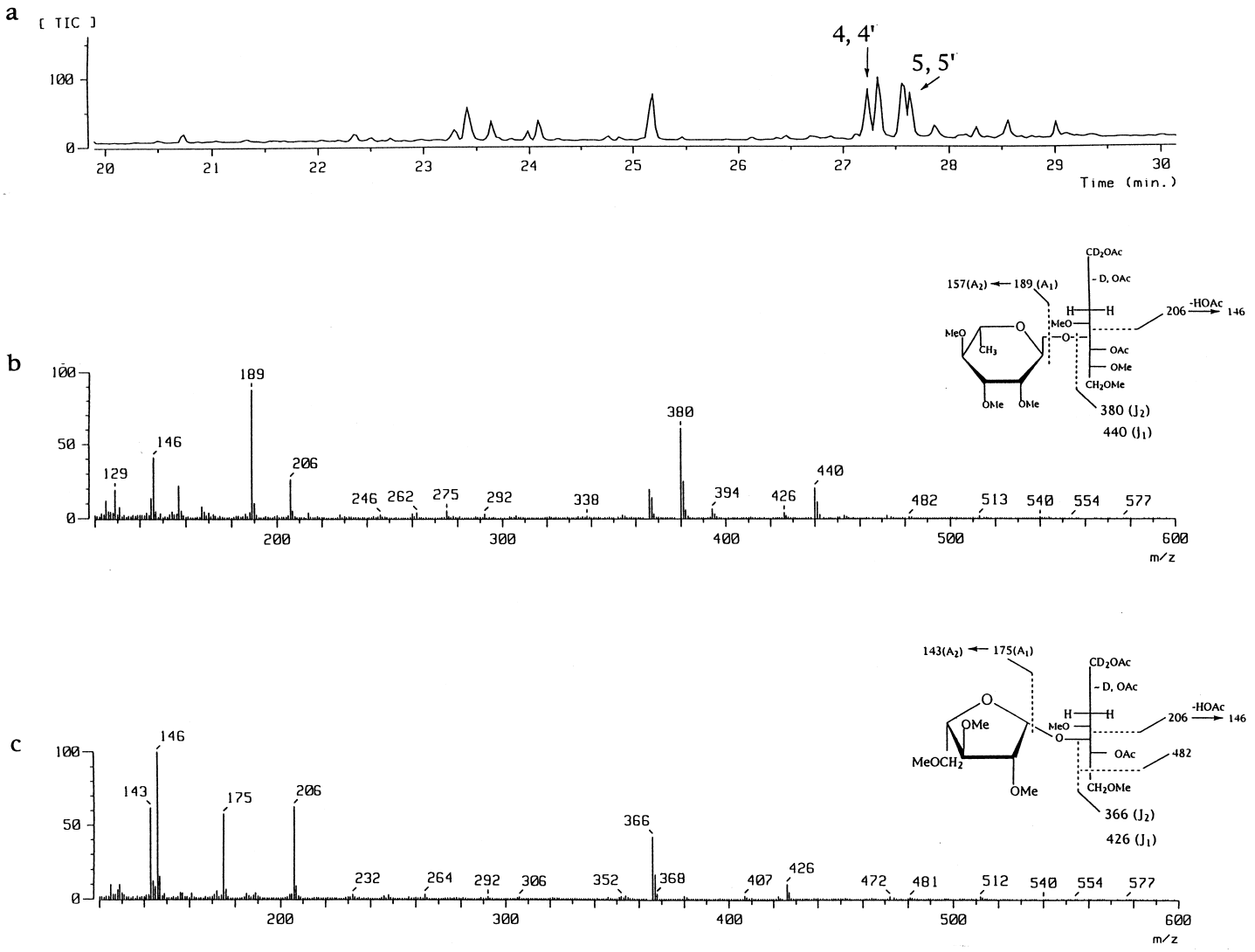
the formation of borate diesters. O'Neill *et al.* [15] and Pellerin *et al.* [16] reported that BRG-IIs from red wine, sycamore and pea also contains 2, 3, 3′-apiosyl residues and have suggested that this residue is cross-linked by borate in the dimer.

3-Deoxy-D-*manno*-2-octuloamic acid (Kdo) and 3-deoxy-D-*lyxo*-heptulosaric acid (Dha) residues are degraded under the conditions normally used for acid-catalyzed hydrolysis of glycosidic linkages [10, 11]. For this reason, the per-*O*-methylated and carboxyl-reduced B-RG-II was hydrolyzed using conditions (0.1 M TFA, 0.5 h, 60°) sufficient to cleave the keto-sidic linkages of derivatized Kdo and Dha residues, but not the glycosidic linkages of furanosyl residues. These conditions did not significantly degrade the Kdo and Dha. The released oligosaccharides were deuterioreduced, acetylated and analyzed by GC-mass spectrometry (Fig. 1). The EI mass spectra of peaks (4 and 4′, Fig. 1b) are consistent with a glycosyl-alditol, derived from 6-deoxyhexose-Kdo [10]. The peaks (5 and 5′) gave fragment ions at m/z 175 (A_1, pentose), m/z 366 (J_2) and 426 (J_1) (alditol from Dha (Figure 2c)). Peaks (5 and 5′) also gave the characteristic ion at m/z 206 due to the fragmentation of the alditol, but they did not give the fragment ion at m/z 161, which confirms that Dha is substituted at C-5 but not at C-4 [11]. Sugar composition analysis indicated that the fraction consisted of *ca* equal amounts of partially *O*-methylated Rha, Ara, Kdo and Dha residues. These results indicated that the disaccharides (4 and 4′) and (5 and 5′) were Rha*p*-(1→5)-D-Kdo*p* (Scheme 1) and shown be Ara*f*-(1→5) Dha*p* (Scheme 1–2), respectively. These two disaccharides had been characterized previously as the disaccharide side chains, released from sycamore RG-II by mild acid hydrolysis [10, 11].

About 35% of the apiosidic linkages of RG-II are hydrolyzed by treatment with 0.1 M TFA for 16 hr at 40° [6]. Thus, purified B-RG-II was subjected to these conditions. Chromatography of the hydrolysate on a Superdex Peptide column yielded three fractions A-C. Glycosyl-residue composition analysis showed that A corresponded to unfragmented RG-II (data not shown).

Glycosyl-residue composition analysis showed that fraction B had Api, Fuc, Ara, Gal, 2-*O*-Me-Fuc and aceric acid residues. Glycosyl-linkage analysis gave 3′-linked Api (1 mol %), terminal Fuc (20 mol %), terminal Ara*p* (22 mol %), 2-linked Rha (26 mol %), 2-linked aceric acid (4 mol %), and 2,4-linked Gal (27 mol %) residues. The negative-ion ES-mass spectrum of B contained intense ions at m/z 951 $[M\text{-}H]^-$ and 993 $[M\text{-}H]^-$. The ^1H NMR spectrum of B showed the presence of *O*-acetyl groups in the oligosaccharide. These results show that it contains two compounds, corresponding to a mono- and di-*O*-acetylated hexasaccharide composed of one Ara, one 2-*O*-Me-Fuc, one Gal, one aceric acid, one Rha and one apiosyl residues. The locations of the *O*-acetyl groups were determined by tandem mass spectrometry (ES-MSMS) analysis. The fragment-ion spectra of di-*O*-acetylated (Figure 2a) and mono-*O*-acetylated (Figure 2b) hexasaccharides both contain ions corresponding to the loss of one acetic acid from the deprotonated $[M]^+$. The di-*O*-acetylated oligosaccharide (m/z 993) gives fragment ions corresponding to the loss of acetic acid or the sequential loss of one Api (m/z 132), one Rha (m/z 146), and one Ara (m/z 132) residues to generate fragments at m/z 861, 715 and 513, respectively (Figure 2a). The ion at m/z 715 corresponds to a fragment composed of a Ara residue (m/z 132), a 2-*O*-Me-Fuc residue (m/z 160), an Ace A residue (m/z 160), a Gal residue (m/z 162), and two *O*-acetyl groups (m/z 84). Fragmentation of the ion at m/z 715 results in the loss of either a mono-*O*-acetylated aceric acid residue or a mono-*O*-acetylated Me-Fuc residue, which in both cases results in an ion at m/z 513, as the acetylated 2-*O*-Me-Fuc and Ace A residues have the same mass. The formation of an ion at m/z 381 from the ion at m/z 513 provides evidence that the Gal-Ara residue is not *O*-acetylated. Thus, these results provide additional evidence that the 2-*O*-Me-Fuc and Ace A residues are *O*-acetylated (**3** in Scheme 1) [8].

The fragmentation pattern of the ES-MSMS quasimolecular-ion spectrum of mono-*O*-acetylated hexasaccharide (M_r 952) (Figure 2b) is similar to that of di-*O*-acetylated hexasaccharide and is consistent with the presence of one *O*-acetyl group on the 2-*O*-Me Fuc or Ace A residue. Whitcombe *et al.* [8] report that sycamore RG-II contains an Ace A containing hexa-, hepta-, octa-, and nonasaccharides side-chains. We


a
[TIC]
4, 4'
5, 5'
Time (min.)
b
189
146
206
129
380
440
m/z
157(A2) ← 189 (A1)
CD2OAc
- D, OAc
-HOAc
206 → 146
380 (J2)
440 (J1)
c
146
143
175
206
366
426
482
143(A2) ← 175(A1)
366 (J2)
426 (J1)

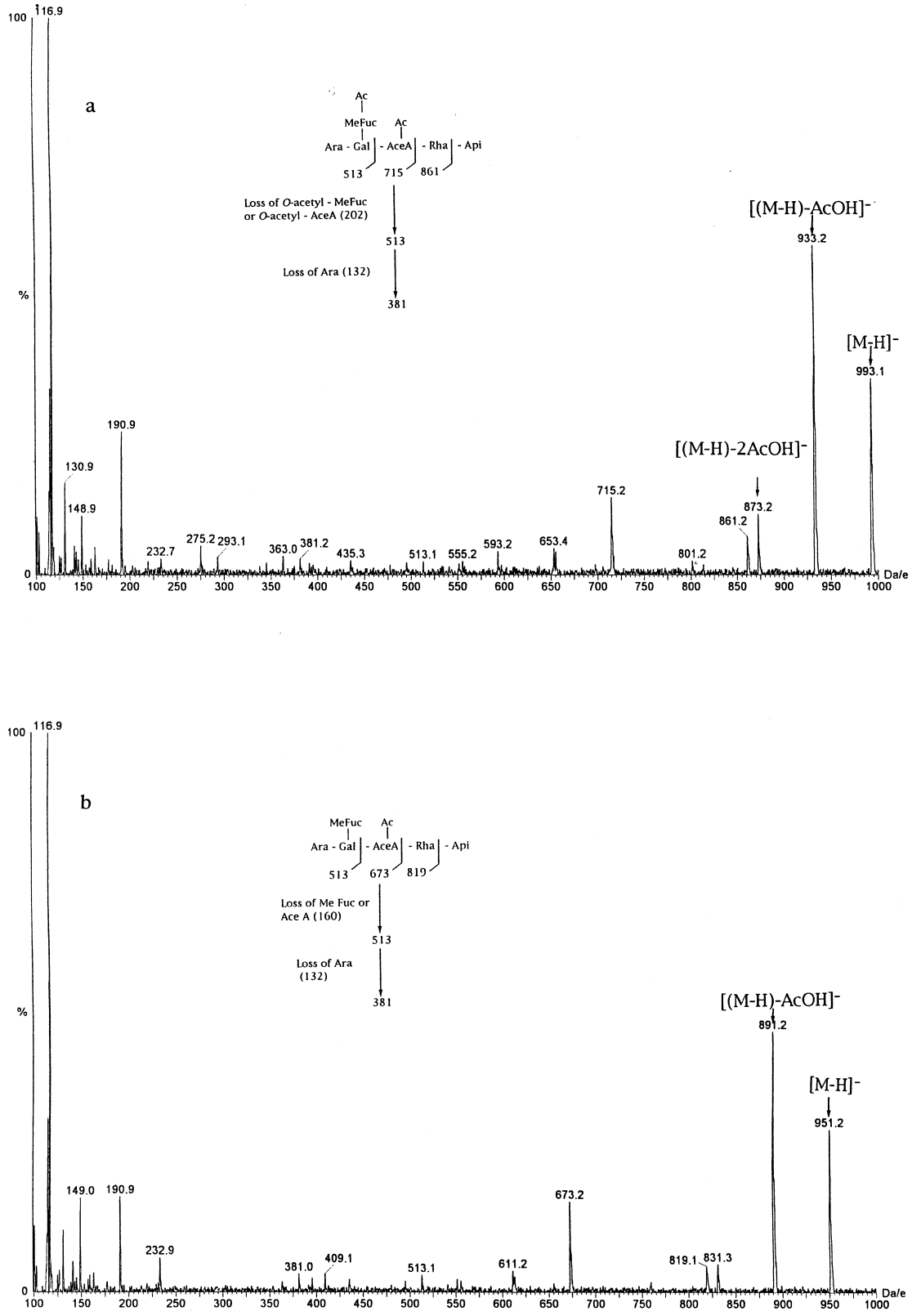
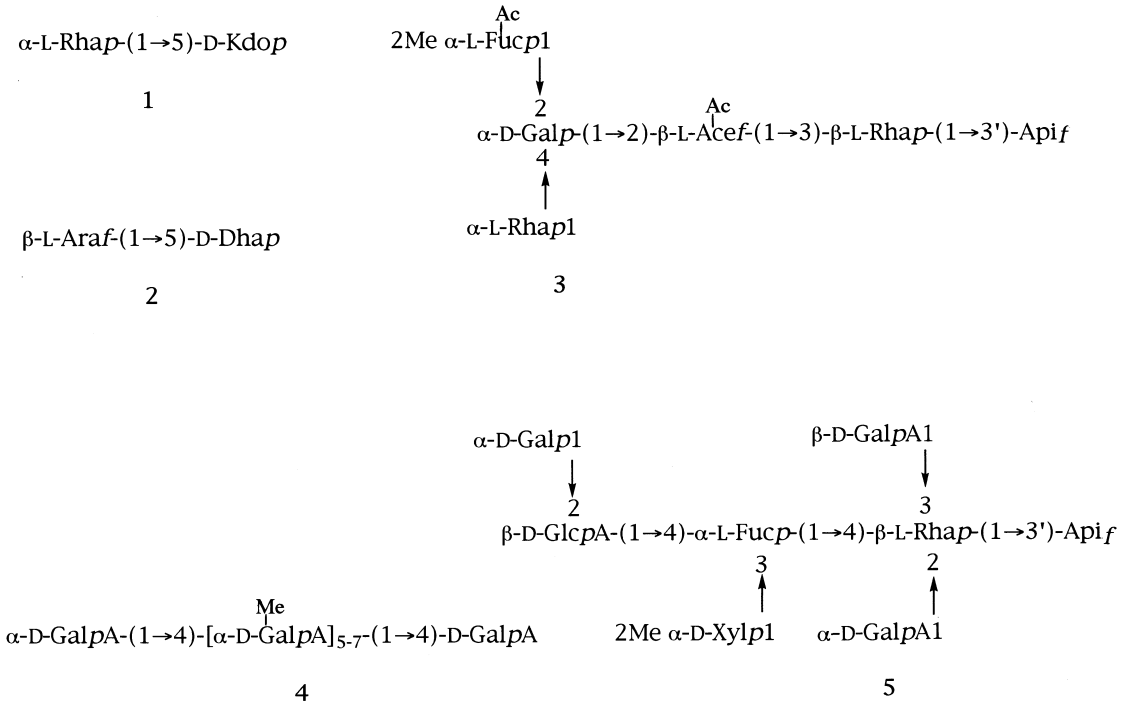


Fig. 2. ESMSMS fragment-ion spectra of partially *O*-acetylated hexasaccharide **5** present in fraction B. a: fragment-ion spectrum of di-*O*-acethylated hexasaccharide 5 (*m*/*z* 993). The insert shows the glycosyl sequence of di-*O*-acetylated hexasaccharide **5** and the proposed origins of the fragment ions. b: fragmention spectrum of mono-*O*-acetylated hexasaccharide **5** (*m*/*z* 951).



Scheme 1. Proposed structure of oligosaccharides generated by partial acid hydrolysis of B-RG-II.

Table 2. ^{1}H and ^{13}CNMR chemical shifts for α-L-Rha*p*-(1→5)-D-Kdo (**4**)

Chemical shifts (ppm) Proton	Residue		(coupling constants)		Carbon		
H-3a	Kdo	1.99	J3a,3e = 12.0	J3a,4 = 10.0	C-1	Kdo	177.08
H-3e	,,	1.73	J3a,4 = 5.4		C-2	,,	97.25
H-4	,,	4.07	J4,5 = 2.3		C-3	,,	34.66
H-5	,,	4.04	J5,6 = 0.9		C-4	,,	68.24
H-6	,,	3.71	J6,7 = 9.2		C-5	,,	73.80
H-7	,,	3.75	J7,8a = 1.9		C-6	,,	70.80
H-8a	,,	3.53	J7,8b = 4.6		C-7	,,	69.90
H-8b	,,	3.64	J8a,8b = 10.7		C-8	,,	63.63
H-1	Rha	5.15	J1,2 = 2.0				
H-2	,,	3.99	J2,3 = 3.0		C-1	Rha	102.09
H-3	,,	3.73	J3,4 = 9.5		C-2	,,	71.11
H-4	,,	3.31	J4,5 = 9.3		C-3	,,	70.73
H-5	,,	3.72			C-4	,,	72.70
H-6	,,	1.16	J5a,6 = 6.2		C-5	,,	71.41

assume that Driselase, a fungal enzyme preparation containing *exo* glycosidase, would cleave some of the Ace A containing side-chains.

The negative-ion FAB-mass spectrum of fraction C contained an intense ion at *m/z* 383. Glycosyl residue composition analysis showed that it contained Rha and Kdo residues. Glycosyl-linkage analysis revealed terminal Rha and 5-linked Kdo residues. 2D ^{1}H and ^{13}C NMR spectroscopy was used to elucidate the complete structure of the disaccharide (Table 2). The ^{1}H NMR spectrum of the disaccharide is consistent with the known *manno* configuration of Kdo [10]. We conclude that C contained α-L-Rha*p*-(1→5)-D-Kdo Scheme 1.

Fraction A was further hydrolyzed with 0.1 M TFA for 1 hr at 100°. Six fractions (D ~ I, Fig. 3) were obtained by size exclusion chromatography. Glycosyl-residue composition was determined (Table 3). Fraction E consisted mainly of GalA residue and was analyzed by high performance anion-exchange chromatography with pulsed amperametric detection. It contained a series of components that cochromatographed with standard 1,4-linked α-D-oligogalacturonides. ES-mass spectrometric analysis

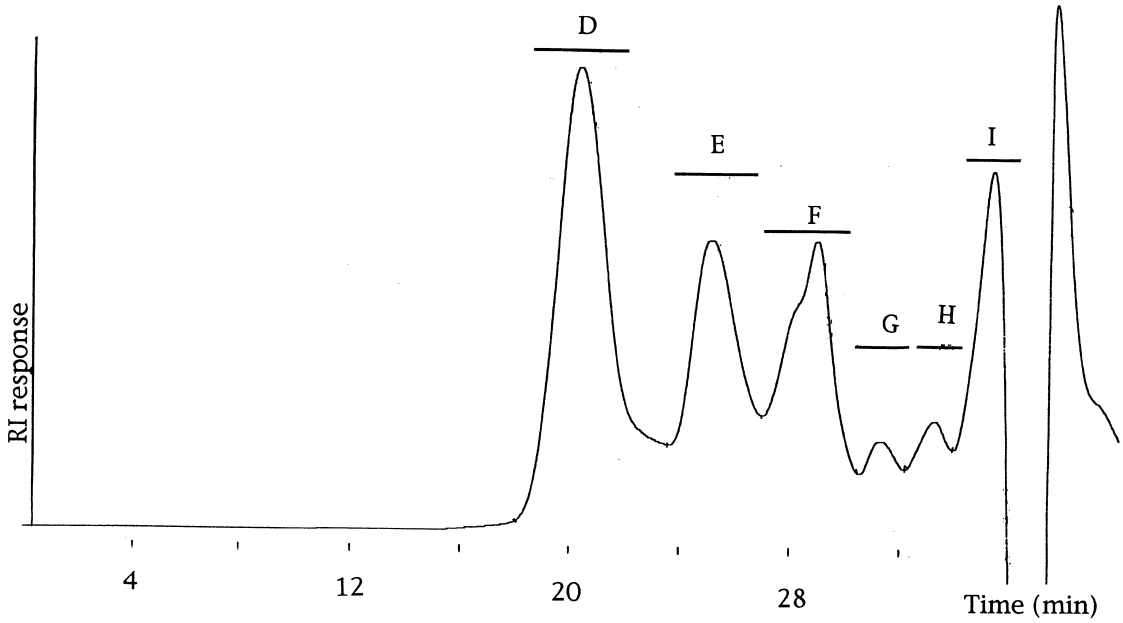


Fig. 3. Superdex Peptide size-exclusion chromatography of the products generated by partial acid hydrolysis (0.1 M TFA, 1 hr at 100°C) of fraction A. Fractions D-I were pooled as shown by the bars.

Table 3. Glycosyl composition of fractions D-I generated by partial acid hydrolysis of B- RG-II

	Fraction mol%					
	D	E	F	G	H	I
2-*O*-Me-Fu	–	–	1	3	5	14
Rha	13	3	5	10	21	28
Fuc	–	–	1	1	4	3
2-*O*-me-Xyl	–	–	2	1	1	20
Ara	1	–	2	5	8	12
Api	–	–	1	2	4	12
Ace A	–	–	1	3	2	1
Gal	2	–	5	10	11	9
Gal A	84	97	65	61	46	13
Glc A	–	–	17	5	–	–

showed that fraction E contained a mixture of partially methyl esterified oligogalacturonides with dps 7–9 (**4** in Scheme 1). For example, the ES-mass spectrum contains an ion at *m/z* 1643, which corresponded to nonagalacturonide with three methyl groups. The distribution of methyl substituents in the oligogalacturonides could not be determined by ES-MSMS, since fragment ion peaks at the high Mr regions were not observed. Pellerin *et al.* [16] reported that wine RG-II contains methyl esters.

Fraction F contained Rha, Gal, GalA, and GlcA residues with smaller amounts of 2-*O*-Me-Fuc, Fuc, 2-*O*-Me-Xyl, Api and Ara residues. Glycosyl-linkage analysis showed the presence of terminal Gal, 3, 4-Fuc, terminal Xyl, 2, 3, 4-Rha, 2, 4-GlcA and terminal GalA residues. These results indicated that this fraction contained 2-*O*-Me-Xyl-containing octasaccharide (**5** in Scheme 1) [9].

Fraction D contained Rha and GalA residues. Methylation analysis of the B-RG-II showed that 2-linked Rha residue was present, indicating it contained RG-I-like polysaccharide as a contaminant. When B-RG-II was saponified with N NaOH and then treated with Driselase, the 2-linked Rha residue was not detected (Table 1). This suggests that ester groups prevented Driselase attack. Fractions G-I were not analyzed further.

The B-RG-II isolated from sugar beet has a partially methyl-esterified α-(1→4) linked oligogalacturonide backbone (dps 7–9) that is substituted with four different side-chains, Ara*f*-(1→5)-Dha, α-Rha*p*-(1→5)-Kdo, an Ace A containing hexa-

saccharide and a 2-*O*-Me-Xyl containing octasaccharide (Scheme 1).

EXPERIMENTAL

Plant material

Sugar beet pulp was refluxed with MeOH for 16 hr. The alcohol-insol. residue (AIR), which contained 43.4 μg Bg^{-1}, was used for the isolation of B-RG-II.

Isolation of B-RG-II

B-RG-II (**1**) was solubilized by Driselase treatment of AIR [13]. It had Mr ~9500 and contains 120 μBmg^{-1}.

Methylation of B-RG-II

Methylation was performed by the procedure described in ref. [11]. The resulting methylated (**2**) and carboxyl-reduced B-RG-II (**3**) was used for identification of 3-deoxy sugar-containing disaccharides and glycosyl-linkage analysis.

GC-MS

The following GC and GC-MS conditions were used: (a) SP-2330 (30 m × 0.25 mm) fused-silica capillary column, split injection (1:50), inj. temp. 250°, oven programmed from 170° (2 min hold) at 4° min^{-1} to 235° with a 15 min final hold, FID-detection; (b) column from (a), splitless injection, oven programmed from 50° (2 min hold) at 30° min^{-1} to 170° then at 4° min^{-1} to 235° with a 10 min final hold, EI MS detection; (c) DB-1 (15 m × 0.25 mm) fused-silica capillary column, splitless injection, oven programmed from 50° (3 min hold) at 30° min^{-1} to 150° then at 6° min^{-1} to 320° with a 10 min final hold, EI MS detection; (d) DB-1 (30 m × 0.25 mm) fused-silica capillary column, split injection (1:50), oven programmed from 140° (2 min hold) at 2° min^{-1} to 200°, then at 30° min^{-1} to 275° with a 10 min final hold, FID detection. MS were recorded at 70 eV. CI-MS was performed using NH_3 as reagent gas (200 eV).

High performance anion-exchange chromatography with pulsed amperometic detection (HPAEC-PAD)

This was performed as described in ref. [20]. An aliquot (~200 μg) of the oligosaccharides generated by partial acid hydrolysis of B-RG-II was analyzed by HPAEC-PAD on a Carbo Pac PA1 column (250 × 4 i.d. mm) eluted at 1 ml min^{-1} and detected by PAD. The following gradient conditions were used. (A): elution with 0.1 M NaOH (0–2 min), followed by a linear gradient (2–30 min) of NaOAc (0–0.2 M) in 0.1 M NaOH. The column was then eluted with a linear gradient (30–54 min) of NaOAc (0.2–1.0 M) in 0.1 M NaOH. The column was washed with 1.0 M NaOAc in 0.1 M NaOH for 5 min and then re-equilibrated with 0.1 M NaOH for 20 min. (B): elution with 0.1 M NaOAc in 0.1 M NaOH (0–5 min), followed by a linear gradient (5–25 min) of NaOAc (0.1–0.5 M) in 0.1 M NaOH. The column was then eluted with a linear gradient (25–50 min) of NaOAc (0.5–0.7 M) in 0.1 M NaOH. The column was washed with 1.0 M NaOAc in 0.1 M NaOH for 10 min and then re-equilibrated in 0.1 M NaOAc in 0.1 M NaOH for 20 min.

*Identification of disaccharides **4** and **5***

A solution of **3** (~2 mg) in 0.1 M TFA (300 μl) was heated for 0.5 hr at 60°. The aq. acid was removed by evapn with a stream of dry air at room temp. A portion of the acid hydrolysates was reduced with $NaBD_4$ and acetylated. GC-MS (condition a) revealed two diastereomers of compound **4** at 27.21 and 27.31 min, and two diastereomers of compound **5** at 27.53 and 27.66 min (see Fig. 1).

Partial acid hydrolysis of B-RG-II

A soln of B-RG-II (25 mg) in 0.1 M TFA (2 ml) was heated for 16 hr at 40°C. The acid was evapd under a stream of dry air. The hydrolysate was fractionated on a Superdex Peptide HR10/30 (300 × 10 mm) column (Pharmacia Biotech. Inc.,) by elution with 25 mM HCO_2NH_4, pH 5.3, at a flow rate of 0.6 ml min^{-1}. The eluent was monitored with a RI detector. Four peaks eluted at 20.8 min (fr. A), 27.5 min (fr. B), 32.7 min (fr. C) and 35.0 min and were collected and freeze-dried. B and C were separately dissolved in 25 mM HCO_2NH_4 buffer and rechromatograped under the same conditions and each peak collected to give compounds **1** and **3** (Scheme 1), respectively. HPAEC-PAD (condition A) analysis showed that both fractions were homogenous. Fr. A was further hydrolyzed with 0.1 M TFA for 1 hr at 100°. The hydrolysate was separated on the same column as described above to give 6 frs (see Fig. 3).

Glycosyl-composition and glycosyl-linkage compositon analyses

The oligosaccharides (100 μg) generated by partial acid hydrolysis of B-RG-II were analyzed for neutral sugars by GC of their alditol acetates (condition A) [22] and for neutral and acid sugars by GC of their per-*O*-TMSi methyl glycoside methyl esters (condition D) [21]. Absolute configurations were determined as described in ref. [22]. The glycosyl-linkage compositions of the oligoglycosyl alditols (200 μg) were determined by methylation analysis and GC-MS (condition B) [22].

Electrospray MS

ES-MS was operated in the positive and negative ion modes. Solns of oligosaccharides (100 μg) in aq. 30% MeOH containing 0.75% HCl (100 μl) were infused into the electrospray source at 4 μl min^{-1}. The ion spray was operated at 5000 V with an orifice potential of 35 V. Ten scans (100–2500 amu) were collected and averaged. ES-MSMS was performed by selecting the appropriate deprotonated molecular ion ($[M-H]^-$) in the first quadrupole MS and then bombarding it with argon gas in the open-structured quadrupole collision cell. The fragment ions generated by collision with the neutral gas molecules were separated in the second quadrupole MS to obtain the fragment-ion mass spectra. Between 50 and 100 scans (100–1500 amu) were collected and averaged.

FAB-MS

Spectra were recorded in the negative- and positive-ion modes, with accelerating voltages of 10 kV. A portion (1 μl) of the oligosscharide in H_2O was mixed with 1 μl glycerol and thioglycerol (1:1, v/v) on the probe tip of the instrument.

NMR spectroscopy

^1H and ^{13}C NMR were recorded at 500 and 125 MHz, respectively. ^1H and ^{13}C chemical shifts (δ) are reported in ppm relative to external Me_2CO (δ 2.04) and dioxane (δ 66.5), respectively.

Acknowledgements—This work was supported by grants from the Ministry of Agriculture, Forestry, and Fisheries (Glyco-Technolog Projects). We thank Mrs Y. Takeda for preparing the manuscript, Mrs N. Kagi of JASCO International Co. Ltd. for determining ESMS, and Dr. M. O'Neill of Georgia University for critical reading of this manuscript.

REFERENCES

1. O'Neill, M., Albersheim, P. and Darvill, A., in *Methods of Plant Biochemistry*, Vol 2, ed. P. M. Dey, Academic Press, London, 1991, p. 415.
2. Darvill, A. G., McNeil, M. and Albersheim, P., *Plant Physiolology*, 1978, **62**, 418.
3. Fisher, M., Wegryzn, T. F., Hallett, I. C. and Redgwell, R. J., *Carbohydrate Research*, 1996, **295**, 195.
4. Thomas, J. T., Darvill, A. G. and Albersheim, P., *Carbohydrate Research*, 1989, **185**, 261.
5. Thomas, J. T., McNeil, M., Darvill, A. G. and Albersheim, P., *Plant Physiology*, 1987, **83**, 659.
6. Spellman, M. W., McNeil, M., Darvill, A. G. and Albersheim, P., *Carbohydrate Research*, 1983, **122**, 131.
7. Doco, T. and Brillouet, J.-M., *Carbohydrate Research*, 1993, **243**, 333.
8. Whitcombe, A., O'Neill, M. A., Steffan, W., Albersheim, P. and Darvill, A. G., *Carbohydrate Research*, 1995, **271**, 15.
9. Melton, L. D., McNeil, M., Darvill, A. G. and Albersheim, P., *Carbohydrate Research*, 1986, **146**, 279.
10. York, W. S., Darvill, A. G., McNeil, M. and Albersheim, P., *Carbohydrate Research*, 1985, **138**, 109.
11. Stevenson, T. T., Darvill, A. G. and Albersheim, P., *Carbohydrate Research*, 1988, **179**, 269.
12. Kobayashi, M. and Matoh, T., Azuma, J., *Plant Physiology*, 1996, **110**, 1017.
13. Ishii, T. and Matsunaga, T., *Carbohydrate Research*, 1996, **284**, 1.
14. Kaneko, S., Ishii, T. and Matsunaga, T., *Phytochemistry*, 1997, **44**, 243.
15. O'Neill, M., Warrenfeltz, D., Kates, K., Pellerin, P., Doco, T., Darvill, A. G. and Albersheim, P., *Journal of Biological Chemistry*, 1996, **271**, 22923.
16. Pellerin, P., Doco, T., Vidal, S., Williams, P., Brillouet, J.-M. and O'Neill, M. A., *Carbohydrate Research*, 1996, **290**, 183.
17. Loumis, W. D. and Durst, R. W., *Biofactors*, 1992, **2**, 229.
18. Hakomori, S., *Jounal of Biochemistry*, 1964, **55**, 205.
19. Sanford, P. A. and Conrad, H. E., *Biochemistry*, 1966, **5**, 1508.
20. Ishii, T., *Plant Physiology*, 1997, **113**, 1265.
21. York, W. S., Darvill, A. G., McNeil, M., Stevenson, T. T. and Albersheim, P., *Methods in Enzymololgy*, 1985, **118**, 3.
22. Gerwig, G. J., Kamerling, J. P., Vliegenthart, J. F. G., *Carbohydrate Research*, 1979, **77**, 1.