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Isolation and structural characterization of β-D-glucosyluronic acid and 4-*O*-methyl β-D-glucosyluronic acid-containing oligosaccharides from the cell-wall pectic polysaccharide, rhamnogalacturonan I

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ABSTRACT

Rhamnogalacturonan I (RG-I), a pectic polysaccharide isolated from the walls of suspension-cultured sycamore cells, was shown by glycosyl-residue composition analysis to contain D-glucosyluronic acid (Glc*p*A) residues (1 mol%) and 4-*O*-methyl-D-glucosyluronic acid (4-*O*-Me-Glc*p*A) residues (0.5 mol%). These monosaccharides were shown, by glycosyl-linkage analysis, to be present in RG-I as terminal nonreducing residues. The glycosyl sequences containing Glc*p*A and 4-*O*-Me-Glc*p*A were determined by structurally characterizing the acidic oligosaccharides released by partial acid hydrolysis of RG-I. Six acidic oligosaccharides were purified by semipreparative high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC–PAD) and characterized by glycosyl-residue and glycosyl-linkage composition analyses, GLC–CIMS, GLC–EIMS, electrospray MS (ESMS), and ^{1}H NMR spectroscopy. We propose that three of the acidic oligosaccharides characterized, 4-*O*-Me-β-D-Glc*p*A-(1 → 6)-D-Gal, β-D-Glc*p*A-(1 → 6)-D-Gal, and β-D-Glc*p*A-(1 → 4)-D-Gal, originate from the galactosyl-containing side chains of RG-I. The three other acidic oligosaccharides characterized, α-D-Gal*p*A-(1 → 2)-L-Rha, α-D-Gal*p*A-(1 → 2)-α-L-Rha*p*-(1 → 4)-α-D-Gal*p*A-(1 → 2)-α-L-Rha, and α-D-Gal*p*A-(1 → 2)-α-L-Rha*p*-(1 → 4)-α-D-Gal*p*A-(1 → 2)-α-L-Rha*p*-(1 → 4)-α-D-Gal*p*A-(1 → 2)-α-L-Rha, were generated by partial hydrolysis of the RG-I backbone. No evidence was obtained for the presence of galactosyluronic acid in the side chains of RG-I. To our knowledge this is the first report that rhamnogalacturonan I contains glycosyluronic acid or 4-*O*-methyl glucosyluronic acid residues.

INTRODUCTION

Rhamnogalacturonan I (RG-I) is a major pectic polysaccharide component of the cell walls of dicots and nongraminaceous monocots[1]. RG-I is present in lesser amounts in graminaceous monocots[1]. The backbone of RG-I, obtained from the walls of suspension-cultured sycamore cells, has been shown to consist of the diglycosyl repeating unit -4)-α-D-Gal*p*A-(1 → 2)-α-L-Rha*p*-(1-. Approximately 50% of the backbone rhamnosyl residues in the RG-I released from sycamore cell walls

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by endo-1,4-α-polygalacturonase (EPG) are substituted at position 4 with side chains composed primarily of arabinosyl and galactosyl residues[2–4]. Although evidence was obtained that the parts of the side chains composed of arabinosyl residues do not contain glycosyluronic acid residues[4], it remained possible that the galactosyl-containing side chains do contain glycosyluronic acid residues[3,4]. We now provide evidence that Glc*p*A and 4-*O*-Me-Glc*p*A residues, but not Gal*p*A residues, are components of the galactosyl-containing side chains of sycamore RG-I.

RESULTS AND DISCUSSION

*RG-I isolated from suspension-cultured sycamore cell walls contains Glc*p*A and 4-O-Me-Glc*p*A.*—RG-I isolated from the walls of suspension-cultured sycamore cells and then purified by gel-permeation and anion-exchange chromatographies was shown, by glycosyl-residue composition analysis, to contain Glc*p*A (1 mol%) and a mono-*O*-methyl hexuronic acid (0.5 mol%); the latter co-chromatographed with authentic 4-*O*-Me-Glc*p*A that was generated by acid hydrolysis of the aldobiouronic acid 4-*O*-Me-β-D-Glc*p*A-(1 → 2)-Xyl*p*. Glycosyl-linkage analysis of methylated and carboxyl-reduced RG-I established that the Glc*p*A and the 4-*O*-Me-Glc*p*A residues were terminal and nonreducing. These two hexosyluronic acids have not been previously reported to be components of sycamore RG-I. Additional evidence that they are indeed part of this primary cell-wall pectic polysaccharide was obtained by the controlled partial acid hydrolysis of sycamore RG-I and structural characterization of the acidic oligosaccharides generated by this procedure.

Partial acid hydrolysis of RG-I.—The glycosidic linkages of hexosyluronic acids are known to be relatively resistant to acid hydrolysis[5]. Indeed, numerous studies have shown that controlled acid hydrolysis of acidic polysaccharides is a convenient method for generating acidic oligosaccharides that can be purified and structurally characterized[6]. Therefore, sycamore RG-I was subjected to partial acid hydrolysis to generate oligosaccharides containing glycosyluronic acid residues. The partial acid hydrolysis conditions required to generate the maximum yield of these acidic oligosaccharides were determined by treating samples of RG-I (500 μg per sample) with 2 M trifluoroacetic acid (TFA) (500 μL) at 100°C for 10 min, 20 min, 40 min, 1 h, 2 h, or 4 h. The acidic components in the hydrolysates were isolated by low-pressure anion-exchange (DEAE-Sephadex) chromatography and then analyzed by HPAEC–PAD. The 1-h TFA hydrolysis generated the disaccharide α-D-Gal*p*A-(1 → 2)-L-Rha, smaller amounts of other oligosaccharides, but only small amounts of free GalA (data not shown). Larger amounts of free GalA were produced when RG-I was hydrolyzed for longer than 1 h. Hydrolysis of RG-I with TFA for less than 1 h resulted in low yields of acidic oligosaccharides.

Acidic oligosaccharides were generated in amounts (200–500 μg) sufficient for structural analysis by treating RG-I (22 mg) with 2 M TFA (2 mL) for 1 h at 100°C.

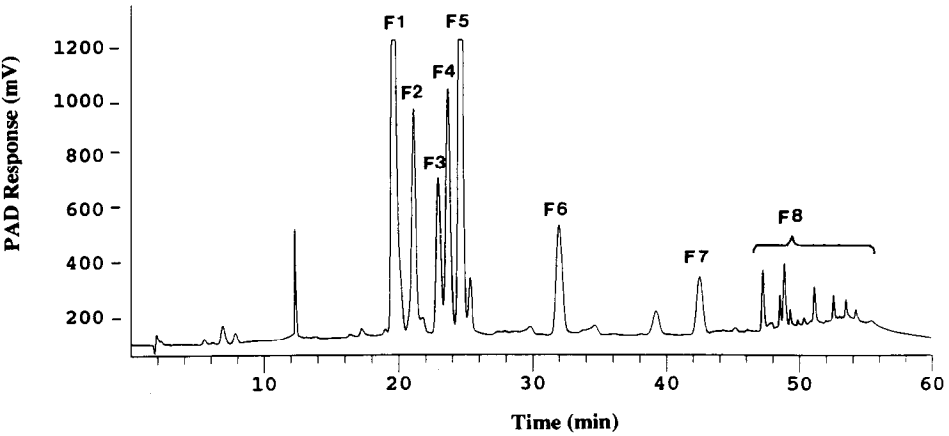

Fig. 1. Semipreparative HPAEC–PAD on a CarboPac PA-1 column (9 mm×25 cm) of the acidic oligosaccharides generated by partial acid hydrolysis of sycamore RG-1. The column was eluted at 5 mL/min with a gradient of NaOAc (0–600 mM) in 100 mM NaOH (see Experimental section for details). The eight fractions (F1–F8) were collected manually, desalted, and then freeze-dried.

The resulting neutral and acidic components were separated by DEAE-Sephadex chromatography. The neutral fraction was shown, by glycosyl-residue composition analysis, to contain Ara (45 mol%) and Gal (43 mol%), and smaller amounts of Rha (5 mol%), Fuc (4 mol%), and Xyl (3 mol%). The acidic fraction was shown, by glycosyl-residue composition analysis, to contain GalA (52 mol%)and Rha (34 mol%), and smaller amounts of Gal (8 mol%), GlcA (5 mol%), and 4-*O*-Me-GlcA (1 mol%).

Semipreparative HPAEC–PAD fractionation of the acidic material released by partial acid hydrolysis of sycamore RG-I.—The acidic material released by partial acid hydrolysis of sycamore RG-I was isolated by anion-exchange chromatography and then fractionated using a semipreparative CarboPac PA-1 column (Fig. 1). Eight fractions (F1–F8, see Fig. 1) were collected, desalted, and then freeze-dried. The components in each fraction were characterized by glycosyl-residue and glycosyl-linkage composition analyses, GC–CIMS, GC–EIMS, ESMS (electrospray mass spectrometry), and ^{1}H NMR spectroscopy.

Structural characterization of the glucosyluronic acid and 4-O-methyl glucosyluronic acid-containing oligosaccharides.—Three of the fractions (F2, F3, and F5, see Fig. 1) purified by semipreparative HPAEC–PAD were shown, by glycosyl-residue composition analyses, to contain oligosaccharides composed of Gal and 4-*O*-Me-GlcA (F2, see Table I) or Gal and GlcA residues (F3 and F5, see Table I). F3 also contained significant amounts of GalA (Table I), which was shown by HPAEC–PAD to be the free monosaccharide. Galacturonic acid eluted in F4, which was only partially resolved from F3. Fractions F2, F3, and F5 together accounted for approximately 9% of the acidic material recovered from partial acid-hydrolyzed RG-I (Table I).

TABLE I

Glycosyl-residue compositions (mol%) of the acidic carbohydrates recovered from partially acid-hydrolyzed sycamore *RG-I*

Residue	HPAE Fractions [a]							
	F1	F2	F3	F4	F5	F6	F7	F8
Rha	49	tr	[b]			48	46	31
GalA	51	5	38 [c]	81 [c]	tr	52	54	59
Gal		53	35	10	47			7
GlcA			27	9	53			
Ara								3
4-Me-GlcA		42						
Mol% of all products recovered	31	3	2	8	4	7	6	40

[a] See Fig. 1 and text for details. [b] Not detected. [c] All the galacturonic acid in F3 and F4 was shown, by HPAEC–PAD and glycosyl-linkage analysis, to be the free monosaccharide.

The ^{1}H NMR spectra of $NaBD_4$-reduced F2 contained a signal for an anomeric proton at δ 4.48 ($J_{1,2}$ 7.5 Hz) that was assigned to a glycosyl residue with the 4-*O*-methyl-β-D-*gluco* configuration. ESMS of $NaBD_4$-reduced F2 gave intense ions at m/z 391 and 396 corresponding to the $[M + NH_4]^+$ and $[M + Na]^+$ ions, respectively, of 4-*O*-Me-GlcA-galactitol. Considerably less intense ions were also present at m/z 553 and 570. These ions correspond to the $[M + NH_4]^+$ and $[M + Na]^+$ ions, respectively, of 4-*O*-Me-GlcA-Gal-galactitol. F2 was shown by glycosyl-residue composition and glycosyl-linkage analyses to contain terminal nonreducing 4-*O*-Me-Glc*p*A and 6-linked galactitol in the ratio 1.0:0.9 (Table II), establishing that β-D-4-*O*-Me-Glc*p*A-(1 → 6)-D-Gal is the major component in F2. The origin of the terminal nonreducing galactosyl residue present in F2 (see Table II) is not known. However, the terminal nonreducing galactosyl residue could have been generated during methylation of F2 by β-elimination of the 4-*O*-Me-Glc*p*A residue in the trisaccharide 4-*O*-Me-GlcA-Gal-galactitol.

The ^{1}H NMR spectra of $NaBD_4$-reduced F3 and F5 both contained a signal for an anomeric proton at δ 4.51 ($J_{1,2}$ 7.5 Hz) that was assigned to a glycosyl residue with the β-D-*gluco* configuration. GLC–MS of $NaBD_4$-reduced and methylated F3 and F5 established that the major component in both eluted in the region for a methyl-esterified, per-*O*-methylated monoglycosyluronic acid hexitol. The GLC–CI mass spectra of F3 and F5 both contained an ion at m/z 503 corresponding to the $[M + 18]^+$ ion of a methyl-esterified and per-*O*-methylated monoglycosyluronic acid hexitol. GLC–EIMS of F3 gave ions at m/z 395 (M-90), 296 ($aldJ_1$), 236 ($aldJ_2$), 233 (aA_1), and 201 (aA_2) that established the sequence HexA-(1 → 4)-hexitol. The abundance ratio of the aA_1 and aA_2 ions ($aA_2 \gg aA_1$) provided additional evidence that the aldobiouronic acid did indeed contain glucuronic acid, as the abundance of aA_2 ions being much greater than the abundance of aA_1 ions is diagnostic of terminal nonreducing methyl-esterified, methylated Glc*p*A, while $aA_2 = aA_1$ is diagnostic of Gal*p*A residues[7]. Glycosyl-linkage composition analysis

TABLE II

Glycosyl-linkage compositions (mol%) of the $NaBD_4$-reduced acidic oligosaccharides recovered from partially acid-hydrolyzed RG-I

Residue	HPAE Fractions [a]							
	F1	F2	F3	F4	F5	F6	F7	F8
2-Rha	[b]					30	36	32
2,4-Rha								4
2-Rhamnitol	34	tr				10	7	tr
T-GalA [c]	66	tr				35	21	14
4-GalA [c]						25	36	30
GalA-ol [d]			50	98				
T-GlcA [c]			30	tr	52			
GlcA-ol [d]					1			
T-4-Me-GlcA [e]		48						
T-Gal		10						9
4-Galactitol			20	2				
6-Galactitol		42			47			
T-Ara*f*								4
5-Ara*f*								1
T-Fuc								2
T-Xyl								3

[a] See Fig. 1 and text for details. [b] Not detected. [c] Detected as their (6,6′-^{2}H)-reduced methylated alditol acetates. [d] The detection of (6,6′-^{2}H)-1,2,3,4,5-penta-*O*-methyl derivatives of galactitol and glucitol confirms that F3 and F4 both contained 50 and 98 mol%, respectively, of free galacturonic acid, while F5 contained 1 mol% of free glucuronic acid. [e] The identity of this glycosyl residue was confirmed by ethylation, carboxyl-reduction, hydrolysis, and acetylation, which gave the (6,6′-^{2}H)-1,5,6-tri-*O*-acetyl-2,3-di-*O*-ethyl-4-*O*-methyl glucitol derivative.

of F3 (Table II) confirmed that the galactitol was 4-linked. The combined results of these analyses establish that the major oligosaccharide component in F3 is β-D-Glc*p*A-(1 → 4)-D-Gal.

GLC–EIMS of reduced and methylated F5 gave ions at *m/z* 351 (M − 134), 296 ($aldJ_1$), 236 ($aldJ_2$), 233 (aA_1), and 201 (aA_2) that establish the sequence Glc*p*A-(1 → 6)-galactitol. The much greater abundance of aA_2 ions than aA_1 ions provided additional evidence that the oligosaccharide contained a terminal nonreducing glucosyluronic acid residue. Glycosyl-linkage analysis of F5 confirmed that the galactitol was 6-linked (Table II). The combined data show that the major oligosaccharide in F5 is β-D-Glc*p*A-(1 → 6)-D-Gal.

Structural characterization of the acidic oligosaccharides released by partial acid hydrolysis of the RG-I backbone.—Three of the fractions (F1, F6, and F7) purified by semipreparative HPAEC–PAD of partially acid-hydrolyzed RG-I were shown by glycosyl-residue composition analyses to contain approximately equimolar amounts of GalA and Rha (see Table I). Fractions F1, F6, and F7 together accounted for approximately 44% of the acidic material recovered from partial acid-hydrolyzed RG-I (Table I).

The ^{1}H NMR spectrum of F1 contained signals for anomeric protons at δ 5.23, 5.05, and 4.94 that were assigned to reducing 2-linked α-L-Rha*p*, terminal nonre-

ducing α-D-Gal*p*A, and reducing 2-linked β-L-Rha*p* residues, respectively. The ^{1}H NMR spectra of F6 and F7 contained signals for anomeric protons at δ 5.27, 5.23, 5.05, 5.01, and 4.94 that were assigned to 2-linked α-L-Rha*p*, 2-linked reducing α-L-Rha*p*, terminal nonreducing α-D-Gal*p*A, 4-linked α-D-Gal*p*A, and 2-linked reducing β-L-Rha*p* residues, respectively. ESMS analyses established that the predominant oligosaccharides in F1, F6, and F7 had the composition Gal*p*A-rhamnitol, (Gal*p*A)$_2$(Rha*p*)-rhamnitol, and (Gal*p*A)$_3$(Rha*p*)$_2$-rhamnitol, respectively.

GLC–EIMS of $NaBD_4$-reduced and methylated F1 gave ions at *m/z* 396 (M − 59), 352 (M − 103), 226 (aldJ$_1$), 233 (aA$_1$), 206 (aldJ$_2$), and 201 (aA$_2$) that established the sequence Gal*p*A-(1 → 2)-rhamnitol. Glycosyl-linkage analysis of F1 (Table II) confirmed that rhamnitol was 2-linked. Thus, the major component in F1 is α-D-Gal*p*A-(1 → 2)-Rha, which is the diglycosyl repeating unit of the RG-I backbone[2].

F6 and F7 were both shown by glycosyl-linkage analysis to contain terminal, nonreducing Gal*p*A, 4-linked Gal*p*A, 2-linked Rha*p*, and 2-linked rhamnitol (Table II). These data and the results obtained by ESMS and ^{1}H NMR spectroscopy establish that F6 contains the tetrasaccharide α-D-Gal*p*A-(1 → 2)-α-L-Rha*p*-(1 → 4)-α-D-Gal*p*A-(1 → 2)-L-Rha, and F7 contains the hexasaccharide α-D-Gal*p*A-(1 → 2)-α-L-Rha*p*-(1 → 4)-α-D-Gal*p*A-(1 → 2)-α-L-Rha*p*-(1 → 4)-α-D-Gal*p*A-(1 → 2)-L-Rha. Both of those oligosaccharides would be generated by incomplete acid hydrolysis of the RG-I backbone. They provide further evidence that the backbone of RG-I is indeed composed of a diglycosyl repeating unit[2].

F8, which accounted for 40% of the acidic material recovered from partial acid-hydrolyzed RG-I (Table I), eluted from the CarboPac column only when the concentration of sodium acetate reached between 400 and 600 mM. Thus, F8 probably contains a mixture of acidic oligosaccharides with dp's > 8. Glycosyl-residue and glycosyl-linkage composition analyses of F8 (Tables I and II) provided evidence that these fragments were also generated from the RG-I backbone by incomplete hydrolysis. In addition, the presence of 2,4-linked Rha*p*, terminal nonreducing Gal*p*, and terminal nonreducing Ara*f* residues (see Table II) suggests that some of these backbone fragments contain portions of the oligoglycosyl side chains[3,4].

GENERAL DISCUSSION

The results of this study have shown that RG-I, released from the walls of suspension-cultured sycamore cells with EPG, contains both β-D-Glc*p*A and 4-*O*-Me-β-D-Glc*p*A residues. This is the first report that RG-I, a defined primary cell-wall pectic polysaccharide, contains either of these hexuronic acids. Glc*p*A acid and 4-*O*-MeGlc*p*A have been shown to be present in various plant polysaccharides including the arabinogalactan-type gum exudates and glycanorhamnogalacturonan-type mucilages[8]. In those polysaccharides, Glc*p*A and 4-*O*-Me-Glc*p*A

usually occur as terminal, nonreducing residues α- or β-linked to position 4 or position 6 of a Gal*p* residue. We have now shown that in sycamore RG-I the terminal nonreducing β-D-Glc*p*A is linked to either position 4 or position 6 of a Gal*p* residue. However, we only found the terminal nonreducing 4-*O*-Me-β-D-Glc*p*A residue linked to position 6 of a Gal*p* residue. No oligosaccharides containing α-D-Glc*p*A or 4-*O*-Me-α-D-Glc*p*A residues were found in the partial acid hydrolysate of sycamore RG-I. Unpublished results of the authors have established that the aldobiouronic acids β-D-Glc*p*A-(1 → 4)-Gal, β-D-Glc*p*A-(1 → 6)-Gal, and 4-*O*-Me-β-D-Glc*p*A-(1 → 6)-Gal are also generated by partial acid hydrolysis of pea cell-wall RG-I. Thus, Glc*p*A and 4-*O*-Me-Glc*p*A residues are minor but probably common components of the primary cell-wall pectic polysaccharide RG-I. However, unequivocal evidence that Glc*p*A and 4-*O*-Me-Glc*p*A are components of RG-I will require the isolation and characterization of oligosaccharides that contain these residues and portions of the RG-I backbone.

The major acidic oligosaccharides generated by partial acid hydrolysis of RG-I, which have the structure [4)-α-D-Gal*p*A-(1 → 2)-α-L-Rha*p*-(1]$_n$, are backbone fragments of the polysaccharide. No oligosaccharides were found that contained consecutive Gal*p*A or Rha*p* residues, providing confirming evidence that the backbone of RG-I is composed of that diglycosyl repeating unit[2].

The results of previous studies have suggested that Gal*p*A residues might be present in the galactosyl-containing side chains of sycamore RG-I[2,4]. We did not detect oligosaccharides composed of Gal*p*A and Gal. Thus, if such structures do occur in sycamore RG-I, they must be present in amounts undetectable by the methods described here.

EXPERIMENTAL

Materials.—4-*O*-Me-β-D-Glc*p*A(1 → 2)-Xyl*p* was obtained from Megazyme (North Rocks, Australia).

Isolation of RG-I.—RG-I was purified as described[9] from the material solubilized by EPG treatment of purified walls of suspension-cultured sycamore cells[10].

Determination of the partial acid hydrolysis conditions that generated, from RG-I, maximum yields of aldobiouronic acids.—Solutions of RG-I (500 μg) in 2 M TFA (500 μL) were heated at 100°C for 10 min, 20 min, 40 min, 1 h, 2 h, or 4 h. The cooled hydrolysates were concentrated to dryness under a flow of air, and the residual acid removed by codistillation with 2-propanol. The residues were dissolved in water (500 μL) and loaded onto columns (0.5 × 1 cm) containing DEAE-Sephadex ($HCOO^-$ form). The columns were washed with water (2 mL), and the acidic material then eluted with aq 10% formic acid (2 mL). The acidic fractions were concentrated to dryness, and the residues were washed with 2-propanol (2 × 500 μL) to remove the remaining acid. Separate solutions of the residues in water (200 μL) were analyzed by HPAEC–PAD using an analytical (4.6 mm × 25 cm) CarboPac PA-1 column (Dionex Corp., Sunnyvale, CA). HPAEC–

PAD was performed with a Dionex metal-free BioLc interfaced to an Autolon Series 400 data system. Carbohydrate was detected with a pulsed electrochemical detector equipped with a gold working electrode. The column was eluted at 1 mL/min with a gradient of NaOAc (0–600 mM) in 100 mM NaOH as follows: 100 mM NaOH (0–1 min), 0–50 mM NaOAc (1–5 min), 50–150 mM NaOAc (5–20 min), 150–250 mM NaOAc (20–40 min), 250–600 mM NaOAc (40–50 min), and 600 mM NaOAc (50–55 min). The column was then reequilbrated in 100 mM NaOH for 15 min prior to the next injection.

Partial acid hydrolysis of RG-I.—A solution of RG-I (22 mg) in 2 M TFA (2 mL) was treated for 1 h at 100°C. The hydrolysate was concentrated to dryness under a flow of air, and the residual acid was removed by codistillation with 2-propanol. A solution of the residue in water (1 mL) was applied to a column (1.0 × 5 cm) of DEAE-Sephadex A-25 ($HCOO^-$ form). The column was washed with water (5 mL) to elute the neutral material and then washed with aq 10% formic acid (5 mL) to elute the acidic carbohydrates. The neutral and acidic fractions were concentrated to dryness under reduced pressure, the residues dissolved in water, and then freeze-dried.

Purification of the acidic oligosaccharides obtained by partial acid hydrolysis of RG-I using semipreparative HPAEC–PAD.—The acidic oligosaccharides were purified using a semipreparative (9 mm × 25 cm) CarboPac PA1 column with a pulsed electrochemical detector equipped with a gold working electrode. The column was eluted at 5 mL/min with a gradient of NaOAc (0–600 mM) in 100 mM NaOH as follows: 100 mM NaOH (0–1 min), 0–50 mM NaOAc (1–5 min), 50–150 mM NaOAc (5–20 min), 150–250 mM NaOAc (20–40 min), 250–600 mM NaOAc (40–50 min), and 600 mM NaOAc (50–55 min). The column was then reequilbrated in 100 mM NaOH for 15 min prior to the next injection. Fractions were collected manually, neutralized with acetic acid, desalted by elution through columns (5 mm × 5 cm) containing Dowex-50W × 12 (H^+) resin, and then freeze-dried.

Glycosyl-residue composition analysis.—Glycosyl-residue compositions were determined by analysis of the per-*O*-trimethylsilylated methyl glycosides by GLC[10]. The derivatives were separated in a J&W Scientific DB-1 column (0.25 mm × 30 m) using an HP-5880 gas chromatograph.

Glycosyl-linkage analysis.—Acidic oligosaccharides were converted to their corresponding acidic oligoglycosyl alditols by reduction with $NaBD_4$ in M NH_4OH as described[10]. Solutions of the acidic oligoglycosyl alditols (100 μg) in Me_2SO (100 μL) were methylated with butyllithium and iodomethane as described[11]. The methyl-esterified and per-*O*-methylated acidic oligoglycosyl alditols were then isolated using SepPak C_{18} cartridges[12]. The glycosyl-linkage compositions of the oligoglycosyl alditols were determined by GLC–MS of the partially methylated alditol acetates as described[9]. Methyl-esterified uronic acid residues were carboxyl-reduced with lithium triethylborodeuteride (Superdeuteride, Aldrich) prior to glycosyl-linkage analysis[10].

GLC–MS analysis of the methylated monoglycosyl alditols.—Methyl-esterified and per-*O*-methylated monoglycosyluronic acid alditols were separated by on-column injection on a DB-1 column (0.25 mm × 15 m) and analyzed by GLC–MS using an HP-5985 GLC–MS instrument in both the CI (ammonia as reagent gas) and EI modes.

Electrospray mass spectrometry.—Electrospray mass spectrometry was performed with an API III Biomolecular Mass Analyzer (PE-Sciex, Thornhill, Canada) interfaced to a Macintosh IIfx data station. The mass spectrometer was operated in the positive-ion mode with an ion-spray voltage of 5000 volts and orifice potential of 35 volts. The uronic acid-containing oligoglycosyl alditols were converted to their ammonium form by elution with water through a Chelex 100 resin (ammonia form) column (0.5 × 5 cm). Solutions (1 μg/μL) of the carbohydrate in aq 20% MeOH were introduced into the electrospray source at 2 μL/min using a Harvard 22 syringe infusion pump. The mass range was scanned from 200–1200 amu. Ten scans were collected and averaged.

^{1}H NMR spectroscopy.—^{1}H NMR spectroscopy was performed with a Bruker AM 500 spectrometer. Spectra were obtained (in D_2O) at 500 MHz. Chemical shifts are reported in ppm relative to the HOD signal (δ 4.76).

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