Carbohydrate Research, 185 (1989) 279–305
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

RHAMNOGALACTURONAN I, A PECTIC POLYSACCHARIDE THAT IS A COMPONENT OF MONOCOT CELL-WALLS*†

JERRY R. THOMAS‡, ALAN G. DARVILL, AND PETER ALBERSHEIM**
Complex Carbohydrate Research Center, School of Chemical Sciences, University of Georgia, U.S.D.A. Russell Research Center, P.O. Box 5677, Athens, Georgia 30613 (U.S.A.)

(Received December 21st, 1987; accepted for publication in revised form, August 19th, 1988)

ABSTRACT

Pectic polysaccharides were solubilized from the primary walls of suspension-cultured cells of maize *(Zea mays)* and rice *(Oryza sativa)*, and shown by glycosyl-linkage analysis to be similar to the pectic polysaccharide rhamnogalacturonan I (RG-I) that had previously been solubilized from walls of suspension-cultured sycamore *(Acer pseudoplatanus)* cells. Maize and rice are monocots; sycamore is a dicot. The backbone of sycamore RG-I had previously been shown to consist of the disaccharide repeating unit →4)-α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→. Side chains containing arabinosyl, galactosyl, and fucosyl residues are attached through O-4 of about half of the backbone rhamnosyl residues. To compare the structures of monocot and dicot RG-I, galactosyluronic acid residues in the maize and rice polysaccharides were selectively cleaved by treatment with lithium in ethylenediamine, and the structural characteristics of the oligoglycosylalditol products compared to those of oligoglycosylalditols previously isolated from sycamore RG-I. The strong resemblance of the glycosyl-linkage compositions of lithium-treated RG-I from maize, rice, and sycamore indicated the overall structural likeness of the three polysaccharides. Spectra obtained by fast-atom-bombardment mass spectrometry (f.a.b.-m.s.) of lithium-treated maize and sycamore RG-I showed that most of the oligoglycosylalditols derived from the side chains of the two polysaccharides were the same. Nine oligoglycosylalditols partially purified from maize RG-I were similar to the corresponding components of sycamore RG-I. F.a.b.-m.s. of the maize reaction-products indicated the presence in maize RG-I of an additional eight oligoglycosylalditols previously isolated from sycamore RG-I. On the other hand, seven oligoglycosylalditols were partially purified from maize RG-I that had not been observed in previous studies of sycamore RG-I, and maize RG-I was shown not to contain fucosyl residues (as does sycamore RG-I). The results described

*Structure of Plant Cell Walls, Part XXV. For Part XXIV, see ref. 1.

†Supported by U.S. Department of Energy Grants 09-85ER13424.000 and 09-85ER13426.000.

‡Current address: c/o Dr. Raymond Dwek, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, Great Britain.
**To whom correspondence should be addressed.

0008-6215/89/$ 03.50

here suggest that, despite the observed differences, many of the structural features of RG-I have been conserved in the primary cell-walls of monocots and dicots.

INTRODUCTION

The pectic polysaccharides of monocots have not been as well studied as those of dicots. The first evidence of rhamnogalacturonans in monocot cell-walls was provided by Aspinall and Cañas-Rodriguez[2]; they extracted from the flesh sisal *(Agave sisalana)* a polysaccharide that contains, *inter alia*, 4-linked galactosyluronic acid, 2-linked rhamnosyl, and 4-linked galactosyl residues. Later, Ray and Rottenburg[3] presented evidence that cell walls of oat *(Avena sativa)* coleoptiles contain the acidic disaccharide sequence α-D-Gal*p*A-(1→2)-L-Rha. More recently, Shibuya and Nakane[4] isolated, from the cell walls of rice *(Oryza sativa)* endosperm, polysaccharides that have glycosyl-linkage compositions similar to that[5] of the sycamore *(Acer pseudoplatanus)* pectic polysaccharide, RG-I. However, detailed information has been presented neither on the structure of the rice-endosperm polysaccharides, nor on any other pectic polysaccharides isolated from monocot cell-walls.

RG-I belongs to a class of pectic polysaccharides that have been isolated from a variety of plant sources[6], including gums and exudates[7]. RG-I is solubilized from the walls of suspension-cultured sycamore cells by an endo-α-1,4-polygalacturonanase (EPGase) isolated from *Colletotrichum lindemuthianum*[5]. The backbone of RG-I consists[8] of the disaccharide repeating unit →4)-α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→. Side chains containing arabinosyl, galactosyl, fucosyl, and, perhaps, galactosyluronic acid residues are attached through O-4 of about half of the rhamnosyl residues in the backbone, *i.e.*, about half of the rhamnosyl residues in the polysaccharide are branched[5]. The distribution of the branched rhamnosyl residues along the backbone appears to be regular[8].

Side chains can be released from the backbone of RG-I by using a recently perfected chemical method[9] that cleaves the backbone while leaving neutral side-chains intact[10]. Glycosidic linkages both to and from galactosyluronic acid residues are selectively cleaved by treatment of the polysaccharide with lithium metal dissolved in ethylenediamine. The resulting oligoglycosylalditol products have an alditol at their "reducing" terminus derived from a glycosyl residue that was glycosidically linked to a galatosyluronic acid residue in the intact polysaccharide. Thus, when sycamore RG-I was treated with lithium–ethylenediamine, all of the abundant side-chains that were isolated had rhamnitol at their "reducing" terminus[10]. In the present study, RG-I was extracted from the walls of suspension-cultured maize *(Zea mays)* and rice *(Oryza sativa)* cells, and purified. In order to compare the structures of monocot and dicot RG-I, oligoglycosylalditols were derived from maize and rice RG-I by treatment with lithium–ethylenediamine, and their structural features compared to those of oligoglycosylalditols previously isolated from sycamore RG-I.

EXPERIMENTAL

Analytical methods. — Glycosyluronic acid and neutral glycosyl residues were quantitated as their per-*O*-trimethylsilyl methyl glycosides, synthesized[11] and analyzed[12] as described. Aceric acid (3-*C*-carboxy-5-deoxy-L-xylose) residues were quantitated as acetylated alditols[12]. Cellulose was determined by Updegraff's method[13]. Total nitrogen and ash determinations were made by Galbraith Laboratories (Knoxville, Tennessee). Protein content was estimated by multiplying total nitrogen values by 6.25. Glycosyl-linkage compositions were determined as described[12,14]. To determine the linkages of glycosyluronic acid residues, per-*O*-methylated polysaccharides were reduced with Li–triethylborodeuteride (M in tetrahydrofuran) for[12] 1 h. Oligoglycosylalditols and polysaccharides were per-*O*-methylated by a modification[12] of the method of Hakomori[15], and purified by using C_{18} cartridges[16]. Oligoglycosylalditols were per-*O*-acetylated[17] using 2:1 (v/v) trifluoroacetic anhydride–acetic acid for 10 min at room temperature; reagents were removed with a stream of filtered air. Absolute configurations were determined as described by Gerwig *et al.*[18], using (+)-2-butanol.

Maize-cell culture and preparation of cell-walls. — Suspension cultures of Black Mexican sweet corn (obtained from C. M. Donovan and derived from stem sections of seedlings) were grown in the medium of Murashige and Skoog[19] containing 2.5 mg of 2,4-dichlorophenoxyacetic acid/L. Cell walls were isolated as described[20], except that a pressure bomb was used to rupture the cells.

Sequential extraction of maize cell-walls. — To a suspension of cell-walls (24.1 g) in 2.4 L of 0.1M K phosphate (pH 7.0) containing 0.01% of thimerosal was added 100 mg of alpha amylase from *Bacillus* species (type II-A, Sigma Chemical). After stirring for 48 h at 25°, the cell walls were collected by filtration, using a sintered-glass funnel. The filtrate was concentrated under diminished pressure at 40°, and the concentrate dialyzed against distilled H_2O, all dialyses being performed using tubing with a nominal molecular-weight cutoff of 12,000 to 14,000. The alpha amylase treatment was repeated twice as described, and the three extracts were combined; lyophilization of an aliquot indicated the yield to be 10% (w/w) of the starting cell-wall material. Prior to dialysis, the filtrates were analyzed for neutral glycosyl residues, using the anthrone method[21], to determine the amounts of starch and β-D-glucan in the starting material that were lost during dialysis of the alpha amylase extracts. (Note that an endo-β-1→3,1→4-glucanase is known to be present in the bacterial enzyme preparation[22].) A total of 8.5% (w/w) of the starting cell-wall material was solubilized by the three alpha amylase treatments as D-glucosyl equivalents.

The alpha amylase-residual wall-material was suspended in 1.5 L of H_2O and stirred for 0.5 h at 95°. Residual wall-material was collected by filtration, and the filtrate was concentrated, dialyzed, and frozen as just described. The procedure was repeated, and the filtrates were combined; the yield was 2.2% (w/w) of the starting material.

The hot-water-residual wall-material was resuspended in 1.5 L of dimethyl sulfoxide, and the suspension was stirred for 72 h at 25°. Residual wall-material was collected as just described, and dialyzed against cold H_2O. The filtrate was dialyzed, concentrated, and frozen. The dimethyl sulfoxide-extracted material accounted for 1.6% (w/w) of the starting cell-wall material.

The wall-material remaining after extraction with dimethyl sulfoxide was suspended in 1.0 L of 0.5% (w/v) ammonium oxalate, and stirred for 1 h at 95°. The residue was collected as described, and resuspended in ammonium oxalate. After three such extractions, the filtrates were combined, dialyzed, concentrated, and frozen. The yield of oxalate-solubilized material was 1.0% (w/w) of the starting material. The residual wall-material was dialyzed against 50mM Na acetate (pH 5.2).

The dialyzed wall-material was suspended in 1.5 L of 50mM Na acetate (pH 5.2) containing 0.02% of thimerosal, and 20,000 units of EPGase (purified as described[23]) was added. After 96 h at 25°, the residue was collected as described, and resuspended in fresh enzyme solution. The residual wall-material was collected, and both filtrates were dialyzed, and lyophilized. The yields of the first and second treatments with EPGase were 0.7 and 0.5% (w/w), respectively, of the starting cell-wall material.

Th EPGase-residual wall-material was resuspended in 1.5 L of 50mM Na_2CO_3 and stirred for 16 h at 4°. After filtration, the residue was resuspended in fresh Na_2CO_3, and stirred for 3 h at 25°. The residue was collected by filtration, and made neutral with acetic acid. The two filtrates were made neutral, concentrated, dialyzed, and lyophilized. The yields of the Na_2CO_3 (4°) and Na_2CO_3 (25°) extracts were 0.5 and 0.3% (w/w), respectively, of the starting cell-wall material.

The Na_2CO_3-residual wall-material was resuspended in 1.0 L of 1.0M KOH containing 1 mg $NaBH_4$/mL, through which N_2 had been bubbled, and stirred for 2 h at 25° with continuous bubbling of N_2. The residue was collected by filtration, and resuspended in 1.0 L of 4.0M KOH containing 1 mg $NaBH_4$/mL, through which N_2 had been bubbled, and stirred for 2 h at 25° with continuous bubbling of N_2. The residue was collected by filtration, and resuspended in 1.0 L of 4M KOH containing 4% boric acid, through which N_2 had been bubbled, and stirred for 2 h at 25° with continuous bubbling of N_2. The residue was collected by filtration, and all filtrates were made neutral, concentrated, dialyzed, and frozen. The yields of extraction with 1.0M KOH, 4.0M KOH, and 4.0M KOH with 4% boric acid were 9.1, 13.8, and 1.5% (w/w), respectively, of the starting cell-wall material. The residue remaining after the final extraction accounted for 3.93 g (16.3%) of the starting cell-wall material.

Gel-permeation chromatography of polysaccharides extracted from maize cell-walls with EPGase. — The Bio-Gel P-10 (3.4 × 64 cm) and Bio-Gel A-5m (2.5 × 45 cm) columns were equilibrated in and eluted with 50mM Na acetate (pH 5.2). Pooled fractions from the columns were passed through Dowex-50W (H^+) ion-exchange resin (Sigma Chemical) prior to lyophilization and analysis. Void and

included volumes were determined by chromatography of blue dextran (Pharmacia) and D-glucose, respectively.

Prior to gel-permeation chromatography, EPGase-extracted polysaccharides were de-esterified and treated with EPGase. Material obtained from the first EPGase treatment (185 mg) was dissolved in 10.0 mL of 0.1M NaOH and incubated for 16 h at 25°. After adjusting the pH of the solution to 5.2 with acetic acid, 5,000 units of EPGase was added, and the solution was incubated for 72 h at 25°. The mixture was applied to the Bio-Gel P-10 column. Sycamore RG-I was treated identically, and applied separately to the Bio-Gel A-5m column, for comparison.

Isolation of RG-I from rice cell-walls. — The isolation of rice RG-I is described in the accompanying article[1].

Lithium–ethylenediamine treatment of RG-I polysaccharides. — The procedure used was the modification of the method of Mort and Bauer[24] reported by Lau *et al.*[9].

H.p.l.c. of the per-O-methylated oligoglycosylalditol products of lithium treatment. — Per-*O*-methylated oligoglycosylalditols were fractionated by reversed-phase h.p.l.c. using a C_{18} column (4.5 × 25 cm). Samples were loaded in 3:17 (v/v) acetonitrile–H_2O, and eluted with concentrations of acetonitrile increasing to 30% after 30 min and to 100% after 70 min.

G.l.c.–m.s. of per-O-methylated oligoglycosylalditols. — Per-*O*-methylated oligoglycosylalditols in h.p.l.c. fractions were analyzed by g.l.c.–m.s. with electron-impact (e.i.) ionization, using a 15-m DB-1 capillary column (J and W Scientific) and on-column injection. The temperature program used for mono- and di-glycosylalditols was 50° for 0.5 min, 30°/min to 170°, and 6°/min to 340°; for larger oligoglycosylalditols, the temperature program was 50° for 0.5 min, 30°/min to 200°, and 12°/min to 340°.

Fast-atom-bombardment mass spectrometry (f.a.b.-m.s.) of per-O-methylated and per-O-acetylated oligoglycosylalditols. — Analysis of per-*O*-methylated oligoglycosylalditols by positive-ion f.a.b.-m.s. was performed by dissolving samples in methanol, loading 1 μL into glycerol containing[25] 0.1M NaCl on a stainless-steel target, and using a VG Analytical ZAB-SE mass spectrometer fitted with an Ion Tech f.a.b. gun. Xenon was the bombarding gas, and the gun was operated at 8 kV, 1mA. Linear, mass-controlled scans were obtained at a rate that covered the mass range from 2000 to 1 mass unit in 30 s. Positive-ion f.a.b.-m.s. of per-*O*-acetylated oligoglycosylalditols was performed by dissolving samples in methanol and adding 1 μL to 1:1 (v/v) glycerol–2-thioglycerol on the target. Spectra were obtained at a scan rate that covered the mass range from 3000 to 1 mass unit in 2 min.

^{1}H-n.m.r. spectroscopy of per-O-methylated oligoglycosylalditols. — One-dimensional spectra of per-*O*-methylated oligoglycosylalditols were recorded with a Bruker AM-500 spectrometer operated at 500 MHz and a temperature of 300K. Samples were dissolved in hexadeuterioacetone (99.997%), and chemical shifts are reported relative to pentadeuterioacetone (δ 2.04).

RESULTS

Analyses of maize cell-wall extracts. — A total of 41.2% of the maize cell-wall starting-material was accounted for by the non-dialyzable material obtained with the ten treatments used in this study (see Table I). Starch and noncellulosic β-D-glucan, which were lost from the alpha amylase extract during dialysis, together accounted for 8.5% of the starting material; protein accounted for 13.5%, cellulose for 21%, and ash for 2.6% of the starting material. Thus, a total of 86.8% of the starting material was accounted for; the remaining 13.2% was probably lost during dialysis of cell-wall extracts, *e.g.*, as galactosyluronic acid oligomers produced during EPGase treatment.

All extracts, except that obtained by EPGase treatment, were rich in xylosyl and arabinosyl residues (see Table I), suggesting the presence of the arabinoxylans reported to be major components of monocot cell-walls[26]. None of the extracts contained significant proportions of glucuronoarabinoxylans[26], because neither glucosyluronic nor 4-*O*-methylglucosyluronic acid residues were detected. The presence of arabinoxylans in the cell-wall extracts was verified by analysis of their glycosyl-linkage compositions (see Table II). All extracts, except the EPGase-extracted material, were rich in the terminal arabinosyl and 4- and 3,4-linked xylosyl residues characteristic of monocot arabinoxylans[26]. In addition to arabinoxylan, the three KOH extracts probably contained xyloglucan, as suggested by the

TABLE I

GLYCOSYL COMPOSITIONS[a] OF MAIZE CELL-WALL EXTRACTS

Extract	*Weight % of starting cell-wall material*[b]	*Glycosyl residue*							
		Rha	*Fuc*	*Ara*	*Xyl*	*Gal*	*Glc*	*GalA*	*GlcA*
		Normalized weight (%)							
Alpha amylase[c]	10.0[d]	3.5	0.6	24.4	29.4	4.4	1.7	33.3	2.7
Hot water[c]	2.2	2.8	[e]	18.6	30.5	7.3	8.6	32.2	
Dimethyl sulfoxide	1.6	2.9	0.3	19.5	30.5	7.3	22.7	16.8	
Ammonium oxalate	1.0	4.3	0.3	20.4	28.1	8.6	6.9	29.5	1.6
EPGase[c,f]	1.2	20.1		28.1	3.3	21.0		27.4	
Na_2CO_3 (4°)	0.5	2.0	0.5	28.6	34.3	4.1	8.5	21.0	1.4
Na_2CO_3 (25°)	0.3	1.5		33.8	42.4	4.4	4.1	13.8	
M KOH	9.1	1.2		33.1	43.4	7.4	4.8	7.5	2.6
4M KOH	13.8	1.2		25.1	40.3	13.5	13.1	6.8	
KOH–borate	1.5	1.2		24.5	36.8	13.5	15.0	9.1	
Total	41.2[d]								

[a]Determined by g.l.c. of methyl per-*O*-(trimethylsilyl)-glycosides. [b]All extracts were dialyzed prior to analysis. [c]These extracts also contained traces of glycosyl residues diagnostic of rhamnogalacturonan II (see text). [d]Value does not include starch and β-linked D-glucan lost during dialysis (see text). [e]Indicates the residue was not detected. [f]Composition is of the first EPGase extract; that of the second was similar to the first.

high levels of terminal and 2-linked xylosyl, terminal galactosyl, and 4- and 4,6-linked glucosyl residues[25]. The absence of terminal fucosyl and 2-linked galactosyl residues indicated that the xyloglucan in these extracts was similar to that isolated from other monocot cell-walls[26].

The hot-water and dimethyl sulfoxide extracts also contained small proportions (<0.1% of the cell wall) of noncellulosic, β-(1→3,1→4)-linked glucan[26], as evidenced by the presence of 3-linked glucosyl residues (see Table II). However, if more of this noncellulosic β-glucan was present in the cell walls, it was either solubilized by the preceding alpha amylase treatments or lost during dialysis of the extracts with high-molecular-weight cutoff tubing (see the Experimental section). Noncellulosic β-glucans have been isolated from the cell walls of maize shoots and coleoptiles (see ref. 27 and references cited therein); there was no evidence of their presence in the walls of other maize tissues.

The traditional methods for removing pectic polysaccharides from plant cell-walls (hot water, dimethyl sulfoxide, ammonium oxalate, and EPGase) produced extracts of maize cell-walls that were rich in galacturonic acid (see Table I). However, only the EPGase extract contained a relatively high proportion of rhamnosyl residues characteristic of rhamnogalacturonans (see Table I). The glycosyl-linkage composition of the EPGase extract resembled that of RG-I isolated from walls of suspension-cultured sycamore cells by EPGase treatment[5]. The glycosyl-linkage composition of sycamore RG-I is included in Table II for comparison. Like sycamore RG-I, the EPGase extract of maize contained rhamnosyl residues that were predominantly 2- and 2,4-linked, arabinosyl residues that were predominantly terminal and 5-linked, and galactosyl residues that were linked at various positions. Furthermore, only 4-linked galactosyluronic acid residues were detected (see Experimental) in the maize polysaccharides (data not shown); the majority of the galactosyluronic acid residues in sycamore RG-I are 4-linked[5]. Interestingly, in contrast to sycamore[5] RG-I, the maize polysaccharide(s) solubilized by EPGase treatment contained no fucosyl residues (see Tables I and II). The absolute configurations of the arabinosyl, rhamnosyl, galactosyl, and galactosyluronic acid residues in the EPGase-extracted material were determined (see Experimental) to be the same as those in the sycamore RG-I, namely, L, L, D, and D, respectively.

Extracts of maize cell-walls obtained by several different treatments (alpha amylase, hot water, ammonium oxalate, and EPGase) contained traces of several glycosyl residues diagnostic of the rhamnogalacturonan II (RG-II) that has been isolated from cell walls of sycamore[28] and rice (see ref. 1). The diagnostic components of RG-II detected as alditol acetates[12] (data not shown) included 2-*O*-methylfucosyl, 2-*O*-methylxylosyl, apiosyl, and aceric acid[29] residues. These residues, as well as another diagnostic glycosyl component of RG-II, namely, 3-deoxy-D-*manno*-2-octulosonic acid, have been detected in walls of the same suspension-cultured maize cells used in this study[30]. Consistent with the very low levels of the glycosyl residues diagnostic of RG-II that were detected, no evidence of RG-II was obtained by glycosyl-linkage analyses of the maize cell-wall extracts, and no poly-

TABLE II

METHYLATION ANALYSIS OF EXTRACTS OF MAIZE CELL-WALLS

Glycosyl residue	*Deduced linkage*[a]	*Alpha amylase*	*Extract*								
			Hot water	*Dimethyl sulfoxide*	*Ammonium oxalate*	*EPGase*[b]	Na_2CO_3 (4°)	Na_2CO_3 (25°)	*M KOH*	*4M KOH*	*KOH–borate*
		Mol%									
Rhamnosyl	terminal	[c]			0.5	0.4 (2.2)[d]					
	2-linked[e]	0.6	0.6	0.9	2.6	5.7 (9.5)	3.2	2.2	1.0	0.7	0.8
	3-							1.7			
	2,4-[e]	0.7	3.4	5.3	6.5	11.8 (9.7)	8.1	1.3	1.5	1.8	3.6
	2,3,4-				1.7	2.1 (0.7)	1.7	0.4	0.7	0.4	0.3
Fucosyl	terminal					1.5 (1.7)					
Arabinosyl	terminal	34.0	25.8	18.4	20.8	14.9 (11.6)	16.4	22.1	26.4	12.1	10.7
	2-linked[e]	0.7	0.7	1.0	1.4	0.8 (2.7)	0.9	2.6	0.6	0.9	1.0
	3-	1.8	1.1	2.0	2.0	2.5 (2.7)	1.5	1.5	1.0	0.8	1.0
	5-	2.6	4.1	4.5	6.9	13.0 (13.6)	6.6	4.3	2.1	2.5	2.4
	2,5-	0.5	1.0	0.8	0.4	1.4 (1.2)	0.9	0.8			
	3,5-	2.1	2.3	2.9	3.1	6.1 (4.3)	3.4	1.7	1.5	2.1	1.0
Xylosyl	terminal	4.6	6.6	6.0	10.1	1.1 (2.4)	7.7	4.6	5.8	10.0	9.1
	2-linked[e]	1.9			3.4		2.2	1.0	2.5	3.2	2.9
	4-[e]	7.6	9.1	7.5	6.7	1.0	4.3	11.3	4.9	3.2	2.9
	3,4-	34.1	29.6	23.0	19.2	1.1	13.6	33.4	23.6	12.3	11.0
Galactosyl	terminal	2.9	3.3	2.5	5.2	14.2 (7.7)	9.0	2.2	3.6	6.3	5.7
	2-linked				0.4	0.4 (0.7)	0.5			0.5	0.4
	3-	0.7			1.3	3.5 (3.3)	2.2	0.8	1.0	2.2	4.4
	4-	0.7	1.3	1.6	2.2	4.8 (10.3)	4.1	1.3	3.1	1.3	1.0
	6-				1.6	4.8 (9.1)	3.6	0.8	0.7	1.3	1.1
	2,4-					0.4 (0.6)	1.2				
	3,4-					2.5	1.2				
	2,6-	0.7		1.5		0.7 (1.5)					
	3,6-					1.9 (1.5)	3.5		2.4	1.0	0.9
	4,6-					2.6 (2.9)	1.8	1.1	1.9	0.9	0.8

Glucosyl	terminal			1.4		1.1		2.3	1.8	1.0
	3-linked[e]	0.7	7.2	5.6			1.4			3.8
	4-	1.6	3.9	2.3	4.4		2.2	6.8	20.3	20.7
	2,3-			6.7						
	3,4-			2.1						
	4,6-					1.2	1.3	6.6	14.3	13.6

[a]Where ambiguous, hexosyl, deoxyhexosyl, and xylosyl residues were assumed to be in the pyranose ring form, and arabinosyl residues in the furanose ring form. [b]Composition is of the first EPGase extract; that of the second was similar to the first. [c]Indicates that the derivative was not detected. [d]Corresponding values for sycamore RG-I are shown in parentheses; values are those summarized by York *et al.*[11]. [e]Two- and 4-linked xylosyl residues coeluted; 2,4-linked rhamnosyl and 3-linked glucosyl residues coeluted; and 2-linked rhamnosyl and 2-linked arabinosyl residues coeluted from the SP-2330 g.l.c. column; relative amounts were estimated by mass-spectrometric analysis.

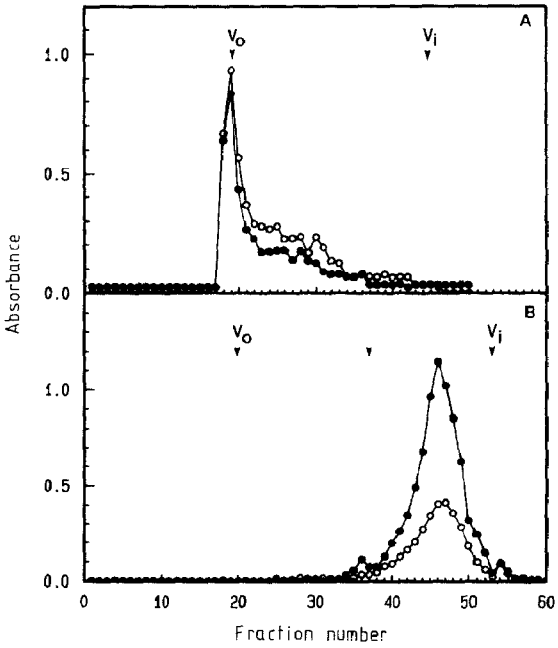

Fig. 1. Gel-permeation chromatography of EPGase-extracted polysaccharides (see the Experimental section for details). A, Chromatography on Bio-Gel P-10 of EPGase-extracted polysaccharides. Column fractions 18 to 21 were pooled for further chromatography on Bio-Gel A-5m. B, Chromatography on Bio-Gel A-5m of Bio-Gel P-10 fractions 18 to 21. For comparison, the elution volume of RG-I isolated from the walls of suspension-cultured sycamore cells is indicated with an arrow at fraction 38. Maize RG-I was eluted as a single peak centered about column fraction 46. Column fractions were assayed for neutral-sugar content by the anthrone method[21] (A_{620}, closed circles) and for uronic-acid content by the *m*-hydroxybiphenyl method[30] (A_{520}, open circles).

saccharide(s) enriched in the diagnostic glycosyl residues was obtained by fractionating each extract by using gel-permeation chromatography (results not shown).

Because the polysaccharide extracted by EPGase from maize cell-walls resembled sycamore RG-I, a closer comparison of the two polysaccharides was made. The maize polysaccharides extracted by EPGase were purified by gel-permeation chromatography. Then, side chains were derived from maize RG-I as oligoglycosylalditols by treatment with lithium–ethylenediamine. Finally, the side chains were fractionated and characterized, and their structures compared to those previously characterized from[10] sycamore RG-I.

Purification by gel-permeation chromatography of RG-I extracted from maize cell-walls with EPGase. — The results of chromatography on Bio-Gel P-10 of material extracted from maize cell-walls by EPGase treatment are shown in Fig. 1A. The EPGase-extracted material was chromatographed on Bio-Gel P-10 after de-esterification and re-treatment with EPGase to insure that all homogalacturonan had been removed from the polysaccharides(s). A 5% aliquot of the material that

voided the P-10 column (fractions 18–21) was chromatographed on Bio-Gel A-5m (see Fig. 1B). All of the carbohydrate was eluted as a single peak from the Bio-Gel A-5m column, and comparison of the elution volume with that of RG-I obtained from walls of suspension-cultured sycamore cells (see Fig. 1B) indicated that maize RG-I was smaller than sycamore RG-I. The material that voided the Bio-Gel P-10 column (fractions 18–21) had glycosyl and glycosyl-linkage compositions (data not shown) very similar to those of the total EPGase extract (see Table II) and was used for the experiments described next.

Lithium–ethylenediamine treatment of maize and rice RG-I

Glycosyl and glycosyl-linkage compositions of the products of lithium treatment. — The glycosyl compositions of the products of lithium–ethylenediamine treatment of maize and rice RG-I were determined by g.l.c. after methanolysis and per-*O*-(trimethylsilyl)ation as already described. A comparison of the relative amounts of galactosyluronic acid residues detected before and after lithium treatment (see Table III) indicated extensive degradation of the galactosyluronic acid residues. Assuming little degradation of neutral glycosyl residues under the reaction conditions that were used[9,10], and comparing the galactosyluronic acid residue compositions of the products and the starting material, we calculated that >90% of the galactosyluronic acid residues in maize and rice RG-I were cleaved.

Residues glycosidically linked prior to methanolysis were detected as their trimethylsilyl methyl glycosides, and they gave rise to multiple signals in g.l.c. analysis; alditols gave rise to single signals distinct from those of the methyl glycosides. Thus, the ratio of alditols to the corresponding glycosidically linked residues could be determined. Alditols are formed during lithium treatment by cleavage of glycosidic bonds to galactosyluronic acid residues, and reduction of C-1

TABLE III

GLYCOSYL COMPOSITIONS OF MAIZE AND RICE RG-I BEFORE AND AFTER TREATMENT WITH LITHIUM IN ETHYLENEDIAMINE

Glycosyl residue	*Maize RG-I*[a]	*Lithium-treated maize RG-I*	*Rice RG-I*	*Lithium-treated rice RG-I*
	Normalized weight %			
Arabinose	28	37 (14)[b]	30	34 (23)
Rhamnose	20	21 (67)	4	8 (92)
Fucose	[c]		tr[d]	1
Xylose	3	6	9	8
Galactose	21	34 (12)	49	49 (9)
Galacturonic acid	27	2	7	tr

[a]Data from Table I. [b]Numbers in parentheses are percentages of that glycosyl residue detected as the alditol, rather than the methyl glycoside, and therefore assumed to be reducing-terminal residues of oligoglycosylalditols (see text). [c]Indicates residue not detected. [d]Indicates residue was present at levels less than 1%.

TABLE IV

METHYLATION ANALYSES OF MAIZE, RICE, AND SYCAMORE RHAMNOGALACTURONAN I BEFORE AND AFTER TREATMENT WITH LITHIUM IN ETHYLENEDIAMINE

Glycosyl residue and deduced linkage[a]	*Maize RG-I*[b]	*Lithium-treated maize RG-I*	*Rice RG-I*[c]	*Lithium-treated rice RG-I*	*Sycamore RG-I*[d]	*Lithium-treated sycamore RG-I*
	Mol %					
Rhamnosyl						
terminal	0.4	0.7			2.2	0.2
2-linked[e]	5.7	0.5	1.1	0.9	9.5	0.4
4-	[f]	3.6				1.4[g]
2,4-	11.8	0.9	5.2		9.7	0.1
2,3,4-	2.1	0.5	0.4		0.7	0.1
Galactosyl						
terminal	14.2	12.1	5.9	14.0	7.7	17.7
2-linked	0.4	0.9	0.5	3.3	0.7	1.8
3-	3.5	4.3	1.9	6.9	3.3	8.4
4-	4.8	3.6	5.8	4.8	10.3	13.3
6-	4.8	6.9	2.0	14.6	9.1	11.9
2,4-	0.4	1.1		1.4	0.6	1.0
3,4-	2.5	2.0	1.0	0.9		0.5
2,6-	0.7	2.1		1.9	1.5	2.8
3,6-	1.9	2.6	7.5	6.5	1.5	4.1
4,6-	2.6	1.6		5.8	2.9	0.5
3,4,6-		0.5				
Arabinosyl						
terminal	14.9	19.4	16.2	7.4	11.6	7.8
2-linked[e]	0.8	1.2	3.2	1.0	2.7	1.8
3-	2.5	4.6	2.3	3.3	2.7	2.5
5-	13.0	13.6	13.3	7.8	13.6	11.2
2,5-	1.4	1.4	1.2	2.8	1.2	5.4
3,5-	6.1	6.8	5.1	2.6	4.3	4.4
Xylosyl						
terminal	1.1	3.2	4.0	4.6	2.4	0.7
4-linked	1.0	2.6	0.9	4.0		
3,4-	1.1	2.7	7.0	0.9		
Fucosyl						
terminal			1.4	1.4	1.7	2.4

[a]For example, 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylrhamnitol was deduced to have arisen from 4-linked rhamnopyranosyl residues. Where ambiguous, galactosyl, deoxyhexosyl, and xylosyl residues were assumed to be in the pyranose ring form, and arabinosyl residues in the furanose ring form. [b]Data from Table II (see text). [c]Data of Thomas *et al.*[1]. [d]Data summarized by York *et al.*[11]. [e]Relative amounts of these two coeluting derivatives were estimated by mass-spectrometric analysis. [f]Derivatives were not detected. [g]These 4-linked rhamnosyl residues were not reproducibly observed (see text).

of the new reducing termini[9,10]. The higher percentages of rhamnosyl residues detected as alditols after lithium treatment indicated that rhamnosyl residues were most often linked to galactosyluronic acid residues in intact maize and rice RG-I, as is the case[8,10] in sycamore RG-I. Significant proportions of arabinosyl and galactosyl residues also appeared to be glycosidically linked to galactosyluronic acid residues in both maize and rice RG-I; lithium treatment of sycamore RG-I results in reduction of 9, 7, and 86%, respectively, of the arabinosyl, galactosyl, and rhamnosyl residues in the polysaccharide[10].

That rhamnosyl residues were most often glycosidically linked to galactosyluronic acid residues in intact maize and rice RG-I was confirmed by analysis of the glycosyl-linkage compositions of the oligoglycosylalditol products of lithium treatment (see Table IV). For comparison, the glycosyl-linkage compositions of intact RG-I from maize, rice, and sycamore, before and after lithium treatment, are shown. There is a striking similarity between the glycosyl-linkage compositions of RG-I from maize, rice, and sycamore, both before and after lithium treatment. A decrease in the relative amounts of specifically linked residues following lithium treatment indicates that they were glycosidically linked to galactosyluronic acid residues before treatment. In each polysaccharide, the 2- and 2,4-linked rhamnosyl residues were almost completely eliminated by lithium treatment. (Note that only the linkages of residues that remained glycosidically linked, and not those of alditols produced by lithium treatment, are included in Table IV.) Thus, the dis-

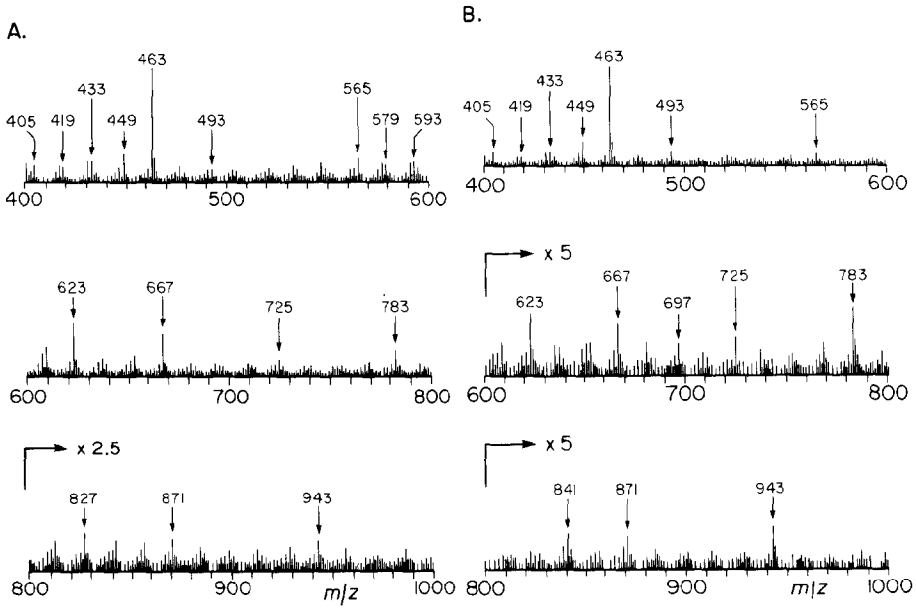

Fig. 2. Fast-atom-bombardment mass spectra of the per-*O*-methylated oligoglycosylalditols derived from RG-I of (A) maize and (B) sycamore by treatment with lithium–ethylenediamine. Reaction and mass spectrometry conditions are described in the Experimental section. Peaks correspond to sodium adducts [$(M + Na)^+$ pseudomolecular ions] (see Table IV).

TABLE V

FAST-ATOM-BOMBARDMENT MASS SPECTROMETRY OF PER-*O*-METHYLATED OLIGOGLYCOSYLALDITOLS DERIVED FROM SYCAMORE AND MAIZE RHAMNOGALACTURONAN I BY LITHIUM TREATMENT

m/z $[(M + Na)^+]$	*Composition consistent with pseudomolecular ion*[a]	*Relative abundance of* $(M + Na)^+$ *ion*	
		Maize RG-I	*Sycamore RG-I*
405	$(Pent)_2$[b]	17	13
419	PentDeoxyhex[b]	14	8
433	$(Deoxyhex)_2$	19	13
449	PentHex[b]	23	21
463	HexDeoxyhex[b]	100	100
493	$(Hex)_2$[b]	11	18
565	$(Pent)_3$	23	16
579	$(Pent)_2$Deoxyhex	17	[c]
593	$Pent(Deoxyhex)_2$	23	
623	PentHexDeoxyhex[b]	46	13
667	$(Hex)_2$Deoxyhex[b]	36	11
697	$(Hex)_3$		7
725	$(Pent)_4$	16	8
783	$(Pent)_2$HexDeoxyhex[b]	23	15
827	$Pent(Hex)_2$Deoxyhex[b]	14	
841	$(Hex)_2(Deoxyhex)_2$		7
871	$(Hex)_3$Deoxyhex	11	7
943	$(Pent)_3$HexDeoxyhex[b]	10	8

[a]Sequences shown are arbitrary, because they cannot be deduced from pseudomolecular ions alone. [b]At least one oligoglycosylalditol characterized, using other methods, had this composition (see text). [c]Indicates that the ion was not detected. Low-abundance ions may have been masked by the background of glycerol-cluster ions.

appearance of glycosidically linked rhamnosyl residues (see Table IV) was consistent with the expected effect of lithium treatment on the backbone[8] of RG-I, and it established the similarity between the overall structures of maize, rice, and sycamore RG-I.

F.a.b.-m.s. analysis of the per-O-methylated oligoglycosylalditols derived from maize RG-I by treatment with lithium–ethylenediamine. — Samples of maize and sycamore RG-I were simultaneously treated with lithium–ethylenediamine. The results of positive-ion f.a.b.-m.s. of the per-*O*-methylated oligoglycosylalditols derived from the lithium-treated polysaccharides are shown in Fig. 2 and summarized in Table V. The f.a.b.-mass spectra of lithium-treated maize and sycamore RG-I strongly resembled each other. Many of the same pseudomolecular ions were observed; the most intense peak observed above *m/z* 200 in each spectrum was at *m/z* 463. Pseudomolecular ions corresponding to fourteen of the nineteen oligoglycosylalditols characterized by Lau *et al.*[10] were present in the f.a.b-mass spectrum of lithium-treated sycamore RG-I (see Fig. 2). In addition, the spectrum contained evidence for an oligoglycosylalditol product of sycamore RG-I (at *m/z* 449) that had not previously been observed. A monoglycosylalditol (Ara → Galitol) with a pseudomolecular ion at *m/z* 449 was isolated from lithium-treated maize RG-I, and was structurally characterized (see later).

A pseudomolecular ion at *m/z* 841, corresponding to the oligoglycosylalditol α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→4)-L-Rhaitol characterized by Lau *et al.*[10], was observed in the spectrum of the sycamore reaction-products, but not in that of the maize products. This fucosyl-containing compound was not anticipated, because maize RG-I contains no terminal fucosyl groups (see Tables II and III). Conversely, three pseudomolecular ions (at *m/z* 579, 593, and 827) were observed in the f.a.b.-mass spectrum of the per-*O*-methylated reaction-products of maize RG-I that were not detected in the corresponding spectrum of sycamore RG-I products. A triglycosylalditol (Gal → Gal → Ara → Rhaitol) corresponding to the ion at *m/z* 827 was subsequently isolated from maize RG-I and characterized (see later). The ions at *m/z* 579 and 593 might have corresponded to the diglycosylalditols, Ara → Ara → Rhaitol and Fuc → Ara → Rhaitol, respectively, that were isolated from sycamore RG-I by Lau *et al.*[10]. The latter compound was not expected in maize RG-I, because maize RG-I had no detectable terminal fucosyl groups; however, terminal rhamnosyl groups were detected in maize RG-I (see Table IV), and might be present in the compound giving rise to the pseudomolecular ion at

TABLE VI

FAST-ATOM-BOMBARDMENT MASS SPECTROMETRY OF PER-*O*-ACETYLATED OLIGOGLYCOSYLALDITOLS DERIVED FROM MAIZE RHAMNOGALACTURONAN I BY LITHIUM TREATMENT

m/z		*Composition consistent with observed ion*[a]
$(M + H)^+$	*A-type fragment*	
	259	Pent[b]
	273	Deoxyhex
	331	Hex[b]
	475	$(Pent)_2$[b]
	547	PentHex[b]
	561	HexDeoxyhex[b]
	619	$(Hex)_2$[b]
	691	$(Pent)_3$[b]
	763	$(Pent)_2Hex$[b]
	777	PentHexDeoxyhex
	907	$(Pent)_4$
	1123	$(Pent)_5$
363		Pentitol
377		Deoxyhexitol
579		$(Pent)_2$-itol
651		PentHex-itol
665		HexDeoxyhex-itol
723		$(Hex)_2$-itol
795		$(Pent)_3$-itol
881		PentHexDeoxyhex-itol
953		$(Hex)_2$Deoxyhex-itol
1011		$(Pent)_4$- or $(Hex)_3$-itol

[a]Sequences shown are arbitrary. [b]At least one maize oligoglycosylalditol was characterized from which an ion with this composition could have been derived.

TABLE VII

LIST OF STRUCTURES AND PARTIAL STRUCTURES OF THE CHARACTERIZED OLIGOGLYCOSYLALDITOLS DERIVED FROM MAIZE RHAMNOGALACTURONAN I BY LITHIUM TREATMENT

Numerical designation	*Per-O-methylated oligoglycosylalditol*
1	β-D-Gal*p*-(1→4)-L-Rhaitol
2[a]	Ara → Galitol
3[a]	Pent → Pentitol
4[a]	Gal → Galitol
5	Ara → Rhaitol
6[b]	β-D-Gal*p*-(1→4)-α-L-Rha*p* → Galitol
7[a]	Gal → Gal-(1→4)-Rhaitol
8	Ara → Gal-(1→4)-Rhaitol
9	α-L-Ara*f* → β-D-Gal*p*(1→4)-L-Rhaitol
10	Ara-(1→3)-Gal → Rhaitol
11	Gal-(1→5)-Ara → Rhaitol
12[a,c]	Ara → Ara-(1→3)-Gal → Rhaitol
13	Gal → Gal → Ara → Rhaitol
14[a,c]	Ara → Ara → Ara-(1→3)-Gal → Rhaitol

[a]Each of these oligoglycosylalditols was eluted from a DB-1 g.l.c. column at more than one retention time, indicating the existence of more than one isomer (see text). [b]The galactitol residue was either 3- or 4-linked; an unambiguous determination could not be made, due to hydrogen reduction at C-1 (see text). [c]The internal arabinosyl residues were 3- or 5-linked, or both (see text).

m/z 593. The terminal deoxyhexosyl group in the sycamore RG-I product isolated by Lau *et al.*[10] may also have been a rhamnosyl group, because those workers did not identify the deoxyhexosyl group.

F.a.b.-m.s. analysis of the per-O-acetylated oligoglycosylalditols derived from maize RG-I by treatment with lithium–ethylenediamine. — The f.a.b.-mass spectrum of per-*O*-acetylated oligoglycosylalditols derived from maize RG-I contained molecular ions, and A-type fragment ions derived from the nonreducing termini (see Table VI). Only four of the fragment ions observed (those at *m/z* 273, 777, 907, and 1123) could not have been derived from oligoglycosylalditols that were subsequently isolated and characterized (see Table VII). The fragment ions at *m/z* 273 were probably derived from terminal rhamnosyl groups [because no terminal fucosyl groups were detected by methylation analysis (see Table IV)]. No oligoglycosylalditols containing a (nonreducing-terminal) rhamnosyl group were isolated from maize RG-I (see Table VII); however, evidence of a component containing a terminal deoxyhexosyl group was seen in the f.a.b.-mass spectrum of the per-*O*-methylated products of maize RG-I (see earlier). Lau *et al.*[10] obtained, from sycamore RG-I, f.a.b.-m.s. evidence of oligoglycosylalditols containing five or more pentosyl residues from which the fragment ions at *m/z* 907 and 1123 (see Table VI) could have been derived.

The deoxyhexitols, evidenced by the pseudomolecular ion at *m/z* 377 (Table VI), were probably derived from unbranched rhamnosyl groups glycosidically

linked to galactosyluronic acid residues in the RG-I backbone. Only the two per-*O*-acetylated pseudomolecular ions at *m/z* 795 and 1011 (Table VI) could not be explained by oligoglycosylalditols that were characterized by other methods (see later). However, corresponding pseudomolecular ions were detected at *m/z* 565 and 725 in the f.a.b.-mass spectrum of the per-*O*-methylated oligoglycosylalditols derived from maize RG-I (see Fig. 2 and Table V).

Characterization of individual per-O-methylated oligoglycosylalditols derived from maize RG-I

Overall scheme. — H.p.l.c. fractions of the per-*O*-methylated products of lithium–ethylenediamine-treated maize RG-I were analyzed by several methods to determine the structure of the component oligoglycosylalditols. H.p.l.c. fractions were collected without a simultaneous means of detection, and so no chromatogram was obtained. Instead, the individual fractions were analyzed by f.a.b.-m.s. to determine which contained only one or two oligoglycosylalditols, and to determine the molecular weights of those components. ^{1}H-N.m.r. spectroscopy of the per-*O*-methylated oligoglycosylalditols was used to determine the anomeric configuration of glycosyl linkages. The oligoglycosylalditols in selected h.p.l.c. fractions were analyzed by g.l.c.–m.s. (e.i.) to produce fragment ions of the A, J, and alditol series[31–33] that served to define the glycosyl-residue sequence of the per-*O*-methylated oligoglycosylalditols and, in some cases, the position of the linkages. Finally, the samples were hydrolyzed, the products reduced, the alditols acetylated, and the per-*O*-methylated alditol acetates characterized by g.l.c. and g.l.c.–m.s. (e.i.) in order to identify and determine the linkage positions of the glycosyl residues.

H.p.l.c. fractions 20 and 22. — The preponderant pseudomolecular ion in the f.a.b.-mass spectrum of h.p.l.c. fraction 20 was at *m/z* 463, consistent with a monoglycosylalditol with a composition deriving from one hexosyl and one deoxyhexosyl residue. Analysis by g.l.c.–m.s. (e.i.) (see Table VIII) indicated the major component of this fraction to be per-*O*-methylated monoglycosylalditol **1** (see Table VII). The ^{1}H-N.m.r. spectrum contained a doublet with a coupling constant of 7.0 Hz at δ 4.28, corresponding to the β-anomeric proton of the galactopyranosyl residues. Glycosyl-linkage analysis of fraction 20 confirmed the presence of component **1**; the quantitatively preponderant components were 4-*O*-acetyl-1,2,3,5-tetra-*O*-methylrhamnitol and 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol, accounting for 19 and 56 mol%, respectively, of all the observed linkages. (Note: 4-*O*-acetyl-1,2,3,5-tetra-*O*-methylrhamnitol is extremely volatile, and was preferentially lost during preparation of the samples.)

Analyses of fractions 20 and 22 by g.l.c.–m.s. (e.i.) (see Table VIII) indicated the presence of other monoglycosylalditols (components **2, 3, 4,** and **5** in Table VII). There were two isomers of arabinosylgalactitol (**2**), three of pentosylpentitol (**3**), two of galactosylgalactitol (**4**), and one of arabinosylrhamnitol (**5**). Identification of the monoglycosylalditols in fractions 20 and 22 was aided by the fact that the only hexosyl residues in the starting material were galactosyl residues, and the only deoxyhexosyl residues were rhamnosyl residues. Furthermore, methylation

TABLE VIII

DIAGNOSTIC ELECTRON-IMPACT G.L.C.–M.S. IONS AND F.A.B.-M.S. PSEUDOMOLECULAR IONS FOR THE PER-*O*-METHYLATED OLIGOGLYCOSYLALDITOLS DERIVED FROM MAIZE RHAMNOGALACTURONAN I BY LITHIUM TREATMENT

Per-O-methylated oligoglycosyl-alditol	*G.c.r.t.*[a]	*L.c. fraction number*[b]	*Electron-impact m.s. ions*[c]											*F.a.b.-m.s. $(M+Na)^+$ pseudomolecular ion*
			aJ_2	aJ_1	bA_1	bA_2	bA_1	bA_2	*Alditol*					
1	11.0	20	205 (31)	265 (12)	219 (10)	187 (24)								463
2	10.2	22	235 (2)	295 (1)	175 (19)	143 (20)								449
	11.4	22	235 (7)	295 —	175 (14)	143 (20)								
3	8.6	20	191 (2)	251 (3)	175 (13)	143 (15)								405
	8.8	20	191 (6)	251 (2)	175 (9)	143 (12)								
	9.4	20	191 (6)	251 (2)	175 (10)	143 (14)			133 (5)	249 (2)	293 (1)			
4	10.2	20	235 (16)	295 (0.5)	219 (11)	187 (43)	175[d] (69)	143[d] (25)						493
	10.4	20	235 (22)	295 (9)	219 (4)	187 (1)								
5	9.7	22	205 (20)	265 (18)	175 (29)	143 (34)								419
			aJ_2	aJ_1	aJ_0	abJ_2	abJ_1	cA_1	cA_2	cbA_1	cbA_2	*Alditol*		
6	21.0	25	235 (82)	295 (27)		409 (9)	469 (1)	219 (28)	187 (102)	393 (0.5)	361 (0.6)	89 (42)	555 599 (0.5) (0.06)	667
7	20.3[e]	26	205 (64)	265 (26)		409 (0.9)	469 (3)	219 (29)	187 (67)	423 (0.9)	391 (0.1)			667
8	20.3[e]	26	205 (64)	265 (26)		409 (0.9)	469 (3)	175 (9)	143 (21)	379 (0.7)	347 (0.2)			623

9	18.3	34	205 (36)	265 (18)		409 —	469 —	175 (41)	143 (48)	379 (1)	347 (1)				623
10	19.4[e]	37	205 (24)	265 (2)	251 (6)	409 (0.4)	469 —	175 (59)	143 (78)	379 (6)	347 (0.8)				623
11	19.4[e]	37	205 (24)	265 (2)		365 (0.6)	425 (0.5)	219 (4)	187 (8)	379 (6)	347 (0.8)				623
			aJ_2	aJ_1	aJ_0	abJ_2	abJ_1	$abcJ_2$	$abcJ_1$	dA_1	dA_2	dcA_1	dcA_2	$dcbA_1$	$dcbA_2$	
12	15.7[e]	43	205 (19)		251 (6)	409 (0.3)	469 (0.04)	569 (0.03)	629 —	175 (65)	143 (57)	335 (5)	303 —	539 (0.5)	507 (0.1)	783
13	15.7[e]	43	205 (19)	265 —		365 (0.4)	425 (0.3)	569 (0.03)	629 —	219 (2)	187 (5)	423 (0.2)	391 (0.06)	583 (0.3)	551 (0.02)	783

[a]Retention time (min); g.l.c. conditions as described in the Experimental section. [b]L.c. fraction in which indicated components were present. [c]Figures in parentheses show ion abundance relative to that of the ion at *m/z* 101. [d]Component 2 was coeluted in this case, contributing ions at *m/z* 175 and 143. [e]Coeluting components contributed ions to the mass spectrum.

analysis of the material in fraction 22 confirmed that the terminal pentosyl groups in **2** and **5** were exclusively arabinosyl groups. In addition, fragment ions arising from alditol cleavages during e.i.-m.s. of the monoglycosylalditols (see Table VIII) indicated that the pentitol in component **3**, eluted at a retention time of 9.4 min, was 5-linked, and therefore was most likely arabinitol [xylosyl residues would have been in the pyranoid ring-form and therefore 4-linked (see Table IV)]. Therefore, the isomer of **3** eluted at 9.4 min may have corresponded to the monoglycosylalditol Ara-(1→5)-Araitol that had previously been isolated[9] from sycamore RG-I.

H.p.l.c. fraction 25. — The preponderant pseudomolecular ion in the f.a.b.-mass spectrum of h.p.l.c. fraction 25 was at *m/z* 667, consistent with a diglycosylalditol having a composition of one deoxyhexosyl and two hexosyl residues. Analysis of fraction 25 by g.l.c.–m.s. (e.i.) (see Table VIII) indicated the presence of diglycosylalditol **6** (see Table VII). The ^{1}H-n.m.r. spectrum of fraction 25 contained a doublet with a coupling constant of 7.4 Hz, at δ 4.55 (corresponding to the β-anomeric proton of a galactopyranosyl residue), and a doublet with a coupling constant of 2.1 Hz at δ 4.96 (attributed to the α-anomeric proton of the rhamnopyranosyl residue[5]). Methylation analysis of the material in fraction 25 yielded 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol, 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylrhamnitol, and either 3-*O*-acetyl-1,2,4,5,6-penta-*O*-methyl- or 4-*O*-acetyl-1,2,3,5,6-penta-*O*-methyl-galactitol (the latter two derivatives were indistinguishable by e.i.-m.s., due to hydrogen reduction of C-1), accounting for 29, 18, and 4 mol%, respectively, of all observed linkages. Thus, the galactitol in component **6** was linked either at O-3 or O-4. Fragment ions at *m/z* 555 and 599 that arose from alditol cleavages (see Table VIII) confirmed the linkage of the galactitol residues through either O-3 or O-4. The presence of non-reduced, 4-linked rhamnosyl residues in compound **6** was consistent with the unexpected observation of 4-linked rhamnosyl residues among the products of lithium treatment of maize RG-I (see Table IV). The formation of compound **6** during lithium treatment of RG-I is discussed later.

H.p.l.c. fraction 26. — The f.a.b.-mass spectrum of fraction 26 contained signals corresponding to compounds **6** and **7** (at *m/z* 667) and **8** (at *m/z* 623). Components **7** and **8** were coeluted during g.l.c.–m.s. with a retention time of 20.3 min (see Table VIII). Consistent with the proposed structures of **7** and **8**, methylation analysis of the material in fraction 26 yielded, *inter alia*, 4-*O*-acetyl-1,2,3,5-tetra-*O*-methylrhamnitol, 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylarabinitol, and 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol, accounting for a trace, 5, and 27 mol%, respectively, of the total linkages. However, the linkages of galactosyl residues in **7** and **8** could not be determined due to the presence of a mixture of internal galactosyl residues, including 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-, 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-, and 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-galactitol that accounted for 6, 15, and 12 mol%, respectively, of the total linkages. The f.a.b.-mass spectrum of h.p.l.c. fraction 26 contained, in addition to signals corresponding to **6, 7,** and **8,** a signal at *m/z* 901 consistent with a triglycosylalditol containing four

galactosyl residues (galactosyl residues were the only hexosyl residues detected in maize RG-I).

H.p.l.c. fraction 34. — The major component of fraction 34 was diglycosylalditol **9** (see Table VII) as determined by g.l.c.–m.s. However, contrary to the results of g.l.c.–m.s., the pseudomolecular ion at *m/z* 623 (corresponding to compound **9**) in the f.a.b.-mass spectrum of fraction 34 was less intense than the pseudomolecular ion observed at *m/z* 667 (corresponding to an isomer of compound **7**, see later). The isomer of diglycosylalditol **9** in fraction 34 had a g.l.c. retention time of 18.3 min, which is distinct from the retention time of 20.3 min of its isomer (**8**) in fraction 26. Analysis of fraction 34 by g.l.c.-m.s. gave diagnostic e.i. fragment-ions (see Table VIII), but the expected abJ_1 and abJ_2 fragment-ions at *m/z* 469 and 409, respectively, were not detected. The ^{1}H-n.m.r. spectrum of fraction 34 contained a doublet with a coupling constant of 7.93 Hz and δ 4.33 (corresponding to the β-anomeric protons of the galactopyranosyl residues), and an unresolved doublet at δ 5.16 (corresponding to the α-anomeric protons of the arabinofuranosyl residues). The results of methylation analysis of the material in fraction 34 were consistent with the proposed partial structure of **9**, and demonstrated the presence of, *inter alia*, 4-*O*-acetyl-1,2,3,5-tetra-*O*-methylrhamnitol and 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylarabinitol that accounted for 8 and 34 mol%, respectively, of the total linkages that were observed. However, the glycosyl linkages of the internal galactosyl residue(s) could not be unambiguously determined, because several differently linked galactosyl residues (*i.e.*, 3-, 4-, and 6-linked) were detected by methylation analysis. Diglycosylalditol **7** was detected as being coeluted with **9** during g.l.c.–m.s. (see Table VIII). The presence of an isomer of **7** in fraction 34 was consistent with the ion at *m/z* 667 observed in the f.a.b.-mass spectrum of that fraction, and with the observation of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalacitol upon methylation analysis (accounting for 17 mol% of the total linkages).

H.p.l.c. fraction 37. — Fraction 37 primarily contained diglycosylalditol **10** (see Table VII). The corresponding pseudomolecular ion at *m/z* 623 was the preponderant ion in the f.a.b.-mass spectrum. Linkage of the internal galactosyl residue through O-3 was indicated by the J_0 fragment ion at *m/z* 251 (see Table VIII) that had previously been shown[32] to be diagnostic for 3-linked hexosyl residues.

Diglycosylalditol **11** was coeluted from the g.l.c. column with **10**; the small proportion of aJ_1 ion at *m/z* 265 (see Table VIII) was concluded to have arisen from oligoglycosylalditol **11**, and not from **10**. Methylation analysis of the material in fraction 37 yielded, *inter alia*, 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylarabinitol, 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol, and 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylgalactitol (accounting for 15, 10, and 23 mol%, respectively, of the total linkages), consistent with the proposed structures of **10** and **11**. The presence of 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylarabinitol, as the preponderant di-*O*-methylarabinitol detected by methylation analysis (14 mol%), suggested that the arabinosyl residue in compound **11** was 5-linked.

H.p.l.c. fractions 43 and 47. — Fractions 43 and 47 both contained different isomers of triglycosylalditol **12** (see Table VII). Interestingly, the f.a.b.-mass spectrum of fraction 43 contained the pseudomolecular ion at *m/z* 623 (corresponding in mass to an isomer of **10** or **11**) as the overwhelmingly preponderant ion, while the ion at *m/z* 783 (consistent with the composition of **12**) dominated the spectrum of fraction 47. No evidence in fraction 43 of a component consistent with the pseudomolecular ion at *m/z* 623 was seen upon g.l.c.–m.s. analysis. The isomers of component **12** in fractions 43 and 47 were eluted during g.l.c.–m.s. at retention times of 15.7 and 14.3 min, respectively (see Table VIII). Selected-ion monitoring during g.l.c.–m.s. was necessary in order to characterize component **12** in fraction 47, due to the small amount of material available (the relative abundances of the diagnostic ions thus obtained are not included in Table VIII). Methylation analysis of h.p.l.c. fractions 43 and 47 yielded, *inter alia*, 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylarabinitol and 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylgalacitol, consistent with the proposed structure of **12**. However, the internal arabinosyl residues in fractions 43 and 47 were predominantly 3- and 5-linked (deduced from the presence of 1,3,4-tri-*O*-acetyl-2,5-di-*O*-methyl- and 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylarabinitol, respectively), suggesting that both of the maize isomers of **12** were different from their sycamore counterpart in which the arabinosyl residues are 2-linked[10].

Fraction 43 also contained a relatively small proportion of triglycosylalditol **13**, coeluted during g.l.c. with component **12** (see Table VIII). Upon methylation analysis of fraction 43, a small amount of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol was detected that could have arisen from component **13**. Also, the presence of component **13** was evident by its pseudomolecular ion at *m/z* 827 in the f.a.b.-mass spectrum of both the crude mixture of oligoglycosylalditols derived from maize RG-I (Table V) and of h.p.l.c. fraction 43. However, a pseudomolecular ion at *m/z* 827 was not seen in the spectrum of the mixture derived from sycamore RG-I, and triglycosylalditol **13** has not been reported to be a component of sycamore RG-I.

H.p.l.c. fractions 50 to 52. — Fractions 50 to 52 were analyzed by f.a.b.-m.s.; in each spectrum a pseudomolecular ion was observed at *m/z* 943 corresponding to the proposed composition of **14** (see Table VII). The three h.p.l.c. fractions were pooled and analyzed by g.l.c.–m.s. with selected-ion monitoring (due to the small amount of material present). Electron-impact fragment-ions diagnostic of **14** were seen at *m/z* 699 ($edcbA_1$), 569 ($abcJ_2$), 409 (abJ_2), 335 (edA_1), 303 (edA_2), 251 (aJ_0), 175 (eA_1), and 143 (eA_2). Furthermore, two isomers of **14** were evident from the elution of compounds giving rise to the same e.i. fragment-ions at different g.l.c. retention times, 16.3 and 16.5 min. Methylation analysis of pooled fractions 50–52 yielded, *inter alia*, 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylarabinitol, and 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylgalactitol (accounting for 13 and 5 mol%, respectively, of the total linkages), consistent with the proposed structure of **14**. Interestingly, 1,2,4-tri-*O*-acetyl-3,5-di-*O*-methylarabinitol accounted for <2 mol% of the total linkages detected, while 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methyl-, 1,3,4-tri-*O*-acetyl-2,5-

di-*O*-methyl-, 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-, and 1,3,4,5-tetra-*O*-acetyl-2-*O*-methyl-arabinitol accounted for 13, 10, 34, and 15 mol%, respectively, of the total linkages. The relatively low level of 2-linked arabinosyl residues in fractions 50–52 suggested that the linear isomers of **14** differed from their sycamore counterpart, in which one of the internal arabinosyl residues was found[10] to be 2-linked. The 3,5-linked arabinosyl residues in fractions 50 to 52 may have been present in a branched isomer of **14**.

H.p.l.c. fractions 30 and 41. — Pseudomolecular ions at *m/z* 885 and 1075 were observed in the f.a.b.-mass spectra of h.p.l.c. fractions 41 and 30, respectively. These ions were not observed in the f.a.b.-mass spectrum of the mixture of reaction products (see Fig. 2), and they may correspond to oligoglycosylalditols (composed of four pentosyl residues, and four hexosyl and one deoxyhexosyl residue, respectively) previously isolated[10] from sycamore RG-I. The amounts of material in h.p.l.c. fractions 30 and 41 were insufficient for further analyses.

DISCUSSION

Characterization of the pectic polysaccharide(s) solubilized from isolated maize and rice cell-walls has shown RG-I to be a component of the cell walls of graminaceous monocots. Evidence of RG-I in the cell walls of rice endosperm had previously been obtained[4]; however, the cell walls of rice endosperm appear not to be representative of the primary cell-walls of grasses[34]. Although the glycosyl-linkage compositions of maize, rice, and sycamore RG-I were very similar overall, the data presented here suggest that the monocot polysaccharides have a higher ratio of branched to unbranched rhamnosyl residues. A high ratio of branched to unbranched rhamnosyl residues is also characteristic of the only other example of monocot RG-I (from rice endosperm) for which complete glycosyl-linkage data are available[4]. In addition, an RG-I extracted from sycamore cell-walls following EPGase treatment has an extremely high proportion of branched and doubly-branched rhamnosyl residues[35]. In this respect, maize and rice RG-I appear to be the same as the RG-I from other plants.

A summary of the structural features of the side chains of maize and sycamore RG-I is presented in Table IX. Evidence was obtained for the existence in maize RG-I of seventeen of the nineteen side-chains of sycamore RG-I that had previously been characterized[10]. Partial or complete structures of nine of the seventeen maize components were obtained in this study (**1, 3, 4, 5, 10, 12, 14,** and two isomers of **7**; see Table VII). As far as has been determined, these nine maize components have the same structures as their counterparts in sycamore RG-I. Of the ten components of sycamore RG-I not isolated from maize RG-I, evidence for eight was obtained by f.a.b.-m.s. analysis of the products of lithium treatment. Only two previously characterized components of sycamore RG-I [Gal → Gal → Galitol and α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→4)-L-Rhaitol] were not detected among the products of lithium treatment of maize RG-I. The most abun-

dant oligoglycosylalditol product of lithium treatment of both maize and sycamore RG-I is β-D-Gal*p*-(1→4)-Rhaitol (**1**).

The absence of α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→4)-L-Rhaitol from maize RG-I was confirmed by the absence of its pseudomolecular ion at *m/z* 841, both in the f.a.b.-mass spectrum of the mixture of lithium reaction products (see Fig. 2A) and in the f.a.b.-mass spectra of every h.p.l.c. fraction examined. In addition, neither fucosyl (see Table III) nor terminal fucosyl (see Table IV) units were detected in maize RG-I. We conclude from these results that maize RG-I

TABLE IX

SUMMARY OF THE NEUTRAL SIDE-CHAINS[a] OF MAIZE AND SYCAMORE RHAMNOGALACTURONAN I

Ara → Arabinitol Gal → Galactitol β-D-Gal-(1→4)-Rhamnitol[b] Ara-(1→3)-Gal → Rhamnitol[b] Ara → Ara-(1→3)-Gal → Rhamnitol[b] Ara → Ara → Ara-(1→3)-Gal → Rhamnitol[b] Gal → Gal → Rhamnitol[b] Gal → Gal → Rhamnitol[b] Ara → Rhamnitol	isolated from both maize and sycamore RG-I
Ara → Ara → Rhamnitol Deoxyhex → Ara → Rhamnitol[c] Gal → Gal → Gal → Rhamnitol[b] Gal → Gal → Gal → Gal → Rhamnitol Ara → Ara → Arabinitol Ara → Ara → Ara → Arabinitol Ara → Ara → Ara → Ara → Arabinitol Gal → Gal → Gal → Galactitol	isolated from sycamore RG-I, and presence in maize RG-I, indicated by f.a.b.-m.s.[d]
Gal → Gal → Galactitol Fuc → Gal → Gal → Rhamnitol[b]	isolated from sycamore, but not maize, RG-I
Ara→ Galactitol[e,f] Gal → Ara → Rhamnitol Gal → Gal → Ara → Rhamnitol Ara → Gal → Rhamnitol α-L-Ara → β-D-Gal-(1→4)-Rhamnitol β-D-Gal-(1→4)-α-L-Rha → Galactitol[g]	isolated from maize, but not sycamore, RG-I

[a]Some of the products of treating RG-I with lithium in ethylenediamine may have resulted from cleavage of galatosyluronic acid residues in side chains, or by cleavage between adjacent, neutral glycosyl residues, or both. [b]Complete structures of these oligoglycosylalditols were obtained by Lau *et al.*[10]. [c]The terminal residues in the component of maize RG-I are undoubtedly rhamnosyl residues, due to the failure to detect terminal fucosyl residues in maize RG-I (see text). In fact, this component of sycamore RG-I may also contain terminal rhamnosyl residues, and not terminal fucosyl residues as reported[10], since the composition was not actually determined. [d]Compositions and sequences of the maize counterparts were not determined. [e]Two isomers of this oligoglycosylalditol were isolated from maize RG-I (see text). [f]A pseudomolecular ion corresponding to this compound was observed in the f.a.b.-mass spectrum of sycamore RG-I after lithium treatment (see text); however, the compound was not observed by Lau *et al.*[10]. [g]Evidence for this compound among the products of lithium-treated sycamore RG-I was obtained by glycosyl-linkage analysis (see text).

does not contain fucosyl residues, and that it therefore differs from sycamore RG-I. Rice RG-I does contain terminal fucosyl residues (see Table IV).

Members of a new structural family, not observed in sycamore RG-I, were found among the side chains of maize RG-I. In this family, represented by Gal → Ara → Rhaitol and Gal → Gal → Ara → Rhaitol (**11** and **13**, respectively), a single arabinosyl residue attached to a rhamnosyl residue is substituted with one or more galactosyl residues. Oligoglycosylalditols with arabinosyl residues glycosidically linked to rhamnitol were observed[10] by Lau *et al.*, but the arabinosyl residues were not substituted with galactosyl residues (see Table IX). The absence of a signal at *m/z* 827 (corresponding to Gal → Gal → Ara → Rhaitol) in the f.a.b.-mass spectrum of lithium-treated sycamore RG-I supports the conclusion that Gal → Gal → Ara → Rhaitol is not a side chain of sycamore RG-I that went undetected[10] by Lau *et al.*, and that it is a feature of maize RG-I, but not of sycamore RG-I.

Two isomers of Ara → Gal-(1→4)-Rhaitol (diglycosylalditols **8** and **9**) were obtained from maize RG-I. Side chains in which terminal arabinosyl residues are linked to galactosyl residues at positions other than O-3 were not observed[10] in sycamore RG-I by Lau *et al.* (see Table IX). The galactosyl residues in two maize RG-I side-chains were not 3-linked, because aJ_1 (and not aJ_0) fragment ions were observed in their e.i.-mass spectra (see Table VIII). It may be concluded that one isomer of Ara → Gal-(1→4)-Rhaitol contains a 4-linked galactosyl residue and the other isomer contains a 6-linked galactosyl residue, because (*1*) h.p.l.c. fractions containing **8** and **9** contained both 4- and 6-linked (but not 2-linked) galactosyl residues, (*2*) it is likely that the anomeric configurations are identical in the two isomers (only α-arabinosyl and β-galactosyl residues were observed in this study, and by Lau *et al.*[10]), and (*3*) the g.l.c. retention times of **8** and **9** were different (see Table VIII). If the anomeric configurations in the two isomers of Ara → Gal-(1→4)-Rhaitol were identical, the difference in retention times must have been due to different linkage positions of the galactosyl residues.

Two isomers of Ara → Galitol (**2**) were isolated from the lithium-reaction products of maize RG-I that were not previously isolated[10] from sycamore RG-I. However, the f.a.b.-mass spectrum of sycamore-derived reaction-products in this study did contain a signal corresponding to these monoglycosylalditols (see Fig. 2B and Table V), that indicated that they are in present in sycamore RG-I. Oligoglycosylalditols with arabinitol or galactitol at their "reducing" ends could result from cleavage between neutral glycosyl residues during lithium treatment, because ~10% of such linkages are cleaved by the reaction conditions used (see ref. 10 and Results). Alternatively, arabinosyl and galactosyl residues could have been linked to galactosyluronic acid residues of the backbone or side chains of RG-I. Indeed, evidence has been obtained[5] for the presence of galactosyluronic acid residues in the side chains of sycamore RG-I.

The origin of the compound β-D-Gal*p*-(1→4)-α-L-Rha*p* → Galitol (**6**) is unknown. Lau *et al.*[10] did not detect it among the products of lithium treatment of sycamore RG-I, nor did they detect unreduced, 4-linked rhamnosyl residues upon

methylation analysis of the reaction products. In this study, we detected unreduced, 4-linked rhamnosyl residues among the products of lithium treatment of both maize and sycamore RG-I. However, the 4-linked rhamnosyl residues were not consistently observed when sycamore RG-I was treated with lithium–ethylenediamine in separate experiments (unpublished data). Because unknown differences between the reaction conditions used by Lau *et al.*[10] and those used in the present study resulted in the formation of compound **6**, its relationship to the structure of RG-I cannot be determined.

F.a.b.-mass spectra of h.p.l.c. fractions provided a reliable indication of which oligoglycosylalditols were present in the fractions. The high sensitivity of f.a.b.-m.s. was essential for determining which h.p.l.c. fractions contained oligoglycosylalditols present in low abundance. This was especially true in the case of oligoglycosylalditol **14**. On the other hand, the relative intensities of pseudomolecular ions did not always correspond to the relative quantities of the corresponding components in h.p.l.c. fractions; relative quantities were estimated from total-ion intensities obtained by electron-impact g.l.c.–m.s. Although no systematic studies have been published on the relationship between sample composition and ionization during f.a.b.-m.s., the generally accepted notion that relative ion-intensities cannot be used to quantify components of mixtures appears to be well founded.

The observed differences in the structures of maize, rice, and sycamore RG-I do not diminish the remarkable similarities between monocot RG-I and dicot RG-I. Both monocot and dicot RG-I have a backbone of alternating rhamnosyl and galactosyluronic acid residues. For the most part, maize and sycamore RG-I have very similar sets of side chains. The conservation of many of the structural features of the polysaccharide in the two subclasses of flowering plants (monocots and dicots) suggests that RG-I has crucial functions in plants.

ACKNOWLEDGMENTS

We thank D. J. Gollin for purifying EPGase, D. J. Gollin and W. S. York for the gift of sycamore RG-I, D. Warrenfeltz for n.m.r. spectroscopy, and K. Moss for editorial assistance.

REFERENCES

1 J. R. THOMAS, A. G. DARVILL, AND P. ALBERSHEIM, *Carbohydr. Res.*, 185 (1989) 261–277.
2 G. O. ASPINALL AND A. CAÑAS-RODRIGUEZ, *J. Chem. Soc.*, (1958) 4020–4027.
3 P. M. RAY AND D. A. ROTTENBURG, *Biochem. J.*, 90 (1964) 646–654.
4 N. SHIBUYA AND R. NAKANE, *Phytochemistry*, 23 (1984) 1425–1429.
5 M. MCNEIL, A. G. DARVILL, AND P. ALBERSHEIM, *Plant Physiol.*, 66 (1980) 1128–1134.
6 M. MCNEIL, A. G. DARVILL, AND P. ALBERSHEIM, *Prog. Chem. Org. Nat. Prod.*, 37 (1979) 191–249.
7 G. O. ASPINALL, *Adv. Carbohydr. Chem. Biochem.*, 24 (1969) 333–379.
8 J. M. LAU, M. MCNEIL, A. G. DARVILL, AND P. ALBERSHEIM, *Carbohydr. Res.*, 137 (1985) 111–125.
9 J. M. LAU, M. MCNEIL, A. G. DARVILL, AND P. ALBERSHEIM, *Carbohydr. Res.*, 168 (1987) 219–243.
10 J. M. LAU, M. MCNEIL, A. G. DARVILL, AND P. ALBERSHEIM, *Carbohydr. Res.*, 168 (1987) 245–274.

11 R. E. CHAMBERS AND J. R. CLAMP, *Biochem. J.*, 125 (1971) 1009–1018.
12 W. S. YORK, A. G. DARVILL, M. MCNEIL, T. T. STEVENSON, AND P. ALBERSHEIM, *Methods Enzymol.*, 118 (1986) 3–40.
13 D. M. UPDEGRAFF, *Anal. Biochem.*, 32 (1969) 420–424.
14 H. BJÖRNDAL, C. G. HELLERQVIST, B. LINDBERG, AND S. SVENSSON, *Angew. Chem., Int. Ed. Engl.*, 9 (1970) 610–619.
15 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
16 T. J. WAEGHE, A. G. DARVILL, M. MCNEIL, AND P. ALBERSHEIM, *Carbohydr. Res.*, 123 (1983) 281–304.
17 E. J. BOURNE, M. STACEY, J. C. TATLOW, AND J. M. TEDDER, *J. Chem. Soc.*, (1949) 2976–2979.
18 G. J. GERWIG, J. P. KAMERLING, AND J. F. G. VLIEGENTHART, *Carbohydr. Res.*, 77 (1979) 1–7.
19 T. MURASHIGE AND F. SKOOG, *Physiol. Plant.*, 15 (1962) 473–497.
20 K. W. TALMADGE, K. KEEGSTRA, W. D. BAUER, AND P. ALBERSHEIM, *Plant Physiol.*, 51 (1973) 158–173.
21 Z. DISCHE, *Methods Carbohydr. Chem.*, 1 (1962) 477–512.
22 D. J. HUBER AND D. J. NEVINS, *Plant Physiol.*, 60 (1977) 300–304.
23 P. D. ENGLISH, A. MAGLOTHIN, K. KEEGSTRA, AND P. ALBERSHEIM, *Plant Physiol.*, 49 (1972) 293–297.
24 A. MORT AND W. D. BAUER, *J. Biol. Chem.*, 257 (1982) 1870–1875.
25 M. BARBER, R. S. BORDOLI, R. D. SEDGWICK, AND A. N. TYLER, *Nature (London)*, 293 (1981) 270–275.
26 M. MCNEIL, A. G. DARVILL, S. C. FRY, AND P. ALBERSHEIM, *Annu. Rev. Biochem.*, 53 (1984) 625–663.
27 D. J. NEVINS, R. YAMAMOTO, AND D. J. HUBER, *Phytochemistry*, 17 (1978) 1503–1505.
28 A. G. DARVILL, M. MCNEIL, AND P. ALBERSHEIM, *Plant Physiol.*, 62 (1978) 418–422.
29 M. W. SPELLMAN, M. MCNEIL, A. G. DARVILL, AND P. ALBERSHEIM, *Carbohydr. Res.*, 122 (1983) 115–129.
30 N. BLUMENKRANTZ AND G. ASBOE-HANSEN, *Anal. Biochem.*, 54 (1973) 484–489.
31 N. K. KOCHETKOV AND O. S. CHIZOV, *Adv. Carbohydr. Chem.*, 21 (1966) 39–93.
32 J. K. SHARP AND P. ALBERSHEIM, *Carbohydr. Res.*, 128 (1984) 193–202.
33 M. MCNEIL, A. G. DARVILL, AND P. ALBERSHEIM, *Plant Physiol.*, 70 (1982) 1586–1591.
34 N. SHIBUYA, R. NAKANE, A. YASUI, K. TANAKA, AND T. IWASAKI, *Cereal Chem.*, 62 (1985) 252–258.
35 T. ISHII, J. R. THOMAS, A. G. DARVILL, AND P. ALBERSHEIM, *Plant Physiol.*, (1988) in press.