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STRUCTURAL CHARACTERIZATION OF OLIGOSACCHARIDES ISOLATED FROM THE PECTIC POLYSACCHARIDE RHAMNO-GALACTURONAN II*,†

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ABSTRACT

Rhamnogalacturonan II (RG-II) is a structurally complex pectic (D-galactosyluronic acid-rich) polysaccharide that is present in the primary (growing) cell-walls of higher plants. RG-II is composed of ~60 glycosyl residues. The isolation and structural characterization of 23 oligosaccharide fragments of the residue of RG-II that remained after removal of hepta- and di-saccharides by partial hydrolysis with acid are reported. In order to obtain the oligosaccharide fragments characterized herein, the carboxyl groups of RG-II were dideuterio-reduced, and the carboxyl-reduced polysaccharide was per-*O*-methylated. The per-*O*-methylated polysaccharide was fragmented by partial hydrolysis with acid, producing partially *O*-methylated oligosaccharides. These derivatized oligosaccharides were reduced, to afford a mixture of partially *O*-methylated oligoglycosyl-alditols, which was then per-*O*-methylated. The structures of the resulting per-*O*-methylated oligoglycosyl-alditols were determined by chemical-ionization mass spectrometry, electron-impact mass spectrometry, fast-atom-bombardment mass spectrometry, ^{1}H-n.m.r. spectroscopy, and analysis of corresponding, partially *O*-acetylated, partially *O*-methylated alditols. Seventeen of the oligosaccharides isolated from RG-II were parts of a single heptasaccharide, namely,

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β-Gal*p*-*6,6*-d_2
1
↓
3
α-Gal*p*-(1→2)-β-Glc*p*-*6,6*-d_2-(1→4)-α-Fuc*p*-(1→4)-β-Rha*p*-(1→3^1)-Apiol*-*1*-*d*.
2
↑
1
α-Gal*p*-*6,6*-d_2

Five of the oligosaccharides were parts of a pentasaccharide, namely,

α-Gal*p*-*6,6*-d_2-(1→4)-α-Gal*p*-*6,6*-d_2-(1→4)-α-Gal*p*-*6,6*-d_2-(1→4)-α-Gal*p*-*6,6*-d_2-(1→4)-Galol-*1,6,6*-d_3.

The final oligosaccharide was Ara*f*-(1→3)-Gal*p*-*6,6*-d_2-(1→4)-Galol-*1,6,6*-d_3. The oligosaccharides of RG-II described herein and previously account for most of the glycosyl residues present in RG-II.

INTRODUCTION

Rhamnogalacturonan II (RG-II) is a complex pectic polysaccharide that is released from the walls of suspension-cultured sycamore *(Acer pseudoplatanus)* cells by the action of an endopolygalacturonase isolated from *Colletotrichum lindemuthianum*[2]. RG-II is homogeneous in size and is composed of ~60 glycosyl residues[2].

When subjected to hydrolysis with acid, RG-II yields at least ten different monosaccharides, including the following seldom-observed sugars, apiose [Api; 3-*C*-(hydroxymethyl)-D-*glycero*-aldotetrose], 2-*O*-methyl-L-fucose, 2-*O*-methyl-D-xylose, 3-deoxy-D-*manno*-2-octulosonic acid[2] (KDO), and aceric acid (AceA; 3-*C*-carboxy-5-deoxy-L-xylose). Aceric acid is the only acidic, branched-chain, deoxy sugar that has been identified in Nature[3]. The monosaccharide constituents of RG-II are interconnected by at least 20 different glycosidic linkages[2] (see Table I).

When RG-II was subjected to mild hydrolysis with acid, a heptasaccharide was isolated[4] that was structurally characterized[4] and found to contain aceric acid. Hydrolysis with still milder acid released a disaccharide that was structurally characterized and found[1] to be composed of L-rhamnose and KDO. After removal of the aceric acid-containing heptasaccharide, a large-molecular-weight residue of RG-II remained. We now report the structural characterization of a portion of this RG-II residue.

*Abbreviations: Apiol, Galol, Rhaol, etc. are abbreviations for Apiitol, Galactitol, Rhamnitol, etc.

EXPERIMENTAL

Isolation of RG-II. — RG-II was isolated from the primary cell-walls of suspension-cultured sycamore cells as described[2,4].

Isolation of the RG-II residue that remained after removal of the previously characterized, aceric acid-containing heptasaccharide and the KDO-containing disaccharide. — RG-II was partially hydrolyzed (in 0.1M TFA for 24 h at 40°), in order to release the previously characterized heptasaccharide, as well as several other smaller oligosaccharides, including one that contained KDO[1,4]. The larger-molecular-weight residue was purified by chromatography on a column of Bio-Gel P-10 as described[4]. We define this material as "the RG-II residue".

Reduction of the carboxyl groups of the RG-II residue. — The carboxyl groups of the RG-II residue (191 mg) were reduced with $NaBD_4$ by the carbodiimide method[5]. The reduced RG-II residue was isolated from the mixture by rotary evaporation. Acetic acid–methanol (1:9) was added 5 times and evaporated, and then methanol was added and evaporated 4 times. A solution of the resulting, borate-free residue in H_2O (20 mL) was applied to a column (1.5 × 58.5 cm) of Bio-Gel P-2, which was eluted with water. The collected fractions (2 mL each) were examined by the orcinol colorimetric method[6], and the orcinol-positive fractions were combined, and freeze-dried (164 mg).

Samples (200 μg each) of the RG-II residue, taken before and after carbodiimide reduction[5], were subjected to methanolysis with M HCl in methanol; the mixture was trimethylsilylated[7] (Sigma Sil-A), and the products analyzed for glycosyluronic acid residues by g.l.c. and g.l.c.–m.s. using an SP-2100 capillary column. The derivatives of the glycosyluronic acid and 6,6-dideuteriohexosyl residues (produced by reduction of the carboxyl groups of the glycosyluronic acid residues) were quantitated, in order that we might calculate the percent reduction of the carboxyl groups. The analyses established that 85% of the carboxyl groups of the galactosyluronic acid residues (the quantitatively dominant glycosyluronic residues of RG-II) had been reduced. The carbodiimide reduction was repeated, and the reduced polysaccharide resulting was purified by passage through a 3-mL column of Dowex 50 (H^+) cation-exchange resin. An aliquot (200 μg) of the freeze-dried, reduced polysaccharide (90 mg) was analyzed for glycosyluronic acid residues by methanolysis, trimethylsilylation, and g.l.c. and g.l.c.–m.s. analyses. Of the galactosyluronic acid residues, 96% had been carboxyl-reduced.

O-Methylation of the carboxyl-reduced RG-II residue. — The carboxyl-reduced residue (90 mg) was dried over phosphorus pentoxide under vacuum for 2 days at 40°, and then *O*-methylated by the Hakomori[8] method.

Dimethylsulfinyl (dimsyl) anion[8] (3.75M, as determined by titration with 0.01M HCl) was added under dry N_2 with stirring, to a solution of the carboxyl-reduced RG-II residue (90 mg) in Me_2SO (10 mL), to give a final concentration of 0.3M dimsyl anion. Dimsyl anion in the reaction mixture was monitored with triphenylmethane, and, when all of the anion had been consumed (~70 min), more

TABLE I

COMPARISON OF THE GLYCOSYL-I INKAGE COMPOSITION OF INTACT RG-II AND OF FRAGMENTS OBTAINED FROM RG-II

Glycosyl residue	*Linkage*	*Number of glycosyl residues per mol of RG-II*[a]							
		Intact RG-II	*Aceric acid-containing hepta-saccharide* (× 4)	*Hepta-saccharide herein* (× 1)	*Hexa-saccharide herein*[b] (× 1)	*Tetra-saccharide herein*[c] (× 1)	*Octa-galactosyl-uronic acid herein*[d] (× 1)	*Tri-saccharide herein; compound* **23** (× 1)	α-L-*Rha*p-(1→5)-*KDO*[e] (× 2)
Galactosyluronic acid	4-linked	12					7	1	
	terminal[f]	2		2	1	1	1		
	3,4-linked	1						1	
Rhamnosyl	terminal	4	4						2
	3-linked	4	4						
	2,4-linked	1			1				
	3,4-linked	1				1			
	2,3,4-linked	1		1					
Arabinosyl	2-linked (pyranose)	4	4						
	terminal (furanose)	2						1	

Galactosyl	2,4-linked	5	4						
	terminal	2		1	1				
	3-linked	1							
Apiosyl	3[1]-linked	4	4	1	1	1			
Aceryl	2-linked	(4)[g]	4						
Fucosyl[h]	3-linked	2				1			
	3,4-linked	1		1[h]	1[h]				
2-*O*-Methylfucosyl	terminal	3	4						
2-*O*-Methylxylosyl	terminal	2							
Glucosyluronic acid	2-linked	2		1	1				
Glucosyl	4-linked	2							
3-Deoxy-D-*manno*-octulosonic acid	5-linked	(2)[g]							2
	Total	62	28	7	6	4	8	3	4

[a]The values were calculated from the glycosyl-linkage composition data reported in ref. 4 and the percentage of KDO reported in ref. 1. The mol percent values were converted into number of glycosyl residues per mol by assigning ~60 glycosyl residues/mol of RG-II; clearly, these numbers are an approximation. [b]This hexasaccharide is outlined with long-dashed lines in Fig. 6B. [c]This tetrasaccharide is outlined with short-dashed lines in Fig. 6B. When this tetrasaccharide is in intact RG-II, the fucosyl residue is 3-linked (see text). [d]This was the largest poly(glycosiduronic acid) detected in this analysis. [e]This disaccharide was characterized in ref. 1. [f]The galactosyluronic acid residues were terminally linked in the isolated oligoglycosyl-alditols, but may be 4-linked or 3,4-linked in intact RG-II. [g]Placing the number of glycosyl residues per mol of RG-II in parentheses signifies that the glycosyl residue does not form a detectable derivative by the standard methylation technique; the presence of the glycosyl residue was ascertained by other methods. Therefore, the numbers in parentheses are estimates. [h]The fucosyl residues were 4-linked in the isolated glycosyl-alditols, but must be 3,4-linked in intact RG-II, because no 4-linked fucosyl residues have been found in RG-II.

dimsyl anion (0.5 mL) was added. After 2.5 h, the mixture was cooled in ice, methyl iodide (215 μL) was added (to give a concentration of 0.3M), the mixture was allowed to thaw, and stirred for 3 h. A second aliquot of dimsyl anion was added to give a concentration of 0.3M. As before, the triphenylmethane test was used to assure that some dimsyl anion remained in the mixture. After 1 h, the solution was cooled in an ice bath, methyl iodide (2.38 mL) was added, to a concentration of 3M, and the frozen mixture was allowed to warm to room temperature, and stirred overnight (11 h).

The solution was cooled in an ice bath, and Waters HPLC grade H_2O (15 mL) was added, to give a final volume of 30 mL. The resulting Me_2SO–H_2O solution was extracted with 6 portions (30 mL each) of dichloromethane, and the extracts combined, and concentrated to 8 mL in a rotary evaporator. Me_2SO (10 mL) and then HPLC grade H_2O (12 mL) were added, resulting in a colloidal suspension which was divided into twenty 1.5-mL parts. Each aliquot was applied to a Sep-Pack C_{18} cartridge[9] that had been preconditioned by washing: first with CH_2Cl_2 (40 mL, spectra grade), then with acetonitrile (2 mL, HPLC grade), and finally with H_2O (6 mL, HPLC grade). The syringe that was used to apply the 1.5-mL sample was washed with 1:1 Me_2SO–H_2O (0.5 mL) which was also applied to the cartridge. Each Sep-Pak cartridge was washed 4 times with H_2O (2 mL; the last wash to dryness), 4 times with 3:17 acetonitrile–water (2 mL; the last wash to dryness), once with 100% acetonitrile (2 mL), and once with CH_2Cl_2 (2 mL). The corresponding fractions from each of the 20 different Sep-Pak cartridges were combined. The orcinol assay showed that per-*O*-methylated polysaccharide was present in both the 100% acetonitrile and the CH_2Cl_2 washes. When combined and evaporated to dryness, these fractions yielded per-*O*-methylated polysaccharide (50 mg by the anthrone assay[6]).

Partial formolysis of the per-O-methylated, carboxyl-reduced polysaccharide. — The per-*O*-methylated, carboxyl-reduced polysaccharide was divided into four ~2.5-mg batches, each of which was dissolved in 88% formic acid (3 mL) and heated for 80 min at 60°. Mass-spectrometric analysis[10,11] showed that, under these conditions, approximately one-third of the glycosidic linkages of the polysaccharide were cleaved. The solvents were evaporated with a stream of filtered air. The resulting, partially *O*-methylated oligosaccharides were dissolved in a solution (1 mL) of 88:4:4:3:1 EtOD–D_2O–H_2O–NH_4OH–$NaBD_4$, and reduction was conducted overnight at room temperature. In order to decompose any remaining $NaBD_4$, glacial acetic acid was added dropwise until effervescence ceased, and the solution was evaporated with a stream of filtered air. Acetic acid–methanol (1:9; ~1 mL) was added and evaporated 5 times, and then methanol (~1 mL) was added and evaporated 4 times. A solution of the residue in 1:1 ethanol–water (0.5 mL) was applied to a column (1 mL) of Dowex 50 (H^+) resin, and eluted with 3 1-mL aliquots of 1:1 ethanol–water. After evaporation of the eluate, a mixture of carboxyl-reduced, partially *O*-methylated oligoglycosyl-alditols remained.

Per-O-methylation of the carboxyl-reduced, partially O-methylated oligo-

glycosyl-alditols. — The partially *O*-methylated oligoglycosyl-alditols prepared from the carboxyl-reduced RG-II residue were evaporated to dryness in a Hypovial, dried overnight under vacuum over phosphorus pentoxide at 40°, and subjected to two per-*O*-methylations (without isolating the methylated carbohydrate between methylations) as already described; the methylated carbohydrate was isolated by use of Sep-Pak C_{18} cartridges as already described. The 100% MeCN fractions, shown by the orcinol method[6] to contain the per-*O*-methylated oligoglycosyl-alditols, were transferred to a Reactivial (Pierce), and evaporated to dryness.

L.c.–m.s. and g.l.c.–m.s. of the per-O-methylated oligoglycosyl-alditols. — The mixture of carboxyl-reduced, per-*O*-methylated oligoglycosyl-alditols was dissolved in 11:9 acetonitrile–H_2O (100 μL) and 30 μL of the solution was injected onto an Altex Ultrasphere-ODS dp 5 μ column 25 cm × 4.6 mm for liquid chromatography (l.c.), a guard column being used. The column was eluted with a linear gradient of 3:17 acetonitrile–H_2O at 0 min to 2:3 at 30 min, and, with a second linear gradient, to 4:1 at 60 min. The flow rate was 1 mL/min. Approximately 2% of the effluent was analyzed by chemical-ionization mass spectrometry (c.i.-m.s.) as described[11]. The rest of the effluent was collected in 0.5-mL fractions. The temperature of the mass spectrometer source was 154°. (In a second l.c.–m.s. analysis, the source temperature was 220°.) All of the data herein were obtained with the source at 154°, except for that noted in Table VII. The solvent in the fractions was allowed to evaporate. The residue in each fraction was dissolved in 1:1 acetone–decane (20 μL). Aliquots of each fraction (0.5 to 1 μL) were analyzed by g.l.c.–m.s. ("on column" injection on a DBU g.l.c. column) as described[11,12].

^{1}H-N.m.r. analysis of the per-O-methylated oligoglycosyl-alditols. — Selected l.c. fractions were evaporated to dryness, dissolved in 0.4 mL of acetone-d_6 (99.96% of D), and transferred to n.m.r. tubes. ^{1}H-N.m.r. spectra were recorded as described[4].

F.a.b.-m.s. of per-O-methylated oligoglycosyl-alditols. — Many l.c. fractions containing larger oligosaccharides were examined by fast-atom-bombardment mass spectrometry (f.a.b.-m.s.) as described[3].

Preparation of partially O-acetylated, partially O-methylated alditols. — Portions of the per-*O*-methylated oligoglycosyl-alditols in the l.c. fractions were converted into partially *O*-acetylated, partially *O*-methylated alditols as described[4,9], except that nitrogen, not air, was used to evaporate the solvent. A modified procedure was introduced in order to prevent loss of the more volatile derivatives (the partially *O*-acetylated, partially *O*-methylated pentitols and deoxyhexitols derived from the reducing ends of per-*O*-methylated oligoglycosyl-alditols) during the evaporation of solvents. The modified procedure involved omitting the reduction with $NaBD_4$. An aliquot of each fraction was hydrolyzed, and then acetylated (the alditols from the "reducing ends" of the per-*O*-methylated oligoglycosyl-alditols did not require reduction). This eliminated the repeated, acidified-methanol evaporations needed to remove borate. Sodium acetate (20 μL of a 50 mg/mL solution) was added as a catalyst for *O*-acetylation, and the solution was evaporated to dryness

before *O*-acetylation and analysis; this procedure allowed much greater recovery of the volatile alditol derivatives.

Preparation of partially O*-acetylated, partially* O*-methylated* L*-fucitol and* L*-rhamnitol standards.* — Standards for g.l.c.–m.s. and g.l.c. of partially *O*-acetylated, partially *O*-methylated L-fucitol and L-rhamnitol were prepared by deliberately incomplete *O*-methylation of methyl α-L-fucopyranoside, methyl α-L-rhamnoside, L-fucitol, and L-rhamnitol (4 mg of each). The L-fucitol and L-rhamnitol were prepared by $NaBD_4$ reduction of L-fucose and L-rhamnose, respectively. Incomplete *O*-methylation of L-rhamnitol and L-fucitol (in contrast to the methyl glycoside) was necessary, in order to produce standards of partially *O*-acetylated, partially *O*-methylated L-fucitol and L-rhamnitol with methoxyl groups on C-1 and C-5, such as are found, for example, in compounds **4, 9,** and **11** (see Fig. 1). In order to achieve incomplete *O*-methylation of the standards, 0.5 equiv. of dimethylsulfinyl anion for 30 min, and then 0.6 equiv. of methyl iodide were used. The samples were purified by use of C_{18} Sep-Paks[9]. The methyl glycosides were hydrolyzed with 2M TFA, the products reduced with $NaBD_4$ in 9:1 EtOH–water containing M NH_4OH, and *O*-acetylated, all as already described. Samples of partially *O*-methylated L-fucitol and L-rhamnitol were prepared as for the methyl glycosides, except that the acid hydrolysis and reduction steps were omitted and sodium acetate (~1 mg) was added to each sample before *O*-acetylation. The patially *O*-acetylated, partially *O*-methylated alditols were carefully evaporated just to dryness. Thus, four separate mixtures of standards were produced, two containing L-rhamnitol derivatives and two containing L-fucitol derivatives. The two mixtures that contained L-rhamnitol derivatives differed, in that the mixtures made by incomplete *O*-methylation of L-rhamnitol, instead of methyl α-L-rhamnoside, contained methoxyl groups on C-1 and C-5 of some of the derivatives; the two L-fucosyl mixtures differed similarly. Individual components were identified by g.l.c.–m.s.; their stereochemistry (*manno* or *galacto*) was clear because of their origin; the arrangement of *O*-methyl and *O*-acetyl groups was determined by mass spectrometry.

The other partially *O*-acetylated, partially *O*-methylated standards required for these studies had been prepared in a way similar to that used in previous experiments in this laboratory[2].

G.l.c. and g.l.c.–m.s. analyses of partially O*-acetylated, partially* O*-methylated alditols.* — These were conducted as described[4,9].

RESULTS

RG-II was released from cell-walls by an endopolygalacturonase, and purified as described previously[2]. This preparation was shown to be homogeneous in glycosyl composition, as it was eluted in the partially included volume of a gel-permeation column and also in a salt gradient from an ion-exchange column[2].

RG-II was treated with mild acid[4], and a residue having a high molecular

TABLE II

DIAGNOSTIC C I.-M S AND E I -M.S IONS FOR THE PER-*O*-METHYLATED MONOGLYCOSYL-ALDITOLS DERIVED FROM THE RG-II RESIDUE

Monoglycosyl-alditol	*R.t.*[a] *in l.c.*	*Chemical ionization-m.s. ions*[b]				*R.t.*[c] *in g.l.c.*	*Electron impact-m.s. fragment-ions*[b]							
		M + 1	aJ_2	$aJ_2 + H_2O$	bA_1		aJ_1	aJ_2	bA_1	bA_2	*Alditol cleavages*			
18	10.75	476	238	256	221	8.28	298	238	221	189	385	384	134	91
		(100)	(10)	(70)	(40)		(20)	(25)	(10)	(50)	(0.4)	(0.06)	(6)	(28)
1	12.75	444	206[d]	224[d]	221[d]	7.28	266	206	221	189				
		(100)					(0.9)	(42)	(4)	(15)				
2	13.75	444	206	224	221	8.55	266	206	221	189				
		(47)	(3)	(100)	(42)		(10)	(4)	(3)	(10)				
3	15.75	398	192	210	189	6.40	252	192	189	157	307	351	352	
		(100)	(16)	(98)	(89)		(22)	(81)	(75)	(17)	(0.9)	(0.1)	(0.1)	
4	19.25	444	206	224	221	7.08	266	206	221	189	353	321		
		(100)	(11)	(84)	(11)		(8)	(29)	(6)	(64)	(0.1)	(0.5)		

[a]Retention time (min) on the ODS l.c. column. L.c. conditions as described in the Experimental section. [b]Figures in parentheses show peak intensity relative to base peak (100). The base peak was often of low mass, and not a diagnostic ion; thus, it is often not included in the Table. [c]Retention time (min) on the fused silica DB1 g.l.c. capillary column. G.l.c. conditions as described in the Experimental section. [d]The mass spectrometer did not scan low enough for these ions, due to an ion overload that occurred while compound **1** was being eluted from the l.c. column.

weight (only slightly less than that[4] of intact RG-II) was purified from the aceric-acid-containing heptasaccharide and other small oligosaccharides, as described[4]. The large RG-II residue was analyzed by the glycosyl-residue sequencing-method developed in this laboratory[10,11]. The RG-II residue was chosen for analysis because removal of the highly acid-labile oligosaccharides from RG-II provided a less complicated polysaccharide. Use of the RG-II residue simplified purification of the 23 oligosaccharide fragments whose structures are presented here, but did not compromise the results. Each oligosaccharide present in the RG-II residue is clearly present in intact RG-II also.

The RG-II residue was carboxyl-reduced, per-*O*-methylated, and partially acid-hydrolyzed. The resulting, partially *O*-methylated oligosaccharides were reduced, and the products per-*O*-methylated, to afford a mixture of per-*O*-methyl-

TABLE III

DIAGNOSTIC C I -M S IONS OF THE PER-*O*-METHYLATED DIGLYCOSYL-ALDITOLS AND PER-*O*-METHYLATED TRISACCHARIDE METHYL GLYCOSIDES DERIVED FROM THE RG-II RESIDUE

Diglycosyl-aldıtol or trisaccharide methyl glycosıde	*R.t.*[a] *in l.c.*	*Chemical ıonization-m.s. ıons*[b]							
		M + 1	*Elim*[c]	aJ_2	$aJ_2 + H_2O$	cA_1	cA_2	cbA_1	cbA_2
5	12 25	631	457	189	207	219	187	425	393
		(n.d.)[d]	(n.d.)	(18)	(n.d.)	(16)	(18)	(50)	(50)
6	14.75	631	457	189	207[e]	219	187	425	393
		(n.d)	(n.d)	(25)	(n.d.)	(20)	(20)	(35)	(35)
19[f]	15.25	665	459	221	239	221	189	427	395
		(4)	(34)	(80)	(1)	(80)	(100)	(6)	(10)
20	18 75	682	476	238	256	221	189	427	395
		(10)	(100)	(9)	(43)	(50)	(52)	(4)	(11)
7	21.50	604	430	192	210	221	189	395	363
		(18)	(18)	(23)	(29)	(93)	(100)	(18)	(78)
8	22.25	604	430[g]	192	210	221	189	395	363
		(7)	(n.d.)	(13)	(17)	(3)	(6)	(100)	(4)
9[h]	22 75	648	442	206	224	219	187	425	393
		(2)	(2)	(13)	(24)	(35)	(30)	(100)	(90)
23[i]	24.25	636	430[g]	238	256	175	143[j]	381	189
		(30)	(n.d.)	(7)	(12)	(84)	—	(14)	(95)

[a]See footnote *a* in Table II. [b]See footnote *b* in Table II. [c]This is the *m/z* value of the ion formed, or expected to be formed, by elimination of the internal glycosyl residue adjacent to the alditol or methyl glycoside (see text). [d]N.d., not detected. [e]207 was a strong background peak. [f]This compound co-chromatographs with compound **10** (see Table V) and with per-*O*-methylated Ara-(1→6)-Galol-*1,6,6-d*$_3$ (see text) Each of the compounds gives characteristic molecular and fragment ions that are absent in the mass spectra of the co-eluting compounds. [g]Not expected, as the elimination reaction occurs infrequently when the internal glycosyl residue is 3-linked[13]. [h]This compound co-chromatographs with compound **11** (see Table V). Both compounds give the same c.i.-mass spectrum, except that compound **11** yielded an M + 1 ion at *m/z* 822. The two compounds were separated, and clearly distinguished, by g.l.c.–m.s. (see Tables IV and VI). [i]Co-elutes with compound **14** (see Table VII); each compound gives characteristic molecular and fragment ions that are absent in the mass spectra of the co-eluting compound. [j]The mass spectrometer was not set to scan as low as *m/z* 143

ated oligoglycosyl-alditols that was separated by l.c. The structures of the isolated per-*O*-methylated oligoglycosyl-alditols were determined by l.c.–c.i.-m.s., g.l.c.–electron-impact(e.i.)-m.s., f.a.b.-m.s., ^{1}H-n.m.r. spectroscopy, and glycosyl-linkage composition analysis. The hydroxyl groups of the per-*O*-methylated polysaccharide[11] that are released by partial hydrolysis with acid are usually *O*-ethylated in the glycosyl-residue sequencing-method used[11]. Because RG-II is so structurally complex, and appropriate *O*-ethylated standards are not readily available, we chose to *O*-methylate the hydroxyl groups released by the partial, acid hydrolysis.

Tables II–IX present the l.c.–m.s., g.l.c.–m.s., f.a.b.-m.s., ^{1}H-n.m.r. spectroscopy, and glycosyl-linkage data obtained from analyzing the per-*O*-alkylated oligoglycosyl-alditols isolated from the RG-II residue. We used these data to determine the primary structures of the per-*O*-methylated oligoglycosyl-alditols presented in Fig. 1.

Some per-*O*-methylated oligoglycosyl-alditols obtained from the RG-II residue were separated by l.c. into individual components; others were separated into simple mixtures of compounds (see Fig. 2). Chemical ionization-m.s. analysis of the per-*O*-methylated oligoglycosyl-alditols in the l.c. effluent identified the l.c.

TABLE IV

DIAGNOSTIC E I -M S. IONS FOR THE PER-*O*-METHYLATED DIGLYCOSYL-ALDITOLS AND PER-*O*-METHYLATED TRISACCHARIDE METHYL GLYCOSIDES DERIVED FROM THE RG-II RESIDUE

Diglycosyl-alditol or trisaccharide methyl glycoside	*R.t.*[a] *in g.l.c.*	*Electron impact-m.s. fragment-ions*[b]								
		aJ_1	aJ_2	abJ_1	abJ_2	cA_1	cA_2	cbA_1	cbA_2	*Alditol cleavages*
5	15.42	249	189	455	395	219	187	425	393	
		(4)	(5)	(0.3)	(0.1)	(3)	(11)	(0.4)	(0.8)	
6	15.08	249	189	455	395	219	187	425	393	
		(2)	(8)	(0.4)	(0.2)	(1)	(9)	(0.4)	(0.7)	
7	12.83	252	192	426	366[c]	221	189	395	363	
		(16)	(88)	(11)	(n.d.)	(37)	(94)	(2)	(6)	
8	13.90	238[d]	192	426	366	221	189	395	157	
		(17)	(98)	(7)	(2)	(43)	(100)	(6)	(45)	
9	15.20	266	206	472	412	219	187	425	393	557
		(16)	(90)	(4)	(n.d.)	(22)	(95)	(5)	(12)	(<0.5)
19	17.17	281	221	487	427	221	189	427	395	
		(16)	(7)	(0.2)	(n.d.)	(7)	(25)	(n.d.)	(0.5)	
20	16.38	298	238	504	444	221	189	427	395	
		(2)	(16)	(6)	(0.2)	(29)	(88)	(0.5)	(0.5)	
23	14.43	284[e]	238	504	444	175	143	381[f]	189	
		(2)	(11)	(0.01)	(0.01)	(33)	(50)	(3)	(9)	

[a]See footnote *c* in Table II. [b]See footnote *b* in Table II and footnote *d* in Table III. [c]366 − MeOH = 334 was present at 7% of base peak. [d]*M/z* 238 is the aJ_0 ion expected from a 3-linked, internal glycosyl residue[12]. The aJ_1 ion was not present, as expected for a 3-linked, internal glycosyl residue[12]. This compound also gave an unexplained ion at *m/z* 322 at 56% of the intensity of the base peak. [e]*M/z* 284 is the aJ_0 ion expected when the internal glycosyl residue is 3-linked. The aJ_1 ion was not detected, the result expected for a 3-linked, internal glycosyl residue. [f]*M/z* 349 (381 − 32) present at an intensity of 0.4.

TABLE V

DIAGNOSTIC C I-M S IONS FOR THE PER-*O*-METHYLATED TRIGLYCOSYL-ALDITOLS AND PER-*O*-METHYLATED TETRASACCHARIDE METHYL GLYCOSIDES DERIVED FROM THE RG-II RESIDUE

Triglycosyl-alditol or tetrasaccharide methyl glycoside	*R.t.*[a] *in l.c.*	*Chemical ionization-m.s. ions*[b]												
		M + 1	*Elim-1*[c]	*Elim-2*[c]	aJ_2	$aJ_2 + H_2O$	dA_1	dA_2	dcA_1	dcA_2	$dcbA_1$	$dcbA_2$	*Unexplained ions*	
10[d]	15.25	805 (n.d.)	631 (0.1)	457 (n.d.)	189 (38)	207 (15)	219 (8)	187 (21)	425 (16)	393 (13)	599 (0.5)	567 (0.1		
11[e]	22.75	822 (0.5)	648 (1)	442 (3)	206 (18)	224 (34)	219 (39)	187 (48)	425 (100)	393 (91)	599 (n.d.)	567 (0.2)		
12	25.25	778 (0.5)	604 (1)	430 (1)	192 (15)	210 (12)	221 (31)	189 (100)	395 (4)	363 (4)	569 (0.4)	537 (2)		
21[f]	25.75	888 (1.5)	682 (9)	476 (24)	238 (10)	256 (31)	221 (72)	189 (100)	427 (9)	395 (9)	633 (0.5)	601 (0.8)		
							cA_1	cA_2	$c'A_1$	$c'A_2$	$cc'bA_1$	$cc'bA_2$		
13	27.50	810 (2)	—	—	192 (13)	210 (22)	221 (73)	189 (100)	221 (73)	189 (100)	601[g] (22)	363 (6)	365 (15)	349 (23)

[a]See footnote *a* in Table II. [b]See footnote *b* in Table II. [c]Elim-1 = the *m/z* value of the ion produced, or expected, by elimination from the M + 1 ion of the internal glycosyl residue adjacent to the alditol or methyl glycoside. Elim-2 = the *m/z* value of the ion produced, or expected, by elimination from the Elim-1 ion of the internal glycosyl residue adjacent to the alditol or methyl glycoside. [d]This compound co-chromatographs with compound **19** (see Table III) and with per-*O*-methylated Ara*f*-(1→6)-Galol-*1,6,6-d*$_3$ (see text); each of the compounds gives characteristic molecular and fragment ions that are absent in the mass spectra of the co-eluting compounds. [e]This compound co-chromatographs with compound **9** (see Table III) Both compounds give exactly the same c.i.-mass spectrum, except that compound **11** yields an M + 1 ion at *m/z* 822. The two compounds were separated, and clearly distinguished, by g.l.c.–m.s. (see Tables IV and VI). [f]Co-elutes with compound **15** (see Table VII); each of the compounds gives characteristic molecular and fragment ions that are absent in the mass spectra of the co-eluting compound. [g]601 − MeOH = *m/z* 569, present at 3% of base peak.

TABLE VI

DIAGNOSTIC E.I.-M.S. IONS FOR THE PER-*O*-METHYLATED TRIGLYCOSYL-ALDITOLS AND PER-*O*-METHYLATED TETRASACCHARIDE METHYL GLYCOSIDES DERIVED FROM THE RG-II RESIDUE

Triglycosyl-alditol or tetrasaccharide methyl glycoside	*R.t.*[a] *in g.l.c.*	*Electron impact-m.s. fragment-ions*[b]														
		aJ_1	aJ_2	abJ_1	abJ_2	$abcJ_1$	$abcJ_2$	dA_1	dA_2	dcA_1	dcA_2	$dcbA_1$	$dcbA_2$	*Unexplained ions*		
10	23.13	249	189	423	363	629	569	219	187	425	393	599	567			
		(9)	(7)	(n.d.)	(2)	(2)	(1)	(8)	(47)	(11)	(18)	(n.d.)	(n.d.)			
11	21.75	266	206	440	380	646	586[c]	219	187	425	393	599	567			
		(19)	(56)	(0.1)	(0.1)	(0.03)	(0.03)	(6)	(63)	(3)	(3)	(0.01)	(0.01)			
12	22.08	252	192	426	366	600	540	221	189	395	363	569	537			
		(1)	(31)	(1)	(2)	(0.1)	(0.02)	(3)	(34)	(0.05)	(n.d.)	(n.d.)	(n.d.)			
21	23.90	298	238	504	444	710	650	221	189	427	395	631	599	379	585	
		(7)	(50)	(1)	(0.5)	(0.09)	(n.d.)	(18)	(50)	(0.3)	(n.d.)	(n.d.)	(n.d.)	(8)	(1)	
				$abc'J_1$	$abc'J_2$	$abcJ_1$	$abcJ_2$	cA_1	cA_2	$c'A_1$	$c'A_2$	$cc'bA_1$	$cc'bA_2$			
13	19.77	252[d]	192	632	572	632	572	221	189	221	189	601	363	336	380	430
		(n.d.)	(34)	(0.4)	(0.05)	(0.4)	(0.05)	(13)	(69)	(13)	(69)	(0.05)	(1)	(2)	(4)	(3)

[a]See footnote *c* in Table II. [b]See footnote *b* in Table II and footnote *d* in Table III. [c]586 − MeOH = *m/z* 554, present at 0.06% of base peak. [d]aJ_1 = *m/z* 252, not expected due to the Gal*p*-6,6-d_2 group on O-3 of the Rha residue.

TABLE VII

DIAGNOSTIC F A B -M S AND C.I -M S IONS OF THE PER-*O*-METHYLATED TETRA-, PENTA-, AND HEXA-GLYCOSYL-ALDITOLS DERIVED FROM THE RG-II RESIDUE

Oligoglycosyl-aldıtol	*R t [a] in t c*	*F.a b [c] (M+18)*	*F a b [c] (M+1)*	*Chemical ionization-m s ions[d]*													
				M+1	*Elim$_1$*	*Elim$_2$*	*Elim$_3$*	*ab'J_2*	*ab'J_2 +H_2O*	*dA_1*	*dA_2*	*dcA_1*	*dcA_2*	*dcbA_1*	*dcbA_2*	*b'A_1*	*b'A_2*	*c'A_1*	*c'A_2*
14[e]	24 25	1028	—	>1000	854 (0 2)	648 (0 2)	—	412 (1 4)	430 (5 5)	219 (41)	187 (20)	425 (13)	393 (12)	599 (n d)	567 (n d)	221 (20)	189 (92)	—	—
								aJ_2	*aJ_2 +H_2O*	*eA_1*	*eA_2*	*edA_1*	*edA_2*	*edcA_1*	*edcA_2*	*edcbA_1*	*edcbA_2*		
15[f]	25 75	982	—	982 (0 2)	808 (0 1)	634 (0 5)	428 (3)	192 (13)	210 (10)	219 (14)	187 (15)	425 (42)	393 (39)	599 (0 2)	567 (0 7)	773 (0 5)	741 (1)	—	—
22[g]	31 00	1094	1111	>1000	888 (0 3)	682 (2)	476 (6)	238 (16)	256 (44)	221 (47)	189 (84)	427 (7)	395 (n d)	633 (0 5)	601 (0 1)	839 (n d)	807 (n d)	—	—
																edcc'bA$_1$	*edcc'bA$_2$*		
16[g]	28 25	1188	1205	>1000	808 (0 1)	634 (n d)	428 (1 3)	192 (12)	210 (21)	219 (35)	187 (100)	425 (28)	393 (83)	599 (0 1)	567 (0 3)	979 (n d)	947 (0 3)	221 (15)	189 (57)
																c''A_1	*c''A_2*		
17[g]	35 00	1394	1411	>1000	—	—	—	192 (9)	210 (14)	219 (33)	187 (87)	425 (24)	393 (70)	599 (0 2)	567 (0 2)	221 (30)	189 (100)	221 (30)	189 (100)

[a]See footnote *a* in Table II. [b]*M/z* value detected in the molecular-ion region of the f.a.b.-mass spectrum [c]The f.a.b.-mass spectra of comounds **16, 17,** and **22** were recorded in the presence of NH_4 ion, leading to (M + 18) ions, as well as (M + 1) ions. [d]See footnote *b* in Table II. (M + 1) ions with an *m/z* value >1000 were not detected, because of the inability of the c i.-mass spectrometer to scan above *m/z* 1000. [e]Co-elutes with compound **23** (see Table III); each of the compounds gives characteristic molecular and fragment ions that are absent in the mass spectra of the co-eluting compound. [f]Co-elutes with compound **21** (see Table V); each of the compounds gives characteristic molecular and fragment ions that are absent in the mass spectra of the co-eluting compound. [g]C i.-m.s. done at a source temp. of 220°.

TABLE VIII

^{1}H-N.M.R CHEMICAL SHIFTS AND COUPLING CONSTANTS OF THE ANOMERIC PROTONS OF SELECTED PER-*O*-METHYLATED OLIGOGLYCOSYL-ALDITOLS[a] AND PER-*O*-METHYLATED OLIGOSACCHARIDE METHYL GLYCOSIDES ISOLATED FROM THE RG-II RESIDUE

Oligoglycosyl-alditol or oligosaccharide methyl glycoside	*Number of protons*	*Chemical shift*	$J_{1,2}$	*Residue*	*Anomeric assignment*	*Other compounds used in order to make assignment*
1	1	5.20	2	T-Gal-*6,6-d*$_2$[b]	α	none
3	1	4.73	2	T-Rha	β	none
4	1	4.51	7.9	T-Glc-*6,6-d*$_2$	β	none
5	1	5.44	3.6	T-Gal	α	none
	1	4.41	7.6	2-Glc-*6,6-d*$_2$	β	**4**
	1	4.72	3.8	4-Fuc	α	none
6	1	5.48	3.3	T-Gal	α	none
	1	4.44	7.4	2-Glc-*6,6-d*$_2$	β	**4**
	1	4.20	6.0	4-Fuc	β	none
7	1	5.01	2.6	T-Gal-*6,6-d*$_2$	α	none
	1	4.77	1.7	2-Rha	β	none
8	1	4.64	<1	3-Rha	β	none
	1	4.44	7.2	T-Gal-*6,6-d*$_2$	β	none
9[c]	1	5.37	3.6	T-Gal	α	none
	1	4.59	7.7	2-Glc-*6,6-d*$_2$	β	**4**
11[c]	1	5.45	3.6	T-Gal	α	**9**
	1	5.11	3.3	4-Fuc	α	none
	1	4.43	7.4	2-Glc-*6,6-d*$_2$	β	**4**
13	1	4.99	2	T-Gal-*6,6-d*$_2$ (c)	α	**7**
	1	4.7	broad	2,3-Rha	β	none
	1	4.49	7.3	T-Gal-*6,6-d*$_2$ (c')	β	**8**
14	1	5.45	3.5	T-Gal	α	**9**
	2	5.20	complex	T-Gal-*6,6-d*$_2$	α	none
				4-Fuc	α	none
	1	4.42	7.9	2-Glc-*6,6-d*$_2$	β	**4**
15	1	5.45	3	T-Gal or 4-Fuc	α	none
	1	5.42	3	4-Fuc or T-Gal	α	none
	1	4.77	2	4-Rha	β	none
	1	4.42	7.8	2-Glc-*6,6-d*$_2$	β	**4**
16	2	5.45	complex	T-Gal	α	none
				4-Fuc	α	none
	1	5.01	2.2	T-Gal-*6,6-d*$_2$	α	**7**
	1	4.77	2.2	2,4-Rha	β	none
	1	4.42	7.9	2-Glc-*6,6-d*$_2$	β	**4**

[a]Per-*O*-methylated oligoglycosyl-alditols **18**, **29**, **21**, and **22** were also analyzed by ^{1}H-n.m.r. spectroscopy. All of the glycosyl residues of all of these compounds were found to be in the α anomeric configuration. [b]T-Gal-*6,6-d*$_2$, etc. represents terminal-Gal-*6,6-d*$_2$. [c]Per-*O*-methylated diglycosyl-alditol **9** was contaminated with triglycosyl-alditol **11** and *vice versa*. the signals arising from the two per-*O*-methylated oligoglycosyl-alditols could be distinguished, because the two compounds were present in different molar proportions.

TABLE IX

GLYCOSYL-LINKAGE COMPOSITION OF PER-*O*-METHYLATED OLIGOSACCHARIDE FRAGMENTS **1–23**

Per-O-methylated oligosaccharide fragments	*Partially O-acetylated, partially O-methylated alditol*[a,b]				
1	2,3,4,6-Gal-*6,6-d*$_2$	1,3,4,5-Rha			
	1	0.3			
2	2,3,4,6-Gal-*6,6-d*$_2$	1,2,4,5-Rha			
	1	0.3			
3	2,3,4-Rha	1,2,3,4-Api			
	1	p[c]			
4[d]	2,3,4,6-Glc-*6,6-d*$_2$	1,2,3,5-Fuc			
	1	1			
5	2,3,4,6-Gal	3,4,6-Glc-*6,6-d*$_2$	2,3-Fuc		
	1	1	1		
6	2,3,4,6-Gal	3,4,6-Glc-*6,6-d*$_2$	2,3-Fuc		
	1	1.2	0.8		
7	2,3,4,6-Gal-*6,6-d*$_2$	3,4-Rha	1,2,3,4-Api		
	1	0.6	0.3		
8	2,3,4,6-Gal-*6,6-d*$_2$	2,4-Rha	1,2,3,4-Api		
	1	0.7	0.4		
9	2,3,4,6-Gal	3,4,6-Glc-*6,6-d*$_2$	1,2,3,5-Fuc		
	1	0.8	0.8		
10	2,3,4,6-Gal	3,4,6-Glc-*6,6-d*$_2$	2,3-Fuc	2,3-Rha	
	2.5	1.4	0.7	0.6	
11	2,3,4,6-Gal	3,4,6-Glc-*6,6-d*$_2$	2,3-Fuc	1,2,3,5-Rha	
	1	0.9	0.9	0.3	
12	2,3,4,6-Glc-*6,6-d*$_2$	2,3-Fuc	2,3-Rha	1,2,3,4-Api	
	1	0.8	0.8	0.5	
13	2,3,4,6-Gal-*6,6-d*$_2$	4-Rha	1,2,3,4-Api		
	4	1	p[c]		
14	2,3,4,6-Gal	2,3,4,6-Gal-*6,6-d*$_2$	3,4,6-Glc-*6,6-d*$_2$	2,3-Fuc	1,3,5-Rha
	1	2	0.5	0.7	0.2

15	2,3,4,6-Gal	3,4,6-Glc-*6,6-d*$_2$	2,3-Fuc	2,3-Rha	1,2,3,4-Api	
	1.5	1	1	1	1.1	
16	2,3,4,6-Gal	2,3,4,6-Gal-*6,6-d*$_2$	3,4,6-Glc-*6,6-d*$_2$	2,3-Fuc	3-Rha	1,2,3,4-Api
	1	1.2	1.2	1.7	1.6	p[c]
17	2,3,4,6-Gal	2,3,4,6-Gal-*6,6-d*$_2$	3,4,6-Glc-*6,6-d*$_2$	2,3-Fuc	Rhaol[e]	1,2,3,4-Api
	1	2.8	1.1	1	0.9	1.1
18	2,3,4,6-Gal-*6,6-d*$_2$	1,2,3,5,6-Gal-*6,6-d*$_2$				
	1	1				
19	2,3,4,6-Gal-*6,6-d*$_2$	2,3,6-Gal-*6,6-d*$_2$				
	1	1				
20	2,3,4,6-Gal-*6,6-d*$_2$	2,3,6-Gal-*6,6-d*$_2$	1,2,3,5,6-Gal-*6,6-d*$_2$			
	1.3	1	0.8			
21	2,3,4,6-Gal-*6,6-d*$_2$	2,3,6-Gal-*6,6-d*$_2$	1,2,3,5,6-Gal-*6,6-d*$_2$			
	1	2.2	0.3			
22	2,3,4,6-Gal-*6,6-d*$_2$	2,3,6-Gal-*6,6-d*$_2$	1,2,3,5,6-Gal-*6,6-d*$_2$			
	1.7	3	0.8			
23	2,3,5-Ara	2,4,6-Gal-*6,6-d*$_2$	1,2,3,5,6-Gal-*6,6-d*$_2$			
	0.6	1.7	1			

[a]The partially *O*-methylated alditol acetates derived from the glycosyl residues of the per-*O*-methylated oligoglycosyl-alditols characterized herein. Most samples contained small proportions of partially *O*-methylated alditol acetates that were not derived from the per-*O*-methylated oligoglycosyl-alditols under consideration. The identity and amounts of these contaminating partially *O*-methylated alditol acetates are not presented in this Table. The contaminants were distinguished from those arising from the per-*O*-methylated oligoglycosyl-alditol under study in three ways. (1) Usually, the contaminating, partially methylated alditol acetates were present in substantially smaller proportions than those of those resulting from the per-*O*-methylated oligoglycosyl-alditols under consideration. (2) The e.i.- and c.i.-mass spectra of the per-*O*-methylated oligoglycosyl-alditols divulged a limit to the partially *O*-methylated alditol acetates that could be present. For example, the e.i.- and c.i.-mass spectrometric analyses of compound **4** showed that a di-*O*-acetyl-1,6,6-trideuterio-tetra-*O*-methylhexitol and a mono-*O*-acetyl-deoxy-tetra-*O*-methylhexitol are the only partially methylated alditol acetates possible in **4** (thus precluding for example, any di-*O*-acetyl-1-deuterio-tetra-*O*-methylhexitols, or any tri-*O*-acetyl-1,6,6-trideuterio-tri-*O*-methylhexitols). (3) When two or more per-*O*-methylated oligoglycosyl-alditols co-eluted, the components of a mixture were usually present in different amounts, which, together with considerations discussed in 2, allowed assignment of the partially *O*-methylated alditol acetate components to each per-*O*-methylated oligosaccharide. [b]The numbers in the abbreviations indicate the positions of *O*-methyl groups. All remaining hydroxyl groups were acetylated. If deuterium atoms were present on C-6, they are noted. Thus, for example, 2,3,4,6-Gal-*6,6-d*$_2$ = 1,5-di-*O*-acetyl-1,6,6-trideuterio-2,3,4,6-tetra-*O*-methylgalactitol. The number in the second line of each entry indicates the molar ratios of the fragments. [c]p = present. In these compounds the volatile alditol was detected by using a procedure described in Methods. When this procedure was used, the ratio of the alditol to the other glycosyl residues of the fragments was not determined. [d]1,5-Di-*O*-acetyl-1,6,6,-trideuterio-2,3,4,6-tetra-*O*-methylgalactitol was present in amounts equal to those of 1,5-di-*O*-acetyl-1,6,6-trideuterio-2,3,4,6-tetra-*O*-methylglucitol. This was true because compound **4** was contaminated with compound **20** (see Fig. 2). Thus, it was theoretically possible that the true structure of **4** is Gal*p-6,6-d*$_2$-(1→4)-Fucol. Because the partially *O*-methylated alditol acetates derived from **20**, obtained by the analysis of the fraction (from the l.c. column) preceding the fraction analyzed for **4**, contained 1,5-di-*O*-acetyl-1,6,6,-trideuterio-2,3,4,6-tetra-*O*-methylgalactitol and no 1,5-di-*O*-acetyl-1,6,6-trideuterio-2,3,4,6-tetra-*O*-methylglucitol, the 2,3,4,6-Gal*p-6,6-d*$_2$ in the fraction that contained **4** was part of compound **20** and the 2,3,4,6-Glc*p-6,6-d*$_2$ was part of **4**. [e]Rhaol = 1,2,3,4,5-penta-*O*-acetylrhamnitol, the derivative expected from rhamnosyl residues substituted at O-2, O-3, and O-4.

1 α-Gal*p*-6,6-d_2-(1→2)-Rhaol-*1-d*
2 Gal*p*-6,6-d_2-(1→3)-Rhaol-*1-d*
3 β-Rha*p*-(1→3^1)-Apiol-*1-d*
4 β-Glc*p*-6,6-d_2-(1→4)-Fucol-*1-d*
5 α-Gal*p*-(1→2)-β-Glc*p*-6,6-d_2-(1→4)-α-Fuc*p*-1Me
6 α-Gal*p*-(1→2)-β-Glc*p*-6,6-d_2-(1→4)-β-Fuc*p*-1Me
7 α-Gal*p*-6,6-d_2-(1→2)-β-Rha*p*-(1→3^1)-Apiol-*1-d*
8 β-Gal*p*-6,6-d_2-(1→3)-β-Rha*p*-(1→3^1)-Apiol-*1-d*
9 α-Gal*p*-(1→2)-β-Glc*p*-6,6-d_2-(1→4)-Fucol-*1-d*
10 Gal*p*-(1→2)-Glc*p*-6,6-d_2-(1→4)-Fuc*p*-(1→4)-Rha*p*-1Me
11 α-Gal*p*-(1→2)-β-Glc*p*-6,6-d_2-(1→4)-α-Fuc*p*-(1→4)-Rhaol-*1-d*
12 Glc*p*-6,6-d_2-(1→4)-Fuc*p*-(1→4)-Rha*p*-(1→3^1)-Apiol-*1-d*

13
```
      c                  b            a
α-Galp-6,6-d2-(1→2)-β-Rhap-(1→3¹)-Apiol-1-d
                          3
                          ↑
                    c'    1
                    β-Galp-6,6-d2
```

14
```
       d          c                 b              a
α-Galp-(1→2)-β-Glcp-6,6-d2-(1→4)-α-Fucp-(1→4)-Rhaol-1-d
                                                  2
                                                  ↑
                                            b'    1
                                            α-Galp-6,6-d2
```

15 α-Gal*p*-(1→2)-β-Glc*p*-6,6-d_2-(1→4)-α-Fuc*p*-(1→4)-β-Rha*p*-(1→3^1)-Apiol-*1-d*

16
```
        e          d                 c                 b            a
α-Galp-(1→2)-β-Glcp-6,6-d2-(1→4)-β-Fucp-(1→4)-β-Rhap-(1→3¹)-Apiol-1-d
                                                       2
                                                       ↑
                                                 c'    1
                                                 α-Galp-6,6-d2
```

17
```
                                          c''
                                          Galp-6,6-d2
                                          1
                                          ↓
  e          d                 c          b 3              a
Galp-(1→2)-Glcp-6,6-d2-(1→4)-Fucp-(1→4)-Rhap-(1→3¹)-Apiol-1-d
                                          2
                                          ↑
                                          c'1
                                          Galp-6,6-d2
```

18 α-Gal*p*-6,6-d_2-(1→4)-Galol-*1,6,6-d*$_3$
19 Gal*p*-6,6-d_2-(1→4)-Gal*p*-6,6-d_2-(1→4)-Gal*p*-6,6-d_2-1Me
20 α-Gal*p*-6,6-d_2-(1→4)-α-Gal*p*-6,6-d_2-(1→4)-Galol-*1,6,6-d*$_3$
21 α-Gal*p*-6,6-d_2-(1→4)-α-Gal*p*-6,6-d_2-(1→4)-α-Gal*p*-6,6-d_2-(1→4)-Galol-*1,6,6-d*$_3$
22 α-Gal*p*-6,6-d_2-(1→4)-α-Gal*p*-6,6-d_2-(1→4)-α-Gal*p*-6,6-d_2-(1→4)-α-Gal*p*-6,6-d_2-(1→4)-Galol-*1,6,6-d*$_3$
23 • Ara*f*-(1→3)-Gal*p*-6,6-d_2-(1→4)-Galol-*1,6,6-d*$_3$.

Fig. 1. The 23 oligoglycosyl-alditol and oligosaccharide-methyl glycoside fragments of RG-II structurally characterized herein. As characterized (data in Tables II–VIII), all oxygen atoms not involved in glycosyl bonds or ring formation were substituted with methyl groups. The only anomeric configurations indicated are those that have been determined by ^{1}H-n.m.r. (see Table VIII).

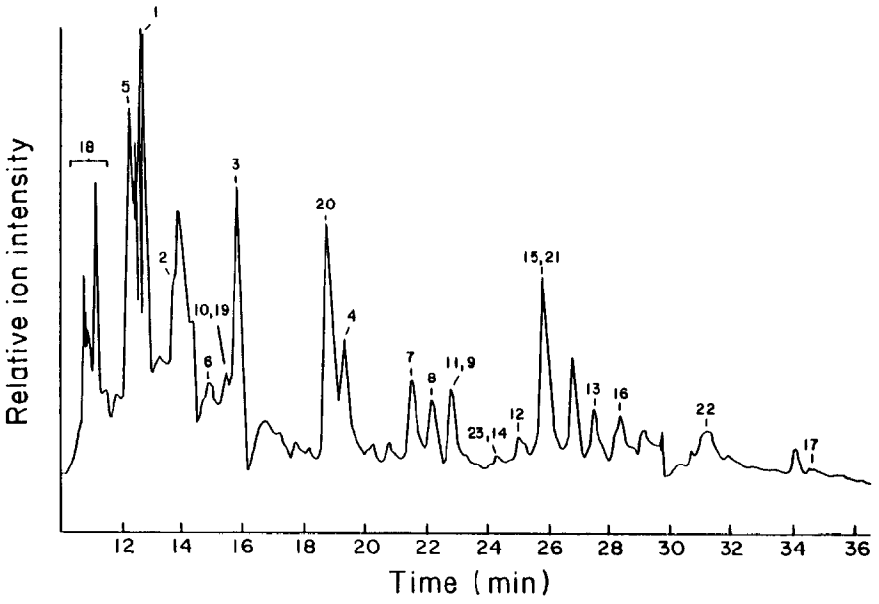

Fig. 2. Reversed-phase, l.c. elution-profile of the per-*O*-methylated oligoglycosyl-alditols and per-*O*-methylated oligosaccharide methyl glycosides derived from RG-II, as detected by c.i.-m.s. The l.c. eluate was split in such a way that 2% could be used for m.s. detection and the remaining 98% could be collected in fractions. Each per-*O*-methylated oligoglycosyl-alditol and oligosaccharide methyl glycoside that has been structurally characterized has been assigned a number (in bold type) indicating the sequence in which it was eluted from the l.c. column (see Tables II–VIII). The components eluted between 10 and 12 min, only, appear to be split, due to an ion overload in the mass spectrometric detection-system during the elution of these peaks.

retention times of each component, and also provided the M + 1 ion and diagnostic fragment-ions (see Tables II, III, V, and VII). It should be noted that, in addition to the per-*O*-methylated oligoglycosyl-alditols expected, several methyl glycosides of per-*O*-methylated oligosaccharides were identified. The origin of these methyl glycosides has not yet been investigated.

The M + 1 ion is the most useful ion obtained by c.i.-m.s. analysis of a per-*O*-methylated oligoglycosyl-alditol (or methyl glycoside), because it reveals the molecular weight[11]. The molecular weight defines the number of the dideuterio-hexosyl (that is, carboxyl-reduced glycosyluronic acid), hexosyl, deoxyhexosyl, and pentosyl residues that constitute the oligoglycosyl-alditol.

Per-*O*-alkylated oligoglycosyl-alditols frequently undergo an elimination reaction during c.i.-m.s. analysis[13]. In this reaction, the glycosyl residue adjacent to the alditol residue is eliminated, and a protonated oligoglycosyl-alditol containing one glycosyl residue less is produced[13]. It has been clearly shown that only the glycosyl residue next to the alditol residue is eliminated[13]; furthermore, the new ion formed by this elimination can undergo another elimination reaction, to form a protonated oligoglycosyl-alditol that contains two glycosyl residues less than the parent oligoglycosyl-alditol. This elimination reaction is illustrated for the per-*O*-

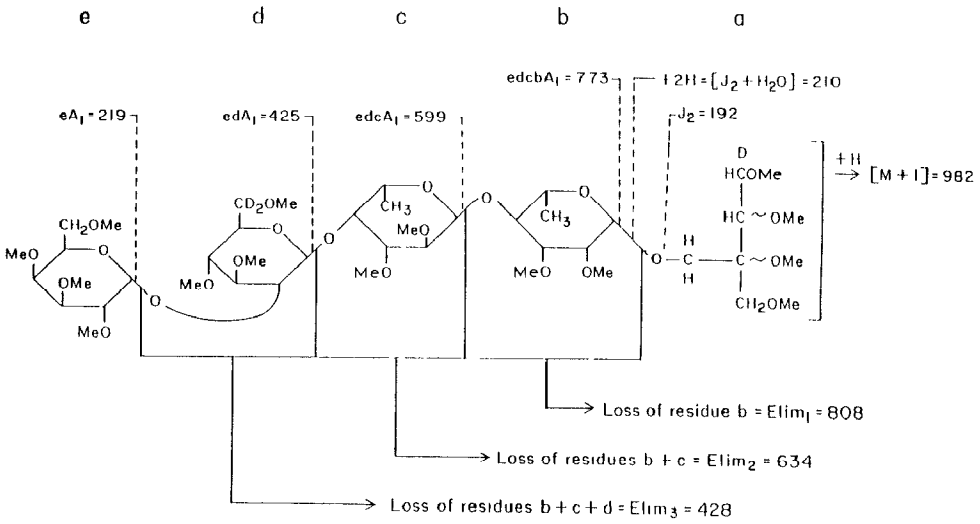

Fig. 3. The production of elimination ions during c.i.-m.s. analysis of compound **15**. The formation of the A series of ions, the J series of ions, and the J + H_2O ions is also illustrated.

methylated tetraglycosyl-alditol **15** in Fig. 3. The fragment-ions that are generated by elimination of internal glycosyl residues from each per-*O*-alkylated oligoglycosyl-alditol are listed in Tables III, V, and VII under the heading "Elim". Per-*O*-alkylated oligoglycosyl methyl glycosides are susceptible to the same elimination reactions[13].

Other c.i.-m.s. fragment-ions are generated from the alditol end of per-*O*-methylated oligoglycosyl-alditols (see Fig. 3). These include the alditol ion (aJ_2) and the alditol ion plus a water molecule ion ($aJ_2 + H_2O$). Chemical ionization also produces an "A" series of fragment ions identical to the series produced from the nonreducing end of per-*O*-methylated oligoglycosyl-alditols during e.i.-m.s.[11].

Some of the per-*O*-methylated oligoglycosyl-alditols co-chromatographed during l.c.; these are noted in the Tables. All such mixtures of per-*O*-methylated oligoglycosyl-alditols, as well as the pure per-*O*-methylated oligoglycosyl-alditols, were analyzed by g.l.c.–e.i.-m.s. The fragment ions produced during g.l.c.–e.i.-m.s. analysis of the per-*O*-methylated oligoglycosyl-alditols are presented in Tables II, IV, and VI. Per-*O*-methylated oligoglycosyl-alditols containing more than five residues could not be analyzed by g.l.c.–m.s., because they were not eluted from the g.l.c. column. The e.i.-m.s. fragmentation-pathways of per-*O*-methylated oligoglycosyl-alditols are well documented[11,14–19], and will not be discussed here (see, however, Figs. 4 and 5).

The mass spectrometer used to analyze the l.c. effluent could not detect ions of m/z >1,000. Per-*O*-methylated oligoglycosyl-alditols having molecular weights >1,000 were analyzed by f.a.b.-m.s., using high-mass instrumentation. The molecular weights of these per-*O*-methylated oligoglycosyl-alditols were obtained from the M + 1 or M + 18 signals, or both, in their f.a.b.-mass spectra, as noted in Table VII.

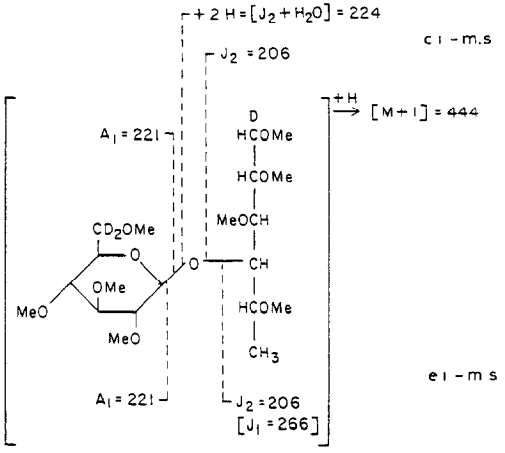

Fig. 4. The formation of the ions produced during the c.i.-m.s. (top) and e.i -m.s. (bottom) of compound **4**.

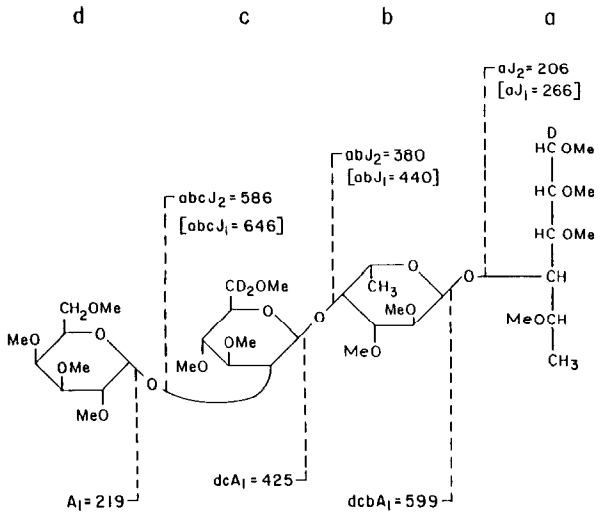

Fig. 5. The formation of the ions produced during e.i.-m.s. of **11**.

The glycosyl-linkage composition of each of the per-*O*-methylated oligoglycosyl-alditols was determined by g.l.c. and g.l.c.–m.s. analysis of the partially *O*-acetylated, partially *O*-methylated alditols derived by hydrolysis, reduction ($NaBD_4$), and acetylation (see Table IX). The partially *O*-acetylated, partially *O*-methylated alditol derived from the apiitol residue located at the former reducing end of some of the per-*O*-methylated oligoglycosyl-alditols (3^1-*O*-acetyl-1,2,3,4-tetra-*O*-methylapiitol) could be identified directly (without a g.l.c. retention-time standard) by its unique e.i.-mass spectrum, because no other branched pentosyl residue is present in RG-II (see Table I). The positions of the *O*-acetyl groups and

O-methyl groups on the alditols derived from 6-deoxyhexitol residues located at the former reducing end of the per-*O*-methylated oligoglycosyl-alditols could be determined by their e.i.-mass spectra. The identities of the deoxyhexitols (rhamnitol or fucitol) were ascertained by comparing their g.l.c. retention times with those of standards prepared by deliberately incomplete *O*-methylation of L-rhamnitol and L-fucitol (see Experimental section). In a similar way, the type of alditol (hexitol, 6,6-dideuteriohexitol, or deoxyhexitol) and the positions of the *O*-acetyl and *O*-methyl groups on the other residues were determined from the e.i.-m.s. fragment-ions. The deoxyhexitols (fucitol or rhamnitol) and hexitols (galactitol or glucitol) were identified by comparing their g.l.c. retention-times to those of appropriate standards.

The anomeric configurations of glycosyl residues present in many of the per-*O*-methylated oligoglycosyl-alditols were determined by ^{1}H-n.m.r. spectroscopy[4,20,21] (see Table VIII). Definitive assignment of the anomeric configurations of some of these per-*O*-methylated oligoglycosyl-alditols required knowledge of the anomeric configuration of the glycosyl residues of some of the smaller per-*O*-methylated oligoglycosyl-alditols, as indicated in Table VIII.

It is not practical to discuss in detail how these data were used to determine the structures of all 23 compounds. Therefore, the structural determination of per-*O*-methylated oligoglycosyl-alditols **4** and **11** will be presented in detail as examples.

The structure of compound **4** was determined as follows. The c.i.-m.s. (Table II and Fig. 4) showed that the molecular weight was 443 (M + 1 ion = *m/z* 444). This corresponded to a mono-*O*-glycosylalditol containing a 6,6-dideuteriohexosyl and a deoxyhexosyl unit (one of which is the alditol and one, the glycosyl unit; the molecular weight is obviously not dependent on which residue is that of the alditol). The c.i. fragment-ions aJ_2 at *m/z* 206 and (aJ_2 + H_2O) at *m/z* 224 (see Fig. 4) established that the alditol is a deoxyhexitol residue. The bA_1 ion at *m/z* 221 confirmed that the glycosyl unit is a 6,6-dideuteriohexosyl group. The e.i.-m.s. (see Fig. 4) confirmed this sequence; the aJ_1 ion at *m/z* 266 and the aJ_2 ion at *m/z* 206 characterized the deoxyhexitol, and the bA_1 ion at *m/z* 221 characterized the non-reducing (terminal) 6,6-dideuteriohexosyl group. The alditol residue was shown to be that of fucitol, linked at O-4, and the 6,6-dideuteriohexosyl group was shown to be 6,6-dideuterioglucosyl by the formation and analysis of the corresponding partially *O*-methylated alditols (see Table IX). ^{1}H-N.m.r. analysis established that the 6,6-dideuterioglucosyl group was in the β configuration (see Table VIII), as shown by the signal at δ 4.51 with a coupling constant of 7.9 Hz. Thus, the per-*O*-methylated glycosyl-alditol **4** is β-Glc*p*-6,6-d_2-(1→4)-Fucol-*1-d*.

Per-*O*-methylated triglycosyl-alditol **11** yielded an M + 1 ion at *m/z* 822. It was composed of a hexosyl, a 6,6-dideuteriohexosyl, and two deoxyhexosyl residues (one of these four residues being the alditol). The remaining ions in the c.i.-mass spectrum of **11** were also present in the c.i.-mass spectrum of **9** (which was co-eluted with **11**). Although these ions are correctly assigned in Table V, they

were not used to determine the structure of compound **11**. Compounds **9** and **11** were separated by g.l.c. during g.l.c.–m.s. analysis (see Tables IV and VI), which allowed an e.i.-mass spectrum of pure **11** to be obtained (see Fig. 5 and Table VI). The presence of the aJ_2 ion at *m/z* 206 established that the alditol in **11** was a deoxyhexitol, and the abJ_2 ion at *m/z* 380, that the next residue ("b" in Fig. 5) was a deoxyhexosyl residue. The $abcJ_2$ ion at *m/z* 586 showed that residue c (see Fig. 5) is a 6,6-dideuteriohexosyl residue. Because the molecular weight of **11** is 821, residue d must be a hexosyl residue; this was confirmed by the dA_1 ion at *m/z* 219. Residue c was confirmed as a 6,6-dideuteriohexosyl residue by the dcA_1 ion at *m/z* 425, and residue b was confirmed to be a deoxyhexosyl residue by the $dcbA_1$ ion at *m/z* 599. The presence of the aJ_1 ion (*m/z* 266) and the abJ_1 ion (*m/z* 440) established that neither of the internal glycosyl residues is 3-linked, as these ions are not produced by 3-linked residues[12]. For 3-linked, internal residues, a J_0 ion (46 mass units greater than the J_2 ion) is produced; see **8** in Table IV. Thus, **11** is Hex-(1→?)-Hex-*6,6-*d_2-(1→?)-Deoxyhex-(1→?)-Deoxyhexol-*1-d*.

The mixture of compounds **9** and **11** was converted into the corresponding, partially *O*-methylated alditol acetates. E.i.-m.s. analysis (as just described for **11**) had established that compound **9** is Hex-(1→?)-Hex-*6,6-*d_2-(1→?)-Deoxyhexol-*1-d*.

The terminal hexosyl group in both compounds was shown to be a Gal*p* group, as 1,5-di-*O*-acetyl-1-deuterio-2,3,4,6-tetra-*O*-methylgalactitol was the only 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol detected in the partially *O*-methylated alditol acetates obtained from **9** and **11**. Therefore, **11** had to yield a 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol, and the only question remaining was the identity of the hexitol. The Hex-*6,6-*d_2 residue in both **9** and **11** was shown to be 2-linked Glc*p-6,6-*d_2, as 1,2,5-tri-*O*-acetyl-1,6,6-trideuterio-3,4,6-tri-*O*-methylglucitol was the preponderant, partially-*O*-methylated hexitol acetate that had two deuterium atoms on C-6, three *O*-acetyl groups, and three *O*-methyl groups. Although smaller proportions of a partially methylated hexitol corresponding to a 4-linked Gal*p-6,6-*d_2 were also detected, the Hex-*6,6-*d_2 in residue **11** was identified as a 2-linked Glc*p-6,6-*d_2, based on quantitative considerations and the structures of other oligosaccharide fragments of RG-II (*e.g.*, **4** and **5**). The deoxyhexosyl residue in **11** was shown to be 4-linked Fuc*p*, as the only partially *O*-methylated deoxyhexitol acetate detected that corresponded to an internal deoxyhexosyl unit (*i.e.*, three *O*-acetyl groups and two *O*-methyl groups) was 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylfucitol. Two partially *O*-methylated deoxyhexitol acetates were detected that corresponded to the alditols of **9** and **11** (one *O*-acetyl group and four *O*-methyl groups). These were 4-*O*-acetyl-1,2,3,5-tetra-*O*-methylfucitol and 4-*O*-acetyl-1,2,3,5-tetra-*O*-methylrhamnitol. The g.l.c.–m.s. and ^{1}H-n.m.r. analyses showed that the diglycosyl-alditol **9** was present in significantly larger proportions than the triglycosyl-alditol **11**. Because there was substantially more of the fucitol derivative it was assigned to **9**, and the rhamnitol derivative to **11**. The pyranose form of the fucosyl residue was established by its presence as a 4-linked fucitol residue in **4** and **9**. Thus, **11** was shown to be Gal*p*-(1→2)-Glc*p-6,6-*d_2-(1→4)-Fuc*p*-(1→4)-Rhaol-*1-d*.

The anomeric configurations were determined by ^{1}H-n.m.r. spectroscopy (see Table VIII). The signal arising from **9** was clearly more intense than those from **11**; thus, the two sets of signals were readily distinguished. Signals corresponding to two α-pyranosyl residues and one β-pyranosyl residue were assigned to **11**. Because the ^{1}H-n.m.r. analysis of **4** showed that the 2-linked Glc*p*-6,6-d_2 residue was in the β configuration, the galactosyl and fucosyl residues must have been in the α configuration. The signals of the anomeric protons of **9** agreed with these assignments. Thus, the complete structure of **11** was determined to be α-Gal*p*-(1→2)-β-Glc*p*-6,6-d_2-(1→4)-α-Fuc-*p*-(1→4)-Rhaol-*1*-*d*.

Similar analyses provided the complete structures of the other per-*O*-methylated oligoglycosyl-alditols. Determining the order of the fucosyl and rhamnosyl residues in per-*O*-methylated oligoglycosyl-alditols **10, 12, 15, 16,** and **17** required knowledge of the glycosyl sequences of the smaller per-*O*-methylated oligoglycosyl-alditols (*e.g.*, compounds **4, 6, 9,** or **11**).

DISCUSSION

The structures of 23 per-*O*-alkylated oligoglycosyl-alditols or methyl glycoside fragments of RG-II were elucidated (see Tables II–VIII and Fig. 1). Seventeen of these (**1–17**) may have arisen as fragments of heptasaccharide B, illustrated in Fig. 6. Indeed, per-*O*-methylated oligoglycosyl-alditol **17** is heptasaccharide B. The structure of heptasaccharide B was investigated by f.a.b.-m.s. analysis of compound **17**, which defined its molecular weight. The knowledge of its molecular weight and glycosyl-linkage composition, and the structures of the overlapping fragments **7, 8,** and **14**, defined the structure illustrated in Fig. 6 (oligosaccharide B). The structures of all other fragments (except fragments **18–23**, which defined different oligosaccharides present in RG-II) confirmed the structure of heptasaccharide B (see Fig. 6).

All of the differently linked glycosyl residues of heptasaccharide B, with one exception, are known constituents of intact RG-II (see Table I). Intact RG-II contains 3,4-linked, not 4-linked, fucosyl residues. The 4-linked fucopyranosyl residue of heptasaccharide B has another glycosyl residue attached to O-3 in RG-II. It seems probable that the glycosidic linkage of the residue attached to O-3 of the fucopyranosyl residue is particularly acid-labile, and could be either a 3^1-apiosyl or a KDO residue.

Heptasaccharide B contains the unusual 2,3,4-linked rhamnopyranosyl residue present in RG-II (see Table I). However, there is only one of these unusually linked rhamnosyl residues per molecule of RG-II (see Table I). There are also one 3,4-linked and one 2,4-linked rhamnosyl residue per molecule of RG-II (see Table I). Thus, it seems probable that RG-II may also contain the hexasaccharide that lacks the galactosyluronic acid residue attached to O-3 of the trisubstituted rhamnosyl residue of heptasaccharide B; this hexasaccharide is outlined (long-dashed line) in Fig. 6.

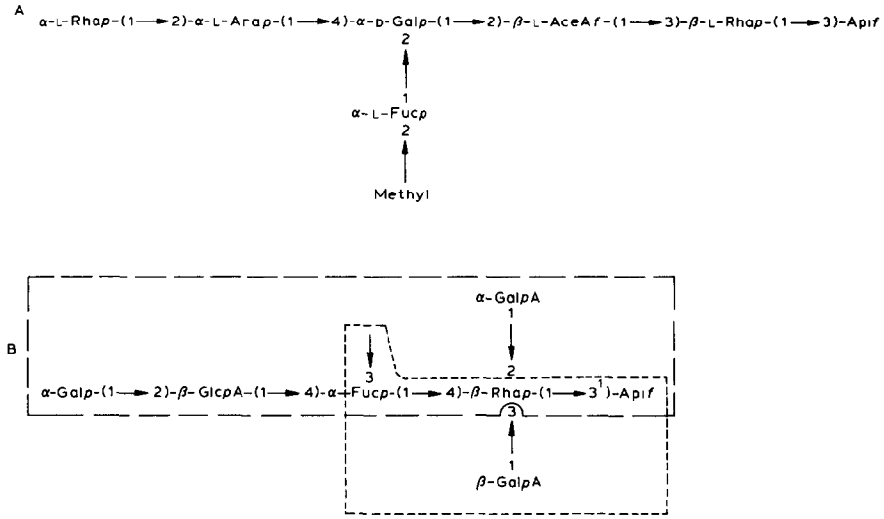

Fig. 6. Two heptasaccharides considered to be present in RG-II. Heptasaccharide A was isolated, and studied, in a previous report[4]. This study presents evidence that heptasaccharide B is also present in RG-II. Two smaller oligosaccharides (parts of heptasaccharide B) that may also be present in RG-II are outlined: a hexasaccharide, with a long-dashed line, and a tetrasaccharide, with a short-dashed line. In RG-II, the fucosyl residue of heptasaccharide B is substituted at O-3 with an unknown residue.

The glycosyl composition of RG-II suggests that other fragments of heptasaccharide B may also be present in RG-II. One of these may be a tetrasaccharide that lacks the terminal galactopyranosyl and the 2-linked glucopyranosyluronic acid, and the terminal galactosyluronic acid residue attached to O-2 of the triply-branched rhamnosyl residue (short-dashed line, Fig. 6). In intact RG-II, an unknown glycosyl residue is probably attached to O-3 of the fucosyl residue of the tetrasaccharide. If this is so, the tetrasaccharide would account for the 3-linked fucosyl residue in the glycosyl-linkage composition of RG-II (see Table I); it would also account for the presence of only two (rather than three) residues of both terminal galactopyranosyl and 2-linked glucosyluronic acid in the glycosyl composition of RG-II. In any case, it appears that, in RG-II, heptasaccharide B is not always present in its complete form, but rather as a mixture of the complete form and several smaller oligosaccharides that contain only part of the heptasaccharide. It must be stressed that the presence of heptasaccharide B in RG-II has been proved, but the presence of such specific, smaller oligosaccharides as those suggested in Fig. 3, although likely, has not been conclusively established.

Per-*O*-methylated oligoglycosyl-alditols **18–23** (see Fig. 1) probably constitute another intermediate-sized oligosaccharide of RG-II. F.a.b-m.s. analysis of the l.c. fractions that contained the per-*O*-methylated oligoglycosyl-alditols eluted between 36 and 60 min (see Fig. 2) demonstrated the presence of oligohexosiduronic segments containing up to eight hexosyluronic acid residues. The presence of an oligogalactosiduronic region in RG-II is supported by com-

pounds **18–22**, as well as by the glycosyl-linkage analysis of RG-II (see Table I). Indeed, the glycosyl-linkage analysis suggests that either there is more than one stretch of 4-linked galactosyluronic acid residues per molecule of RG-II, or that the stretch is longer than eight residues. The glycosyl-linkage composition of RG-II (see Table I) indicates that there are only two terminal galactosyluronic acid residues per molecule of RG-II. Thus, there may be only a single chain of galactosyluronic acid residues in the molecule. Another possibility is that some of the terminal galactosyluronic acid residues observed in the characterized fragments of the RG-II residue (including heptasaccharide B, shown in Fig. 3) possess, in intact RG-II, other glycosyl residues attached to O-4 of the terminal galactosyluronic acid residues.

The structure of per-*O*-methylated oligoglycosyl-alditol **23** shows that an arabinofuranosyl residue is attached to O-3 of one of the galactosyluronic acid residues of RG-II. This could be the terminal arabinosyl residue indicated in the glycosyl-linkage composition analysis of RG-II (see Table I). The 3-linked galactosyluronic acid residue of **23** is likely to be the 3,4-linked galactosyluronic acid residue known to be present in RG-II (see Table I). The glycosyl residue originally attached to O-4 of the 3,4-linked galactosyluronic acid residue still remains unidentified.

One unusual per-*O*-methylated monosaccharide-alditol was characterized. This monosaccharide-alditol had an arabinofuranosyl residue attached to O-6 of a 6,6-dideuteriogalactitol residue. Although the alditol must have originated from a galactosyluronic acid residue (because of its containing the two deuterium atoms), it was surprising to observe a glycosyl residue attached to O-6 after carboxyl reduction. This compound was carefully characterized by l.c.–c.i.-m.s. [*m/z* 430 (55), 238 (19), 256 (12), and 175 (100)]; g.l.c.–e.i.-m.s. [*m/z* 298 (0.7), 238 (7), 175 (20), 143 (25), and 178 (7)], and, most importantly, by analysis of the partially *O*-acetylated, partially *O*-methylated alditols produced by hydrolysis, reduction, and acetylation of the per-*O*-methylated monosaccharide-alditol. The compound 6-*O*-acetyl-1,6,6-trideuterio-1,2,3,4,5-penta-*O*-methylgalactitol was unequivocally identified by g.l.c.–m.s. [*m/z* 251 (0.1), 207 (4.3), 178 (19), 163 (30), 146 (43), 119 (100), 103 (78), 101 (78), and 90 (42)]. The origin and significance of this unexpected compound are not yet known. The compound may be an artifact of the chemical procedures used, or it may result from an unexplained, structural component of RG-II. If this disaccharide fragment is a component of RG-II, it contains the second terminal arabinofuranosyl residue.

Comparison of the glycosyl-linkage composition of intact RG-II with those of oligosaccharides that have now been characterized as, or suggested to be, parts of RG-II makes it clear that there are few glycosyl residues left unaccounted for in RG-II (especially if a few assumptions about the points of interconnection between the oligosaccharides are made; see Table I). Although the following is only an estimate, it indicates the progress being made in characterizing RG-II. It may be postulated that RG-II contains four molecules of the aceric acid-containing hepta-

saccharide (heptasaccharide A; see Fig. 6A), one molecule each of the hepta-, hexa-, and tetra-saccharides illustrated in Fig. 6B, one molecule of the octagalactosyluronic acid oligosaccharide detected, one molecule of the trisaccharide in which a terminal α-L-arabinofuranosyl residue is attached to O-3 of a galactosyluronic acid residue (**23**; see Fig. 1), and two molecules of the disaccharide α-L-Rha*p*-(1→5)-KDO previously characterized[2]. This model accounts for 60 of the ~62 glycosyl residues of RG-II. The only residues in the glycosyl-linkage composition of RG-II that remain unaccounted for are two residues each of terminal 2-*O*-methylxylosyl and 4-linked glucosyl, and one residue of 3-linked galactosyl.

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