

# Structure of Plant Cell Walls

## VIII. A NEW PECTIC POLYSACCHARIDE<sup>1</sup>

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ALAN G. DARVILL, MICHAEL MCNEIL, AND PETER ALBERSHEIM<sup>2</sup>  
*Department of Chemistry, University of Colorado, Boulder, Colorado 80309*

### ABSTRACT

This paper describes the isolation and characterization of rhamnogalacturonan II, a hitherto unobserved component of the primary cell walls of dicotyledonous plants. Rhamnogalacturonan II constitutes 3 to 4% of the primary cell walls of suspension-cultured sycamore (*Acer pseudoplatanus*) cells. Rhamnogalacturonan II is a very complex polysaccharide yielding, upon hydrolysis, 10 different monosaccharides including the rarely observed sugars apiose, 2-O-methylxylose, and 2-O-methylfucose. In addition, rhamnogalacturonan II is characterized by the rarely observed glycosyl interconnections of 2-linked glucuronosyl, 3,4-linked fucosyl, and 3-linked rhamnosyl residues. These glycosyl linkages have never previously been detected in primary sycamore cell walls. Evidence is presented which suggests that polysaccharides similar to rhamnogalacturonan II are present in the primary cell walls of the three other dicotyledonous plants examined.

A structural model of the primary cell wall of suspension-cultured sycamore (*Acer pseudoplatanus*) cells has been proposed previously (7, 20, 27). A continuing study of the pectic polymers of the primary walls of sycamore cells has now revealed the presence in these walls of a complex polysaccharide which had not been detected previously. This paper describes the extraction and purification of this polysaccharide and the unusually linked glycosyl residues of which it is comprised.

### MATERIALS AND METHODS

**Isolation of Cell Walls.** The walls were prepared from suspension-cultured sycamore (*Acer pseudoplatanus*) cells as previously described (27).

**Enzyme Purification.** Endo- $\alpha$ -1,4-polygalacturonase from *Colletotrichum lindemuthianum* was purified as previously described (14). The purified enzyme was shown to be free of  $\beta$ -1,4-galactanase,  $\beta$ -1,4-glucanase, and arabanase by the inability of a large excess of the endopolygalacturonase to degrade a  $\beta$ -1,4-galactan (22), CMC-cellulose (Hercules Inc.), and beet araban (18).

**Enzymic Extraction of Pectic Polymers.** A large proportion of the sycamore cell wall pectic polysaccharides were extracted by subjecting the walls three successive times to the action of the *C. lindemuthianum* endopolygalacturonase. This procedure was carried out by first washing 3.6 g of the walls three times with 200 ml of 10 mM Na-acetate (pH 5.2). The washed walls were suspended in 200 ml of this buffer and 8,400 units of the purified endopolygalacturonase was added. The wall-enzyme suspension was incubated at 30 C for 3 hr, after which the walls were removed from the solubilized material by centrifugation at 10,000g for 15 min.

This extraction procedure was repeated with another 8,400 units of the endopolygalacturonase and, finally, with 4,200 units of the enzyme. The solubilized material from the three enzymic extracts was combined (total volume 600 ml) and filtered through GF/C glass filter paper. This solution was dialyzed exhaustively against distilled H<sub>2</sub>O and then lyophilized to yield 12% of the mass of the original cell walls. Small oligosaccharides, consisting mainly of mono-, di-, and trigalacturonic acid (27), were lost during dialysis. This was the only significant amount of carbohydrate lost during the fractionation and purification procedures.

**Gel Filtration.** Gel filtration was accomplished on an agarose 5m column (3 × 50 cm) and then on a Bio-Gel P-10 column (2 × 23 cm). Both columns were equilibrated and eluted with 50 mM Na-acetate (pH 5.2). The void and included volumes of the columns were determined with blue dextran (Sigma) and NaCl, respectively.

**Colorimetric Techniques.** Neutral sugar concentrations were determined by the anthrone method of Dische (12), and uronic acid concentrations were determined by the *m*-hydroxydiphenyl method of Blumenkrantz and Asboe-Hansen (10).

**Analysis of Glycosyl and Glycosyl Linkage Compositions.** Glycosyl compositions were determined by the alditol acetate method of Albersheim *et al.* (1). Glycosyl linkage compositions were determined by combined gas chromatographic-mass spectrometric analysis of the partially methylated alditol acetate derivatives (9, 24). Polysaccharide methylations were performed using a modification (27) of the procedure reported by Hakomori (15). *K*-dimethyl sulphanyl anion was used instead of *N*-dimethyl sulphanyl anion because, in our hands, the *K* anion works more rapidly and results in a more complete methylation. The *K* anion is made as reported for the *Na* anion (15) except for the use of *K* hydride instead of *Na* hydride and the omission of heating during anion preparation. The hydrolysis of the methylated polysaccharide was accomplished by heating at 100 C for 5 hr in 88% formic acid followed by heating at 121 C for 1 hr with 2 M trifluoroacetic acid. Partially methylated aldoses were reduced with *Na*-borodeuteride rather than *Na*-borohydride to aid in mass spectral analysis.

The uronosyl derivatives of RG-II<sup>3</sup> were analyzed by reduction to the corresponding 6,6-di-deutero hexoses; the reduction was accomplished by the method of Taylor and Conrad (28) using *Na*-borodeuteride and deuterium oxide instead of *Na*-borohydride and water. The samples were then analyzed for glycosyl residues and glycosyl linkages as described above. The quantities of unlabeled hexose and of dideutero-labeled hexose, resulting from the reduction of the corresponding uronic acid, were determined by quantitation of the appropriate fragment ions obtained by mass spectrometric analysis of the alditol acetate derivative.

Methylation with trideuteromethyl iodide was used to determine the glycosyl linkages of the endogenously methylated glycosyl residues of RG-II.

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<sup>2</sup> To whom correspondence should be addressed.

<sup>3</sup> Abbreviations: RG-II: rhamnogalacturonan II; GC-MS: gas chromatography-mass spectrometry.

**GD-MS.** GC analysis of the alditol acetates was performed on column A, which contained 0.2% ethylene glycol succinate, 0.2% ethylene glycol adipate, and 0.4% XF-1150 on Gas-chrom P (1). GC analysis of the partially methylated alditol acetates was performed on column B, on column C, which contained 0.3% OV-275 and 0.4% XF-1150 on Gas-chrom Q (11), and on column C, a 25-m open tubular glass capillary column containing SE 30 (LKB, Broma, Sweden). All GC-MS analyses were carried out on a Hewlett-Packard GC-MS system (model 5980A) coupled to a Hewlett-Packard (model 5933A) data system.

The GC flame-ionization detector peaks were quantitated by a computer-assisted electronic technique (H. Albert and P. Albersheim, unpublished results). All of the GC flame-ionization responses to partially methylated alditol acetates were corrected to mol responses as described by Sweet *et al.* (26).

## RESULTS

The endopolygalacturonase-solubilized cell wall material was applied to a DEAE-Sephadex A-25 ion exchange column (1.5 × 12 cm) that had been preequilibrated with 10 mM K-phosphate (pH 7.0). The column was then washed with 2 column volumes of the phosphate buffer. The material that adsorbed to the column was removed using a linear 0 to 0.5 M NaCl gradient in the phosphate buffer (Fig. 1). The total gradient volume was 300 ml. Three carbohydrate-containing peaks were eluted in the salt gradient. The column fractions constituting the third carbohydrate-containing peak (fractions 28–40) were pooled, dialyzed against distilled H<sub>2</sub>O, and lyophilized.

The polysaccharide in DEAE-Sephadex fractions 28 to 40 was dissolved in 0.5 ml of distilled H<sub>2</sub>O and was further fractionated by gel filtration on agarose 5m yielding the two carbohydrate-containing peaks illustrated in Figure 2. The agarose 5m column fractions 41 to 49 were combined, dialyzed against distilled H<sub>2</sub>O, and lyophilized. The polysaccharides present in fractions 41 to 49 were dissolved in 0.5 ml of distilled H<sub>2</sub>O and applied to a Bio-Gel P-10 gel filtration column (Fig. 3). A single carbohydrate-containing peak eluted from this column ( $V_e/V_o = 1.8$ ). Column fractions 18 to 26, which contained the carbohydrate peak, were pooled, dialyzed against distilled H<sub>2</sub>O, and lyophilized. The polysaccharide in this peak is RG-II; RG-II represents 3 to 4% of the starting cell wall material by weight.

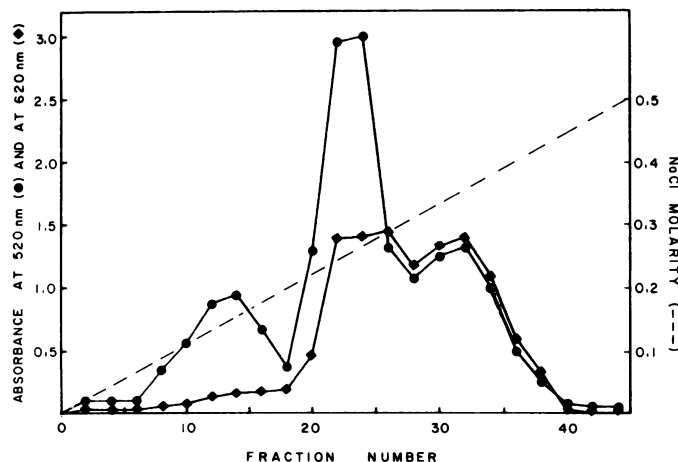


FIG. 1. Chromatography of endopolygalacturonase-solubilized cell wall material on a DEAE-Sephadex column (1.5 × 12 cm). After sample loading, the column was washed with 2 column volumes of 10 mM K-phosphate (pH 7.0). Material that adsorbed to the column was eluted using a linear NaCl gradient (0 to 0.5 M) in the phosphate buffer. Collected fraction volume was 4 ml. Column fractions were assayed for neutral glycosyl residues by the anthrone method (12) (absorbance at 620 nm) and for detection of uronosyl residues by the *m*-hydroxydiphenyl method of Blumenkrantz and Asboe-Hansen (10) (absorbance at 520 nm).

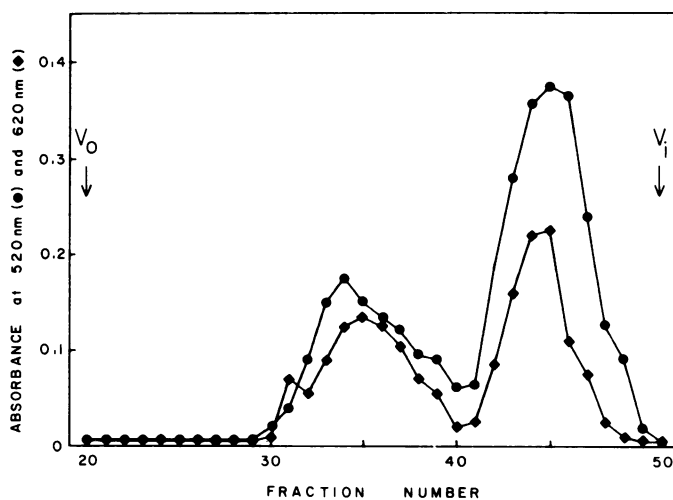


FIG. 2. Chromatography, on agarose 5m column (3 × 50 cm), of material in fractions 28 to 40 of the DEAE-Sephadex column (Fig. 1). Collected fraction volume was 2.5 ml. Column fractions were assayed as described in the legend of Figure 1.

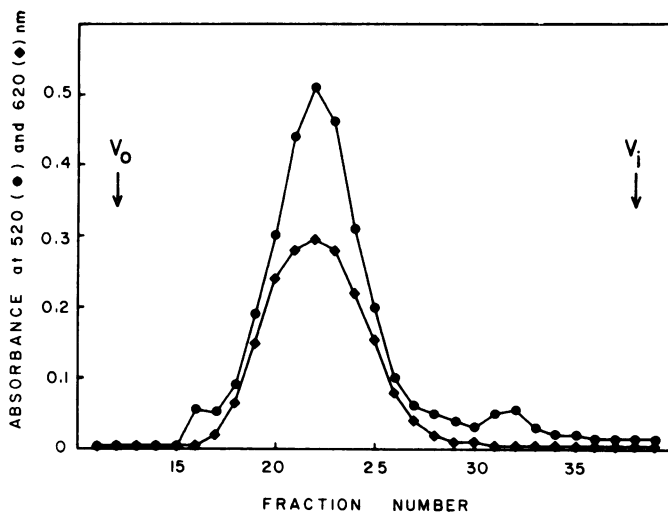


FIG. 3. Chromatography, on Bio-Gel P-10 column (2 × 23 cm), of material in fractions 41 to 49 from the agarose 5m column (Fig. 2). Collected fraction volume was 1.1 ml. Column was assayed as described in legend of Figure 1. In a separate experiment, material present in the Bio/Gel P-10 column fractions 18 to 26 was reappplied to the same Bio-Gel P-10 column. Column fractions 19, 21, and 25 were individually assayed by the alditol acetate method (1) for their neutral glycosyl residue compositions (Table II).

Up to 15% of the total RG-II is present in fractions 18 to 26 of the DEAE-Sephadex ion exchange column (Fig. 1). The RG-II in fractions 18 to 26 is separated from other polysaccharides present in this fraction by gel filtration on Bio-Gel P-10. The RG-II of DEAE fractions 18 to 26 coelutes on the Bio-Gel P-10 with the RG-II of DEAE Sephadex fractions 28 to 40.

**Glycosyl Residues of RG-II.** The glycosyl composition of RG-II is given in Table I. The nonmethylated glycosyl residues were identified by comparing the retention times of their alditol acetate derivatives with the retention times of standards on columns A and B (1, 11) and by GC-MS electron impact (9) and chemical ionization analyses (24) (an authentic sample of apiose was kindly supplied by P. Kindel of Michigan State University). The electron impact fragmentation pattern obtained from the alditol acetate derived from apiose is given in Figure 4. The electron impact fragmentation ions of the alditol acetates of 2-O-methyl fucose (*m/e* 118, 275, 215) and 2-O-methyl xylose (*m/e* 118, 261) are

Table I. Glycosyl Residue Composition of Rhamnogalacturonan II

Sugar Residue	Mole %
Galacturonic acid	29 <sup>a</sup>
Rhamnose	18
Arabinose	14
Galactose	12
Apiose	7
2-O-Methyl Fucose	5
Fucose	4
2-O-Methyl Xylose	4
Glucuronic Acid	3
Glucose	2

<sup>a</sup>The galacturonosyl content of RG-II varies +20% from preparation to preparation.

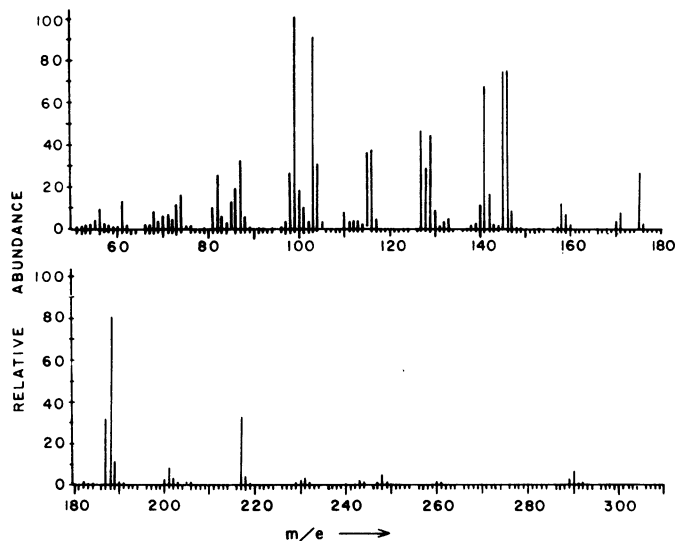


FIG. 4. Electron impact mass spectra of 1,2,3,3',4 penta-O-acetyl apitol. Mass spectrometer was set to scan the mass range of 50 to 350. All acetylated alditols (including the apiose derivative) give a base peak at  $m/e$  43 which is not presented in this spectra.

characteristic of the known fragmentation patterns of the acetylated alditols derived from these monomethylated glycosyl residues (9). These alditol acetates also give chemical ionization mass spectra showing the expected  $M + 1$  ions of  $m/e$  350 and 336, respectively. The relative retention times (compared to acetylated *myo*-inositol) of the alditol acetates of 2-O-methyl fucose, 0.32, and of 2-O-methyl xylose, 0.45, are consistent with the retention times expected for the alditol acetates derived from monomethylated derivatives of these aldoses (27). The identity of these monomethylated glycosyl residues was confirmed by trideutero-methylation analysis of RG-II.

Two unidentified peaks (retention times on column A relative to fully acetylated *myo*-inositol of 0.35 and 0.75) are present in the gas chromatogram of the alditol acetates derived from RG-II. The unidentified components do not give electron impact fragmentation patterns identifiable as alditol acetates. The components each represent about 2% of RG-II.

**Purity of RG-II.** One method of assessing the purity of a polysaccharide is to determine the glycosyl residue composition in various portions of a peak of that polysaccharide which has eluted from a chromatography column. This technique not only gives information about the purity of the polysaccharide but also indicates whether quantitatively minor constituents are contaminants (by the ratio of the glycosyl residue in question to total carbohydrate or by the ratio to other glycosyl residues in the peak).

Purified RG-II was reappplied to both DEAE-Sephadex (Fig. 5) and Bio-Gel P-10 (Fig. 3) columns. Fractions were taken for neutral sugar analysis at various locations in the carbohydrate peaks which eluted from each of the columns (see Figs. 3 and 5).

The ratios of all of the neutral glycosyl residues, with the exception of the xylosyl residues, were the same in all of the fractions examined (Table II). The two unidentified peaks, accounting for a total of 4% of RG-II, chromatographed in a constant ratio to the neutral glycosyl residues on both the ion exchange and gel filtration columns. Using colorimetric assays, the ratio of neutral glycosyl residues to uronosyl residues remained constant across the elution profiles of both columns (Figs. 3 and 5).

**Glycosyl Linkage Composition of RG-II.** The glycosyl linkage composition of RG-II is given in Table III. The composition presented in Table III includes the hexosyl derivatives obtained from the uronosyl residues by reduction of the carboxyl groups to dideutero-labeled primary alcohols prior to methylation. Indeed, complete methylation of RG-II was achieved only following reduction of the uronosyl carboxyls. All of the partially methylated

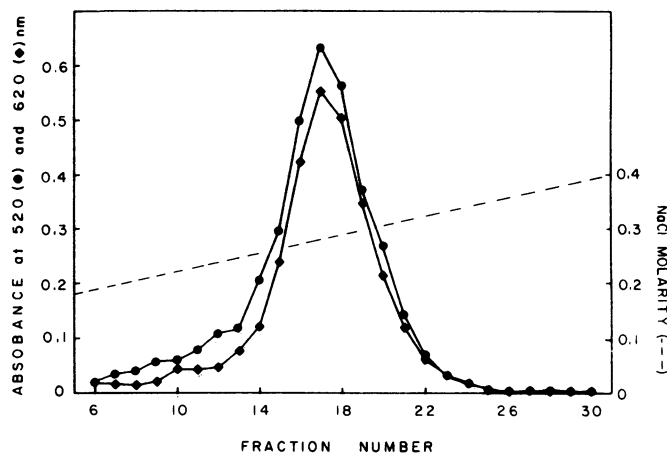


FIG. 5. Material in fractions 18 to 26 of Bio-Gel P-10 column was reappplied in 10 mM K-phosphate (pH 7.0), to a DEAE-Sephadex column (1.5 x 3.5 cm). The column was washed with 60 ml of 10 mM K-phosphate containing 140 mM NaCl. The carbohydrate was removed from the column in a linear 180 mM to 400 mM NaCl gradient in the phosphate buffer. The column was assayed as described in the legend of Figure 1. Fractions 15, 17, and 20 were individually assayed by the alditol acetate method (1) for their neutral glycosyl residue compositions (see Table II).

Table II. Glycosyl Residue Composition Analysis.

A comparison of the glycosyl residue composition (excluding the uronosyl residues and unidentified components) of fractions selected from the leading edge, the center, and the trailing edge of RG-II as this polysaccharide eluted from DEAE Sephadex (Fig. 5) and from Bio-Gel P-10 (Fig. 3) columns.

DEAE Sephadex Column	mole %		
Fraction	15	17	20
2-O-Methyl Fucose	8	8	8
Rhamnose	27	27	26
Fucose	8	8	8
2-O-Methyl Xylose	6	6	6
Arabinose	23	21	21
Apiose	7	7	6
Galactose	17	17	15
Glucose	5	5	5
Xylose	0	2	5
Bio-Gel P-10 Column	mole %		
Fraction	19	21	25
2-O-Methyl Fucose	8	8	8
Rhamnose	27	30	26
Fucose	5	6	6
2-O-Methyl Xylose	7	5	5
Arabinose	24	23	21
Apiose	7	7	8
Galactose	18	18	15
Glucose	3	3	4
Xylose	1	1	8

Table III. Glycosyl Linkage Composition<sup>a</sup> of Rhamnogalacturonan II.

Sugar Residue	Determined Position of 0-Methyl Groups	Deduced Glycosidic Linkage	Mole %
Galacturonic Acid	2,3,4,6	terminal	3.5
	2,3,6	4	19.6
	2,6	3,4	1.7
Rhamnose	2,3,4	terminal	6.8
	2,4	3	6.4
	3	2,4	1.7
	2	3,4	1.5
		2,3,4	1.4
Arabinose	2,3,5	terminal	2.7
	3,4	2 (pyranose) <sup>c</sup>	6.8
Galactose	2,3,4,6	terminal	3.5
	2,4,6	3	1.6
	3,6	2,4	8.0
Apiose	2,3	3 <sup>d</sup>	6.6
2-O-Methyl Fucose	2,3,4	terminal	4.8
Fucose	2,4	3	4.1
	2	3,4	2.5
2-O-Methyl Xylose	2,3,4	terminal	1.7 <sup>e</sup>
Glucuronic Acid	3,4,6	2	3.1
Glucose	2,3,6	4	3.7

<sup>a</sup> Any derivative which represents less than 1 mole % of RG-II has been omitted from the table; in total, these quantitatively minor components account for 8 mole % of RG-II.

<sup>b</sup> The terminal arabinosyl residues were shown by the methylation analysis to be in the furanosyl linkage. All other sugars were either shown by the methylation analysis to be or were assumed to be in the pyranose ring form.

<sup>c</sup> This derivative is a 3,4-di-0-methyl-1,2,5-tri-0-acetyl pentitol as identified by E.I. and C.I. mass spectrometry. Although this derivative has a similar retention time to 3,4-di-0-methyl-1,2,5-tri-0-acetyl xylitol, the amount of the derivative (6.8 mole %) is considerably greater than the total amount of xylosyl residues in RG-II. The only other possibility is that this constituent is derived from arabinosyl residues. Indeed, the amount of this derivative (6.8 mole %), when added to the amount of terminal arabinosyl (2.7%), is required to account for the arabinose content (14%) of RG-II (Table I). The retention time of this derivative is the same as that expected of the retention time of 3,4-di-0-methyl-1,2,5-tri-0-acetyl-arabinitol (19). Thus, we tentatively conclude that this derivative is derived from 2-linked arabinopyranosyl residues.

<sup>d</sup> The identity of this derivative must still be considered tentative because of the lack of an authentic standard.

<sup>e</sup> The amount of this derivative has varied from 1.5 to 4% in the preparations examined.

alditol acetates were identified by retention times and by both electron impact (9) and chemical ionization (24) MS of the gas chromatographic effluent.

Methylation of RG-II with trideuteromethyl iodide established both the identity and the linkage of the two monomethyl glycosyl residues. The mass spectra of the partially methylated, partially trideuteromethylated, acetylated deoxyhexitol and the partially methylated, partially trideuteromethylated, acetylated deoxyhexitol and the partially methylated, partially trideuteromethylated, acetylated pentitol showed that each of the endogenously methylated derivatives is terminally linked. The derivatives both co-eluted on column A with a retention time of 0.71 compared to 1,5-di-0-acetyl-2,3,4,6-tetra-0-methyl mannitol. This retention time indicates that the monomethyl deoxyhexose is, in fact 2-O-methyl fucose (27) and that the monomethyl pentose is, in fact, 2-O-methyl xylose (27).

**Distribution of RG-II.** All of the RG-II present in the sycamore cell walls is solubilized by endopolygalacturonase. This was shown by the fact that the cell wall material remaining after endopolygalacturonase treatment no longer contained detectable levels of apiosyl, 2-O-methyl xylosyl and 2-O-methyl fucosyl residues.

Primary cell walls of pea, pinto bean, and tomato seedlings were analyzed and found to contain 0.15 and 0.3% apiosyl residues, and between 0.1 and 0.2% of both 2-O-methyl xylosyl and

2-O-methyl fucosyl residues. The amounts of each of these glycosyl residues in each of these dicots are similar to the amounts of these glycosyl residues in the suspension-cultured sycamore cell walls. The primary cell walls of oat internodes, which were extracted by P. Kaufman of the University of Michigan (Ann Arbor), were also analyzed for these three glycosyl residues. Trace amounts (less than 0.04% of the wall) of 2-O-methyl xylosyl and 2-O-methyl fucosyl residues were detected in this tissue. No apiosyl residues were detected, but the retention times of the alditol acetates of apiose and xylose (present in large amounts in the monocot cell walls) are so similar that small amounts of apiosyl pentaacetate would have been masked by xylitol pentaacetate.

## DISCUSSION

RG-II, which constitutes from 3 to 4% of the primary cell walls of dicotyledons, has not been previously observed. The polysaccharide is called rhamnogalacturonan II to distinguish it from the rhamnogalacturonan previously described (27). Clearly, the name rhamnogalacturonan II is somewhat arbitrary in that, although rhamnosyl and galacturonosyl residues are present in RG-II in larger amounts than any other glycosyl residues, RG-II also contains eight other glycosyl residues. Two glycosyl components of RG-II, apiose and 2-O-methyl xylose, have not previously been detected in sycamore cell walls (7, 20, 27) and three other glycosyl constituents, 3-linked rhamnose, 2-linked glucuronic acid, and 3,4-linked fucose, have not previously been reported to be present in primary cell walls. The reason for the absence of these glycosyl residues in previous cell wall reports is likely to be the absolute requirement for pre-reduction of the uronosyl residues of RG-II prior to methylation to prevent undermethylation and, therefore, loss of detection of the constituents of this polysaccharide. The small amount of each of the individual components of RG-II in the wall (0.5% or less) would also tend to mask the presence of the polysaccharide in total wall analyses.

There are reports of the existence of apiosyl, methylated fucosyl, and methylated xylosyl residues in a variety of plant tissues (5), although these three sugar residues have not been recognized previously to be the constituents of a single polysaccharide. Apiosyl residues have been shown previously to be covalently linked to galacturonosyl-containing polysaccharides (8-13, 16, 17). There are reports of the presence of 2-O-methyl fucosyl and 2-O-methyl xylosyl residues as trace components of pectic polysaccharides (2-4, 6).

Ninety-two per cent of the weight ( $\pm 7\%$ ) of purified RG-II is accounted for by the identified carbohydrates. The weight per cent of the neutral glycosyl residues was determined by the alditol acetate method (1). This weight per cent of the neutral glycosyl residues was confirmed colorimetrically using the anthrone assay (12). The response factors of the individual glycosyl residues in this assay and the ratio of the neutral glycosyl residues in RG-II (determined by the alditol acetates method) (1) were used for this determination. The weight per cent of uronosyl residues was determined by the *m*-hydroxydiphenyl assay (10). The purified polysaccharide contains no detectable protein (23), hydroxyproline (21), or aminoglycosyl residues. The aminoglycosyl residues were looked for by preparation (1) of their alditol acetates following an 18 hr, 6 M HCl hydrolysis of RG-II. Chitin and *n*-acetyl glycosamine were used as positive controls. Proton NMR spectrometry failed to indicate the presence of any components other than the identified sugars.

Methylation analysis of RG-II detected somewhat more terminally linked glycosyl residues than branched glycosyl residues (23% terminal to 18% branched). The reason for this minor discrepancy is unknown.

Purified RG-II, when reapplied to either DEAE-Sephadex or Bio-Gel P-10 columns, gives a symmetrical peak in which the uronosyl and all of the glycosyl residues, apart from the xylosyl

residues, are in a constant ratio to each other across the eluted carbohydrate-containing peak. Xylosyl residues, which account for only 1.4% of the total carbohydrate in purified RG-II, represent a greater proportion of the carbohydrate in the trailing RG-II-containing fractions (7.5% of the total) than in the leading (0.5%) or middle (0.9%) RG-II-containing fractions (Table II). These results strongly indicate that the xylosyl residues are not part of RG-II but rather are part of a contaminating xylan.

RG-II is slightly included in agarose 5m (Fig. 2), but the results of gel filtration chromatography on Bio-Gel P-10 suggest that RG-II should be totally included in agarose 5m. If the elution pattern of RG-II on Bio-Gel P-10 is correct, this indicates that RG-II is a fairly small polymer (25–50 glycosyl residues). The reason for the anomalous elution pattern on either the Bio-Gel P-10 or the agarose column is not known. The sharpness of the peak of RG-II as it elutes from both of these gel filtration columns indicates that RG-II is a relatively size-homogeneous polysaccharide. This observation might be interpreted as suggesting that RG-II is a small molecule with a discrete structure.

RG-II has been isolated with equal success from seven different preparations of suspension-cultured sycamore cell walls. As noted in Table I, the amount of the galacturonosyl residues present in RG-II varies from preparation to preparation. This variation could result from differing amounts of methyl esterification of the uronosyl residues in different cell wall preparations; the endopolygalacturonase cannot hydrolyze methylated uronosyl residues and would leave varying amounts of galacturonosyl residues attached to the polymer.

Characteristic glycosyl residues of RG-II have been found in similar quantities in the cell walls isolated from seedlings of the three other dicotyledons studied, namely, pea, pinto bean, and tomato. This suggests that RG-II or a similar polysaccharide is present in the cell walls of all dicotyledons. This finding not only demonstrates the widespread occurrence of RG-II but demonstrates once again that the structure of the walls of suspension-cultured cells is similar to the structure of the walls of intact plants (25). Trace amounts of two of the three diagnostic glycosyl residues of RG-II were found in the one monocot studied.

The discovery of RG-II adds to our knowledge of the primary cell walls of dicots, whose total structure remains a goal of this laboratory.

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