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CHARACTERISATION OF THE EXTRACTABLE PECTINS AND HEMICELLULOSES OF THE CELL WALL OF CARROT*

PATRICE MASSIOT, XAVIER ROUAU, AND JEAN-FRANCOIS THIBAULT
Institut National de la Recherche Agronomique, Laboratoire de Biochimie et Technologie des Glucides, Rue de la Géraudière, F-44072 Nantes (France)

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ABSTRACT

Pectic and hemicellulosic polysaccharides were successively extracted from an alcohol-insoluble residue (AIR) from carrot root by the actions of Pronase, hot dilute acid, cold dilute alkali, and concentrated alkali in yields corresponding to 12.6, 13.5, 21.7, and 6.7% of AIR, respectively. The first two products were fractionated further by ion-exchange chromatography. Carrot pectins contained 61.3–66.0% of galacturonic acid and 16.0–19.9% of neutral sugars, mainly galactose, arabinose, and rhamnose. Except for the alkali-soluble pectins, the degrees of methylation were high (62.9–67.1) and there was a significant degree of acetylation (7.2–13.5). Pectin fractions were homogeneous in gel-filtration chromatography with viscosity-average molecular weights varying between 36,200 and 56,500. Methylation analysis indicated the presence of arabinogalactans in the pectins extracted during the proteolysis, and fairly long chains of (1→4)-linked galactan with a branched arabinan in the two other pectic fractions. The hemicellulose fraction was mainly composed of (1→4)-linked glucan, (1→4)-linked mannan, (1→4)-linked xylan, and small but significant amounts of pectic polysaccharides. The possible association of cell-wall polymers is discussed.

INTRODUCTION

The preceding paper[1] described a structural investigation of the cell-wall polysaccharides of carrot. An alcohol-insoluble residue (AIR) was treated with Pronase to give a cell-wall material (CWM) which was fractionated with hot dilute acid and cold dilute alkali in order to solubilise the major part of pectic substances, and with concentrated alkali in order to solubilise the hemicellulose polymers.

The materials resistant to the graded extraction contained mainly cellulose (25% of the CWM) with associated xyloglucans, xylans, and mannans, and an abundant pectic matrix (~50% of the CWM). The cell walls of carrot are rich in galacturonic acid (34.3% of the CWM); pectins are 50–60% methyl-esterified[2–4]

*Structural Study of the Cell Wall of Carrot (*Daucus carota* L.), Part II. For Part I, see ref. 1.

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and slightly acetylated[1]. We report now the isolation, composition, and characterisation of the fractions solubilised by the above extraction scheme.

EXPERIMENTAL

Extraction of carrot AIR. — CWM was isolated[1] from AIR by treatment with Pronase in 0.2M sodium acetate buffer (pH 5.0) at 25°. The soluble fraction (PS) was recovered. Sequential extractions with 0.05M HCl at 85° and with 0.05M NaOH at 4° gave an acid-soluble fraction (HS) and a dilute-alkali-soluble fraction (OHS), respectively. A final treatment with 4M NaOH released a "concentrated-alkali-soluble fraction" (COHS) (see the preceding paper[1]).

The pH of the soluble fractions was brought to 4.5–5.0 with 2M NaOH for HS and 2M acetic acid for OHS and COHS. The solutions were extensively dialysed against distilled water, concentrated under reduced pressure (at 40°), and freeze-dried.

Purification of the pectic polysaccharides. — Aqueous solutions (100 mL) of PS and HS (400 mg) were each loaded onto a column (3.2 × 20 cm) of DEAE-Sephacel (Pharmacia) and eluted with 0.05M sodium acetate buffer (pH 4.8, 350 mL at 50 mL/h). Fractions (10 mL) were assayed for galacturonic acid and neutral sugars. Appropriate fractions were combined, extensively dialysed, and freeze-dried.

Analytical chromatography. — Aqueous solutions (2 mL) of PS, HS, and OHS (~4 mg) were each loaded onto a column (10.0 × 1.6 cm) of DEAE-Sephacel and eluted with 0.05M sodium acetate buffer (pH 4.8, 50 mL at 50 mL/h). Bound material was then eluted by a linear gradient 0.05→1M (90 mL) of sodium acetate, at pH 4.8. Fractions (5 mL) were assayed for galacturonic acid and neutral sugars. Chromatograms based on these assays were recalculated on the basis of actual recoveries of galacturonic acid (2 mg for HS, 1 mg for OHS).

COHS (7 mg) was dispersed in 0.01M Tris-HCl buffer (pH 8.0) for 12 h at 20°. Insoluble residue (1 mg) was removed by centrifugation, and the soluble fraction was loaded onto a column (12.0 × 1.6 cm) of DEAE-Sephacel and eluted with the same buffer at 50 mL/h. The bound material was eluted by a linear gradient 0.01→1M (90 mL) of Tris-HCl, at pH 8.0. Fractions (5 mL) were analysed for galacturonic acid and neutral sugars.

Gel-permeation chromatography was performed on a column (97 × 1.5 cm) of Sepharose CL-2B (Pharmacia) by ascending elution with 0.1M sodium acetate buffer (pH 4.0) at 20 mL/h on samples (4 mg) of pectins purified by ion-exchange chromatography. Fractions (4 mL) were analysed as above. The void (V_o) and total (V_t) volumes of the column, 66.3 and 183 mL, respectively, were determined with a high-molecular-weight apple pectin and galacturonic acid, respectively. Results are expressed as a function of $K_{av} = (V_e - V_o)/(V_t - V_o)$, V_e being the elution volume of the fraction. Chromatograms were recalculated for 4.0 mg of galacturonic acid recovered.

Viscosity measurements. — Intrinsic viscosity values ([η], mL/g) were obtained from experiments at 25° with an Automatic Fica Viscometer (solvent flow time = 175.2 s). Solutions of pectins (3 mg/mL) in 0.155M sodium chloride were used. The viscosity-average molecular weights ($\overline{M}_v$) were calculate according to Owens *et al.*[5].

Analytical methods. — All values were calculated on a moisture-free basis. "Anhydrogalacturonic acid" and total neutral sugars (expressed as "anhydrogalactose") were determined by the automated 3-hydroxybiphenyl[6] and orcinol[7] methods, respectively, the latter being corrected for interfering galacturonic acid. T.l.c. of the COHS hydrolysate was performed[1] for the identification of the uronic acids.

Neutral sugars were released from polysaccharides by hydrolysis with M H_2SO_4 (2 h, 100°) and analysed[1] as their alditol acetates by g.l.c.

Methyl, acetic, and phenolic esters were determined as described in the preceding paper[1]. The degrees of methylation and acetylation of pectins were calculated (mol/mol × 100) from the contents of methanol and acetic acid, respectively, and galacturonic acid.

Nitrogen was determined by an automated Kjeldahl method and protein contents were taken as N × 6.25.

Polysaccharides were methylated by a modification of the Hakomori method[8] and, after hydrolysis with 2M trifluoroacetic acid, the products were converted into partially methylated alditol acetates which were analysed by g.l.c.[1] on OV1, CPSIL 88, and SP 1000 capillary columns.

RESULTS AND DISCUSSION

Extraction and purification of soluble fractions. — More than half of the alcohol-insoluble residue (AIR) was solubilised by the four successive extractions (Table I). PS, HS, and OHS accounted for the major part of the galacturonic acid-containing material (47.8% of the AIR), corresponding to 93.5% of the

TABLE I

YIELDS OF SOLUBLE FRACTIONS OBTAINED FROM ALCOHOL-INSOLUBLE RESIDUES (AIR)[a]

Fraction	*Treatment*	*Yield*	
		% of AIR	*% of total crude pectins*
PS	Pronase in acetate buffer	12.6	26.4
HS	0.05M HCl	13.5	28.2
OHS	0.05M NaOH	21.7	45.4
COHS	4M NaOH	6.7	
Total of extracted polysaccharides		54.5	

[a]Percentages are on the basis of dry matter.

galacturonic acid initially present[1], whereas COHS contained only 1.8% of the total galacturonic acids. Proteolysis and mild acid treatment solubilised 12.6 and 13.5%, respectively, of pectin-containing material, but the major part (21.7%) was found in OHS. However, the concentrated alkali treatment still solubilised 6.7% of the AIR.

The yield of AIR from the fresh carrot was 2.4%; thus, the pectic polysaccharide-containing material represented ~1.1% of the fresh carrot. Direct extraction of AIR with 0.05M HCl at 85° gave 26.7% of an acid-soluble fraction equivalent to the sum (26.1) of PS and HS, indicating that treatment with Pronase did not interfere in the subsequent extractions. Nevertheless, extractable and dialysable material was more important with direct extraction with acid, since 50.9% of AIR was recovered instead of 57.0% with the two successive steps, indicating more degradation of cell-wall polysaccharides.

In contrast to commercial pectins, which are extracted from apple and citrus wastes by a hot acid process[9,10], the bulk of carrot pectins was preferentially solubilised by treatment with 0.05M NaOH at 4°, suggesting that most of the pectic polysaccharides of carrot cell wall probably came from the protopectin and were bound by alkali-labile linkages to such cell-wall components as cellulose, lignin, other polyphenols, or proteins. Nevertheless, the isolation of the cell wall, especially the preparation of the AIR with extensive dehydration of the plant material, could alter the association between the cell-wall polymers and lead to a collapse of the capillary structures, thereby modifying the extractability of polysaccharides.

The isolation of the CWM by treatment with Pronase eliminated ~25% of the AIR[1], whereas the non-dialysable polymers recovered in the PS fraction accounted for only 12.6% of the AIR, indicating the probable removal of amino acids, peptides, phenolic components, inorganic material, *etc.* Carbohydrate fractions, such as the arabinose-containing polysaccharides, could be also partially solubilised as monomers or oligomers by the acetate buffer[11]. The solubilisation of the polysaccharides in PS may be explained by the use of acetate buffer[1] or by the action of Pronase leading to disruption of pectin–protein complexes as detected in the cell walls of various plant tissues[2,12]. This hypothesis is supported by the fact that Pronase in acetate buffer solubilised more pectic components than the buffer alone[1]. Nevertheless, the possibility of β-elimination cannot be ruled out[13] in spite of the low pH and temperature used for the extraction.

The three first fractions (PS, HS, and OHS) were submitted to chromatography on DEAE-Sephacel (Fig. 1) in order to assess their purities. The recoveries of galacturonic acid were 87, 90, and 32% for PS, HS, and OHS, respectively. The last fraction was de-esterified during the extraction and remained bound, largely irreversibly, to the gel. Some materials (PS1, HS1, and OHS1) did not bind to the gel in 0.05M sodium acetate buffer (pH 4.8), and contained neutral polysaccharides not covalently linked to the pectins. These materials represented 5.1, 18.2, and 0.8% of the initial weights of PS, HS, and OHS, respectively. Most of the injected

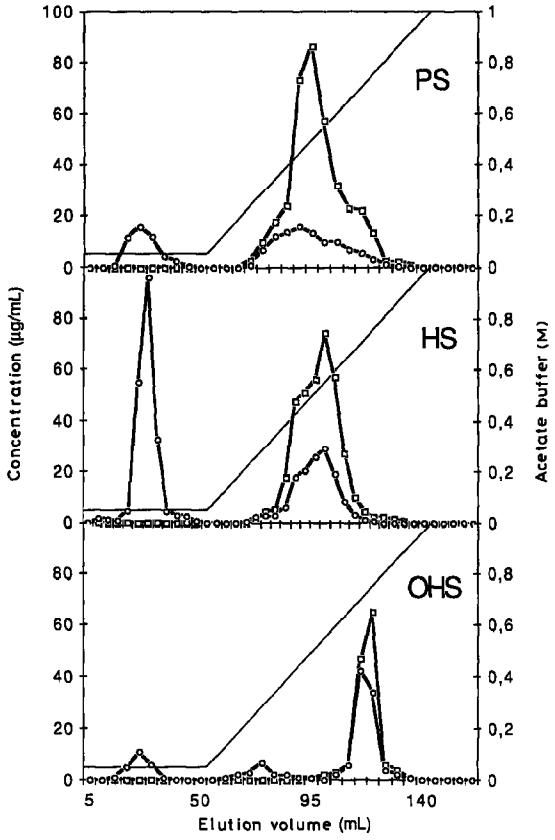

Fig. 1. Elution pattern of PS, HS, and OHS fractions of carrot AIR from DEAE-Sephacel with sodium acetate buffer (pH 4.8) (see Experimental): —□—, galacturonic acid; —○—, neutral sugars; determined by the 3-hydroxybiphenyl and orcinol methods, respectively.

material was bound to the column (PS2, HS2, OHS2) and was eluted with the sodium acetate gradient; PS2 and OHS2 exhibited a single peak of galacturonic acid eluted with 0.5 and 0.75M acetate, respectively, whereas HS2 exhibited two peaks, not fully resolved, eluted with ~0.5 and ~0.6M acetate, respectively.

For further characterisation, ~400-mg amounts of PS and HS were purified on DEAE-Sephacel with yields of galacturonic acid in the range 75–80%. Subfractions PS1 and HS1 were obtained corresponding to the materials washed from the column with 0.05M acetate buffer (pH 4.8), and purified pectins PS2 and HS2 eluted with M acetate buffer (pH 4.8). Fraction OHS was not subjected to ion-exchange chromatography because the neutral unbound material amounted to <1% of the injected material.

The intrinsic viscosities, Huggins coefficients, and the viscosity-average molecular weights ($\overline{M}_v$) of PS2, HS2, and OHS are given in Table II. $\overline{M}_v$ values

TABLE II

VISCOSIMETRIC CHARACTERISTICS AND VISCOSITY-AVERAGE MOLECULAR WEIGHTS OF PECTINS FROM AIR OF CARROT

	Pectins		
	PS2	*HS2*	*OHS*
$[\eta]$ (mL/g)	332	231	326
Huggins coefficient	0.17	0.45	0.90
$\overline{M}_v$	57,000	43,600	56,500

were ~36,200, ~43,600, and ~56,500 for PS2, HS2, and OHS, respectively. Thus, the polysaccharides of OHS were not degraded extensively by the treatment with dilute alkali since the $\overline{M}_v$ values remained high; the low temperature (4°) probably prevented degradation by β-elimination. In contrast, the PS2 pectins exhibited low $\overline{M}_v$ values.

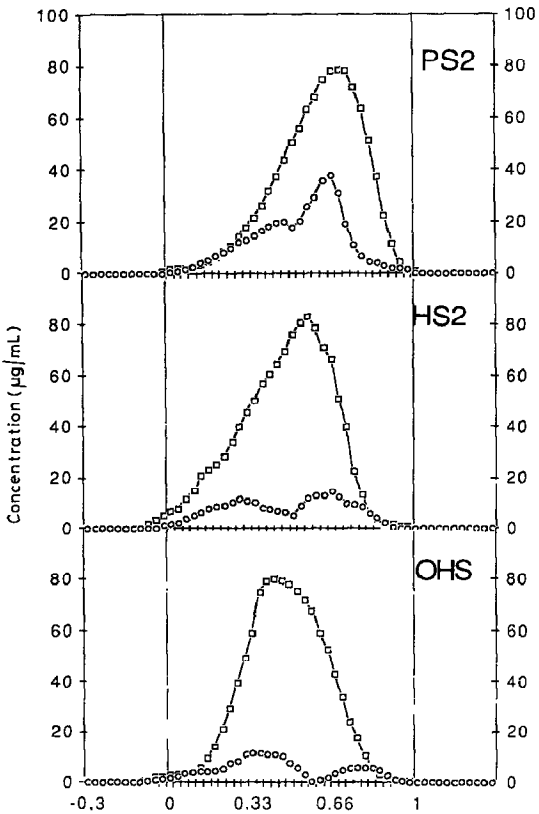

Fig. 2. Elution pattern of PS2, HS2, and OHS fractions of carrot AIR from Sepharose CL-2B (see Experimental): —□—, galacturonic acid; —○—, neutral sugars; determined as in Fig. 1.

Gel-filtration chromatograms of PS2, HS2, and OHS (Fig. 2) showed a homogeneous distribution of galacturonic acids in contrast to the elution pattern of neutral sugars which exhibited two populations of molecules. This fact may be ascribed to the presence of two types of side chains associated to the pectic backbone. The neutral fraction of PS2 was larger, suggesting the occurrence of long side-chains and/or highly branched pectin. The range of the K_{av} values of the peaks of galacturonic acid in the three fractions were in agreement with the $\overline{M}_v$ values, as suggested by the fact that the ionic strengths of the solvents were similar.

The COHS material was chromatographed on DEAE-Sephacel (Fig. 3) equilibrated at pH 8.0 with 0.1M Tris-HCl buffer. This pH was chosen for a better solubilisation of the hemicellulosic fraction. However, 14.3% of the polysaccharides remained insoluble. The soluble fraction was chromatographed with a recovery in total sugars of 67%, so that the results should be considered with caution. The major fraction, 58% of the material and composed only of neutral polysaccharides, was not bound to the gel and corresponded to "typical" hemicellulose. The gradient released two peaks containing only minor amounts of uronic acid, suggesting the occurrence of two acidic populations.

Attempts to precipitate a part of the COHS material by acidification[14], to pH 4.8 at 4°, of a solution in 4M NaOH failed to provide hemicelluloses of type A. Addition of 4 vol. of ethanol to the solution gave a light precipitate corresponding to hemicelluloses of type B. The fact that no A-type hemicelluloses were found suggested that hemicellulosic polysaccharides had low molecular weights.

Composition of the soluble fractions. — As expected, the purification procedure increased the galacturonic acid content of the pectins (Table III). PS2, HS2, and OHS contained 66.0, 61.3, and 63.8% of galacturonic acid, respectively. These pectins contained similar amounts of neutral sugar (19.9, 16.0, and 18.3%, respectively), mainly galactose, arabinose, and rhamnose together with minor amounts of xylose and glucose. Galactose was the most abundant sugar, accounting

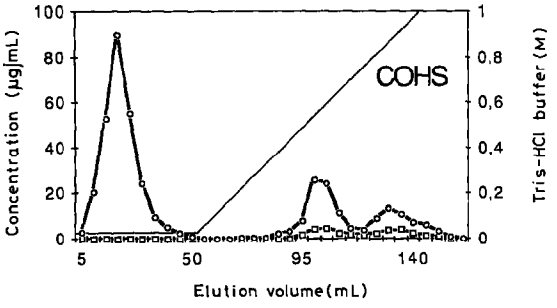

Fig. 3. Elution pattern of concentrated-alkali-soluble fraction (COHS) of carrot from DEAE-Sephacel with Tris-HCl buffer (pH 8.0) (see Experimental): —□—, galacturonic acid; —○—, neutral sugars determined as in Fig. 1. Chromatogram was recalculated for the 4 mg of material injected onto the column.

TABLE III

COMPOSITION[a] OF SOLUBLE FRACTIONS OBTAINED FROM AIR FROM CARROT AND OF SUB-FRACTIONS OBTAINED BY ION-EXCHANGE CHROMATOGRAPHY

Fraction	*PS*	*PS1*	*PS2*	*HS*	*HS1*	*HS2*	*OHS*	*COHS*
Uronic acids	42.0	2.0	66.0	54.7	3.0	61.3	63.8	8.6
Total neutral sugars[b]	17.7	32.6	19.9	26.1	55.7	16.0	18.3	79.1
Rhamnose	1.3	0.2	2.1	3.8	2.2	4.3	4.0	0.6
Fucose								0.7
Ribose	0.8	0.2		0.2			0.1	
Arabinose	5.3	12.4	5.7	11.7	37.3	3.5	3.0	3.0
Xylose	0.1	0.4	0.1	0.2	0.1	0.3	0.3	19.1
Mannose	0.8	5.6	0.1	0.7	2.6			16.4
Galactose	8.2	7.0	11.4	8.3	9.8	7.9	10.6	4.1
Glucose	1.2	6.8	0.5	1.2	3.7		0.3	35.2
Methanol[c]	5.5 (74.3)	n.d.[d]	7.8 (67.1)	5.7 (59.2)	n.d.	6.8 (62.9)		
Acetic acid[c]	4.2 (29.8)	n.d.	1.6 (7.2)	2.3 (12.5)	n.d.	2.8 (13.5)	0.1 (0.5)	
Phenolic acids	0.4	n.d.	tr	tr	n.d.	0.1	0.1	0.1
Protein (N × 6.25)	22.1	n.d.	3.1	1.7	n.d.	1.4	1.0	2.2

[a]As % of dry matter. [b]Neutral sugars determined by g.l.c. of the alditol acetates, and expressed as "anhydrosugars". [c]Values in parentheses are the degrees of methylation and acetylation for methanol and acetic acid, respectively. [d]Not determined.

for 57.3, 49.4, and 57.9% of the total neutral sugars in the three extracts, respectively, whereas the arabinose content was lower (28.6, 21.8, and 16.4%, respectively). These results suggested the presence of galactose- and arabinose-containing polysaccharides linked to the pectic backbone, probably through rhamnosyl residues which are known as branching points of side chains[15].

The fractions PS1 and HS1, corresponding to the material not bound to the DEAE-Sephacel column, contained 2 and 3% of galacturonic acid, respectively. Almost all of the glucose, mannose, and xylose, and only part of the arabinose (21.0%) and galactose (7.7%), found in PS and HS were recovered in PS1 and HS1; they corresponded to neutral polysaccharides, and, especially for PS1, they may arise from glycoproteins. The relatively high ribose content in PS probably originated from cytoplasmic material, *e.g.*, nucleic acids.

The nitrogen detected mainly in the PS fraction (22.1%) may result from non-dialysable degradation products of proteins. However, some proportion of proteins could be associated with pectic substances, as pointed out in previous work[2,16] and as suggested by their presence in purified pectins PS2 and HS2. The abundance of protein could explain the low carbohydrate content of PS1 fraction, although the amount was not determined.

The main phenolic acids detected after h.p.l.c. were *p*-hydroxybenzoic and ferulic acid: 0.10 and 0.15% in PS, 0.04 and 0.03% in HS2, and 0.08 and 0.01% in OHS, respectively. Only traces were detected in PS2. The low contents of phenolic acids (1.1%, in the initial AIR) in the soluble fractions indicated they were eliminated during the dialysis step. The u.v. spectra of the crude pectins showed a single peak at 375 nm at pH 10, and peaks at 285 and 325 nm at pH 2.5. This bathochromic shift is indicative of esters of cinnamic acid-type phenols[17]. In contrast, the spectra of purified pectins exhibited no u.v. absorption peak, indicating the absence of phenolic components. These results suggested that ferulic acids, also detected in AIR, were extracted as esters of dialysable material.

The pectic fractions of carrot contained acetic acid in amounts similar to that of apricot pectins[18] but less than for sugar-beet pectins[19]. The differences between the degrees of acetylation of PS and PS2, 29.8 and 7.2, respectively, proved that some of the acetyl groups did not originate from the pectic polysaccharides. On the other hand, HS and HS2 had similar degrees of acetylation, 12.5 and 13.5, respectively, suggesting that acetyl groups came from pectin material.

The degrees of methylation were relatively high, 67.1 and 62.9 for PS2 and HS2, respectively, but near zero for OHS because of almost complete de-esterification during extraction. The degrees of methylation were in good agreement with previously published data[3,4,20] and they explained the elution pattern of the soluble fractions on DEAE-Sephacel; the increasing ionic strength necessary to elute PS2, HS2, and OHS, in this order, from DEAE-Sephacel (Fig. 1) corresponded to a decrease of the degrees of methylation, the OHS fraction being partly irreversibly bound to the gel.

The composition of COHS was very different from those of the preceding

TABLE IV

PARTIALLY METHYLATED ALDITOL ACETATES DERIVED FROM METHYLATED POLYSACCHARIDE-FRACTIONS OF CARROT AND IDENTIFIED BY G.L.C.

Derivatives[a]	*PS1*	*PS2*	*HS1*	*HS2*	*OHS*	*COHS*
2,3,4,6-Me_4-Gal[b]	4.4[c]	3.2	1.0	6.4	8.4	0.9
2,3,4-Me_3-Gal	0.5	17.0	5.5	5.0	9.1	0.3
2,3,6-Me_3-Gal	6.8	7.0	9.6	32.2	33.5	1.6
2,4,6-Me_3-Gal	2.1	9.5		4.8	2.7	
2,3-Me_2-Gal	5.6	19.4	0.5	3.2	3.8	0.7
2,4-Me_2-Gal	0.9	0.7				
2,6-Me_2-Gal	0.6					
Total[d]	20.9 (21.5)	58.8 (59.1)	16.6 (17.6)	51.6 (49.8)	57.5 (58.4)	3.5 (5.1)
2,3,5-Me_3-Ara	13.8	18.4	23.4	7.8	5.8	3.1
2,3-Me_2-Ara	3.2	5.0	22.8	7.2	10.5	0.4
2,5-Me_2-Ara	20.6	3.9	3.8	3.8		
2-Me-Ara	tr[e]	2.9	13.5	2.2	2.2	
Ara	0.7	0.7	3.0	2.0	0.6	
Total	38.3 (28.3)	30.9 (29.4)	66.5 (67.3)	23.0 (21.9)	19.1 (16.5)	3.5 (3.8)
3,4-Me_2-Rha		1.5	1.3	12.2	7.8	1.7
3-Me-Rha		4.1	1.7	8.7	12.4	
4-Me-Rha		1.1		2.5		
Rha		tr		0.5	3.1	
Total	−(0.7)	6.7 (8.0)	3.0 (3.7)	23.9 (26.9)	23.3 (21.9)	1.7 (1.6)
2,3,4,6-Me_4-Glc						2.1
2,3,4-Me_3-Glc						0.9
2,3,6-Me_3-Glc	21.0	2.8	8.7		tr	28.3
2,4,6-Me_3-Glc	2.2					1.3
2,3-Me_2-Glc						3.4
2,6-Me_2-Glc						0.3
3,6-Me_2-Glc						3.4
Total	23.2 (20.9)	2.8 (2.3)	8.7 (6.6)		(1.6)	39.7 (44.5)
2,3,4-Me_3-Xyl		0.5				2.5
2,3-Me_2-Xyl	1.1	0.5		tr	tr	20.9
2-Me-Xyl						3.0
3-Me-Xyl						0.6
Total	1.1 (1.3)	1.0 (0.6)	−(0.1)	−(1.6)	−(1.6)	27.0 (24.2)
2,3,6-Me_3-Man	15.4		4.5			23.7
2,3-Me_2-Man	1.0		1.0			0.9
Total	16.4 (17.3)	−(0.6)	5.5 (4.7)			24.6 (20.8)

[a]Some minor components, the identities of which were not confirmed, are omitted. [b]2,3,4,6-Me_4-Gal denotes 1-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol, etc. [c]% of total area of the identified peaks. [d]Values in parentheses are based on analysis of alditol acetates. [e]Trace.

fractions (Table III). This alkali-soluble fraction contained an array of neutral polysaccharides mainly composed of glucose, xylose, and mannose, with minor amounts of galactose and arabinose. A small proportion of uronic acid, 2% of the total uronic acids of AIR, was recovered in COHS. T.l.c. of a hydrolysate of this fraction revealed only galacturonic acid. Nevertheless, Aspinall *et al.*[3] detected in a similar fraction an acidic xylan with single 4-*O*-methyl-α-D-glucopyranosyluronic acid residues as substituents. The COHS fraction contained a small amount of fucose as already encountered in side chains of xyloglucans.

Methylation analysis. — The methylation analysis data for the soluble fractions PS1, PS2, HS1, HS2, OHS, and COHS are given in Table IV. The amounts of sugars calculated from the analyses of alditol acetates and partially methylated alditol acetates were generally in good agreement. Except in PS and OSH, the sums of "branch points" and of terminal residues were equivalent.

The PS2 polysaccharides were mainly arabinose- and galactose-containing polymers, together with rhamnogalacturonan. Some methylated derivatives corresponded to (1→6)- and (1→3,6)-linked galactosyl residues and to terminal and (1→3)-linked arabinosyl residues, which was consistent with the presence of type II arabinogalactan[21]. Large amounts of 2,3-di-*O*-methylgalactitol derivatives were detected as already reported[1,2] for carrots and field-bean hulls[22]. However, appreciable proportions of (1→4)-linked galactosyl residues and (1→3,5)- and (1→5)-linked arabinosyl residues were also detected. As expected, rhamnose was (1→2)-linked and substituted mainly at position 4, but also at position 3, *i.e.*, as kinks in the pectic backbone and as branching points of side chains. Minor amounts of xylose as terminal and (1→4)-linked residues were detected as observed in similar fractions of carrot cell wall[2].

The association between xyloglucan and purified pectic polymers as suggested in apple cell wall[23] could explain the presence of glucose and xylose residues in pectic fractions. On the other hand, if the xyloglucan is hydrogen-bonded to cellulose fibres, this could partly explain the presence of pectic polymers in the final residue.

PS1 was composed of similar families of neutral polysaccharides, except for the presence of (1→4)-linked mannosyl residues and of (1→4)-linked glycosyl residues resulting either from a glucan with mixed (1→3),(1→4)-linkages or a (galacto)glucomannan, or from residual starch (0.3% of AIR).

As indicated by the significant proportion of rhamnitol derivatives, the HS2 fraction was composed of a rhamnogalacturonan backbone with rhamnosyl residues being the branching point of side chains, mainly at positions 3 and 4; half of the rhamnose was branched. It can be calculated that, in this pectin, ~8.2 kinks of rhamnose occurred per 100 galacturonic acid residues. The associated neutral polysaccharides were galactan with a fairly high d.p. as evidenced by the high proportion of 2,3,6-tri-*O*-methylgalactitol derivatives, and probably small amounts of arabinan or type II arabinogalactan.

The polysaccharides of HS1 were neutral polymers not covalently linked to

the pectic backbone, or degraded side-chains of pectin containing large amounts of arabinose. They were formed mostly of a highly branched arabinan as indicated by the high proportion of 2,3,5-tri-*O*-methyl-, 2-*O*-methyl-, and unmethylated-arabinitol derivatives obtained. Minor amounts of rhamnose were detected, probably resulting from rhamnogalacturonan characterised by a low galacturonic acid content and a galacturonic acid/rhamnose ratio of ~1.1. These results suggest that these rhamnogalacturonan fragments had many potential branching points and probably projected from hairy regions of the pectic backbone. The small amounts of 2,3,6-tri-*O*-methylglucitol could have been derived from similar glucan or (galacto)glucomannan found in PS1 or from residual starch.

The neutral polysaccharides of OHS were similar to those of HS2, suggesting that these pectic fractions had a common origin in the cell wall. Their major features were the presence of large proportions of (1→4)-linked galactan and (1→5)-linked arabinan, and important amounts of (1→2)-linked and (1→2,4)-linked rhamnosyl residues arising from the rhamnogalacturonan. It can be calculated that 69% of the rhamnosyl residues carried side chains.

For the COHS polysaccharides, the results of methylation analysis were consistent with the presence of (1→4)-linked xylopyranosyl residues with possible side-chain attachment predominantly at position 3. The formation of 2,3,6-tri-*O*-methyl-mannitol derivatives was evidence of the presence of a linear mannan; the high amount of (1→4)-linked glucosyl residues accorded with the presence of gluco-mannans[3], but a large part of these glucose residues could arise from xyloglucans, which are generally the main hemicellulosic polysaccharides of dicotyledonous primary cell-walls and already observed in carrot cell wall[2].

A residual pectic backbone was also detected by the simultaneous presence in this fraction of 3,4-di-*O*-methylrhamnitol and small amounts of galacturonic acid. Significant amounts of 2,3,5-tri-*O*-methylarabinitol could have been derived either from the side chains attached at position 3 of a xylan backbone[15] and/or from neutral polysaccharides associated with pectins and not removed by the preceding extractions.

The main results of this study, particularly the composition and structure of the cell-wall material, are in good agreement with those of previous work[2,3] on cell-wall polysaccharides of carrot. The treatment with hot acid extracted more than half of the pectic material of the cell wall (0.65% of the fresh carrot). These pectins had medium viscosity-average molecular weights and a high degree of esterification. The presence of phenolic acids suggested by Stevens and Selvendran[2] was confirmed by the detection of *p*-hydroxybenzoic and ferulic acids in the soluble fractions.

The purified pectin obtained during the proteolysis of AIR was characterised by a high content of galacturonic acid (66.0%), a high degree of methylation (67.1%), and the presence of acetyl groups. The neutral polysaccharides found in this fraction were mainly arabinan and/or arabinogalactan which may be associated with proteins; these arabinogalactan–protein complexes are readily solubilised

from AIR with dilute aqueous solutions of buffer as found in previous work[24], and occur in various dicotyledons[25].

The acid- and alkali-soluble pectins were similar in composition and structure, but had different degrees of methylation and viscosity-average molecular weights. Their neutral polysaccharides were similar, namely, long chains of galactan and branched arabinan. Substantial amounts of rhamnosyl residues, two-thirds of which were branched, occurred along the rhamnogalacturonan backbone. These pectic fractions had similar ratios of galacturonic acid/rhamnose (~12–13), which were lower than those of apple[26] or beet[19] pectins. These polysaccharides probably resulted from a common material in the cell wall.

The hemicelluloses in COHS represented a minor part (~9%) of cell-wall material and were composed mainly of the xylans, xyloglucans, and glucomannans classically found in most of the parenchymatous tissues of dicotyledons. These polymers may be tightly associated with cellulose fibres since strong alkali was required to release them, but the small but significant content of protein (2.2%) indicated possible hemicellulose–protein association, as detected in similar extracts[2].

There was a good accord between the composition and structure of the insoluble residues previously analysed[1], those of the soluble fractions, and the recoveries from the AIR of carrot. This overall analysis allowed a better understanding of the nature of the cell-wall complex.

The α-cellulose and the lignin, which constitute the final residue, formed the cell-wall core (~30%). They were strongly associated with xyloglucans, probably by hydrogen bonds, and with minor amounts of acidic polysaccharides; 0.3% of protein rich in hydroxyproline was also present. Part of the xyloglucans and xylans, and all of the mannans, soluble in 4M NaOH, were complexed with the former polymers through more labile cross-links.

The cell-wall matrix accounted for approximatively half of the cell-wall material and was composed of a rhamnogalacturonan backbone with more than half of the rhamnosyl residues carrying side chains; the location and exact structure of these chains on the pectic backbone are unknown, but they are galactose- and arabinose-containing polysaccharides. The larger part of this pectic material apparently was bound to the cell-wall framework through alkali-labile linkages. The cell-wall complex also contained readily extractable pectins together with arabinogalactans some of which were associated with protein[16]. Nevertheless, some hydroxyproline-rich proteins were found in all the insoluble fractions, suggesting a structural origin[27,28]. Phenolic components, in ester forms, were present in carrot cell wall, but a minor fraction appeared to be linked covalently to polysaccharides.

This structural scheme of carrot cell wall, typical of dicotyledonous tissue, can differ in its polymer proportions with type of tissues, stage of differentiation, and plant variety.

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