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STRUCTURAL FEATURES OF CELL-WALL POLYSACCHARIDES OF THE CARROT *Daucus carota*

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ABSTRACT

Cell-wall material was isolated from the alcohol-insoluble residue of carrot by treatment with Pronase, phenol–acetic acid–water, and aqueous 90% methyl sulphoxide. Some pectic material was solubilised, but the major component was a highly esterified, acidic arabinogalactan. The purified cell-wall material, which contained ~1% of protein, was sequentially extracted with water at 80°, ammonium oxalate at 80°, and M and 4M KOH at 20°, to leave a residue of α-cellulose, which contained some pectic material. From the hot-water-soluble fraction, a major pectic polymer was isolated by anion-exchange chromatography. Methylation analysis showed that it was a rhamnogalacturonan, probably having highly branched arabinan and slightly branched galactan side-chains linked to O-4 of rhamnopyranosyl residues. An unusual feature of this pectic polymer is that it contained a small but significant proportion of 1,4-linked xylopyranosyl residues. From the alkali-soluble fractions, a range of pectic polymers associated with various amounts of xylans and possibly xyloglucans was isolated. The main linkages present in these complexes were 1,4-linked galactopyranosyluronic acid, 1,4-linked galactopyranosyl, and 1,5-linked arabinofuranosyl residues, terminal arabinofuranosyl and galactopyranosyl groups, and, in some fractions, 1,4-linked xylopyranosyl residues. The possible association of some of these polymers with proteins and phenolics is discussed.

INTRODUCTION

The composition and structure of cell-wall polymers of aerial organs from a range of dicotyledonous plants have been extensively studied[1–5] but few, if any, detailed studies have been made of polymers of the primary cell-walls of storage root tissues. Carrots have attracted attention as sources of pectin[6] and dietary fibre[7,8]. The composition of the alcohol-insoluble residue of carrot used as a source of dietary fibre in clinical feeding trials has been studied with a view to obtaining a better understanding of the physiological effects observed[9]. The results of a more detailed investigation into the structural features of the cell-wall polymers of carrot are reported here.

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TABLE I

SUGAR COMPOSITION OF ALCOHOL-INSOLUBLE RESIDUE (AIR) OF CARROT AND OF MATERIAL SOLUBILISED DURING STAGES OF PURIFICATION OF CELL-WALL MATERIAL

Stages of purification	*Proportion of AIR solubilised (% dry weight)*	*Sugar composition (μg/mg)*[a]							
		Rha	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*	*Total sugars*
Initial AIR	—	27	66	12	16	98	230	366	815
Ball milling									
EtOH insoluble[b]	13.8	28	63	3	3	122	6	616	841
EtOH insoluble fraction *A*[c]	2.2	20	49	4	5	74	28	461	641
EtOH insoluble fraction *B*[d]	9.4	24	60	3	2	114	4	677	884
Pronase	13.4	9	23	1	2	22	6	262	325
Methyl sulphoxide	5.2	48	46	10	25	63	92	593	877

[a]Neutral-sugar values after Saeman hydrolysis[21]; other values after hydrolysis with M H_2SO_4. [b]Material precipitated by ethanol (90%, v/v) from the supernatant solution after ball milling. [c]Residue insoluble in phosphate buffer (0.01M, pH 6.4), removed prior to chromatography on DEAE-Sephacel. [d]Eluted from DEAE-Sephacel with NaCl.

RESULTS AND DISCUSSION

Isolation of cell-wall material (CWM). — The alcohol-insoluble residue of carrot contained coprecipitated intracellular proteins, starch, and pigments which had to be removed. The purification treatments solubilised 32.4% of the initial residue as polymeric material, including 21% of the arabinose originally present, 27% of the galactose, and 39% of the uronic acid-containing material. Some of the carbohydrate polymers solubilised during the purification stages may have been of intracellular origin, and these were isolated and their carbohydrate compositions determined (Table I). It appears that pectic substances constituted most of the material solubilised. Of the total non-dialysable material extracted, 60% of the arabinose, 73% of the galactose, and 56% of the uronic acids were from polysaccharides isolated from the supernatant solution obtained after wet ball-milling. Most of the initial protein content (10%) was removed by the Pronase and phenol–acetic acid–water treatments.

The material precipitated by ethanol from the supernatant solution after ball-milling was dispersed in phosphate buffer, an insoluble residue (*A*, Table I) removed by centrifugation, and the soluble material chromatographed on a column of DEAE-Sephacel, to yield one acidic fraction (*B*, Table I).

The carbohydrate recovery of fraction *A* was relatively low (Table I), probably because it contained the bulk of the soluble protein (10.8%) present in the original supernatant. However, some of the proteins may be covalently linked to carbohydrates[5]. The results in Table I indicate that fraction *B* is a pectic polysaccharide containing arabinose and galactose as the preponderant neutral sugars. Because of its extractability in cold water and relatively high degree of esterification (49%), it was probably derived from the middle lamella.

The fraction solubilised by the Pronase treatment contained 33% of carbohydrate[9] and accounted for 26% of the extractable uronic acid (Table I). The treatment with methyl sulphoxide, which was included to remove residual starch and pigments, also solubilised a small proportion of pectic material. The residue remaining after these treatments was designated as purified CWM and was white, showing the virtual absence of pigments, *etc.*, which coloured the original alcohol-insoluble residue.

Composition of CWM. — Analysis of CWM[9] (Table II) showed that galactose, arabinose, and uronic acids were the main sugars present in the non-cellulosic polysaccharides, indicating a high level of pectic substances. The protein content of the CWM is ~1% and it is probable that a small proportion was solubilised by the Pronase treatment. The amino acid composition of the remaining wall-protein is shown in Table III. Studies with cabbage[3] have shown that Pronase solubilises some of the cell-wall proteins.

Fractionation of CWM. — To obtain a clearer indication of the types of polymers constituting the cell-wall complex, the CWM was sequentially extracted with hot water, hot ammonium oxalate, and M and 4M KOH, to leave a residue of

TABLE II

SUGAR COMPOSITION OF CELL-WALL MATERIAL OF CARROT AND OF FRACTIONS OBTAINED BY SEQUENTIAL EXTRACTION WITH INORGANIC SOLVENTS

Fraction	*Recovery (%)*	*Sugar composition (μg/mg)*[a]							
		Deoxyhexose	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*	*Total sugars*
Cell-wall material	—	29	81	20	20	126	409(43)	203	888
Hot-water-soluble[b]	9.3	54	134	10	2	155	21	456	832
Hot-oxalate-soluble[c]	4.0	41	103	1	2	135	11	554	847
M KOH-soluble	13.1	49	184	13	7	331	20	217	821
4M KOH-soluble	9.5	46	70	206	65	109	295	96	887
Final residue	64.1	18	49	6	26	74	626	106	905

[a]After Saeman hydrolysis[21]; values in parentheses are after hydrolysis with M H_2SO_4. The values for uronic acid were determined by a colorimetric method[21,22]. [b]Degree of esterification, 29%; calculated from methanol content determined colorimetrically[23]. [c]Degree of esterification, 49%.

TABLE III

AMINO ACID COMPOSITION OF FRACTIONS OBTAINED DURING PURIFICATION AND SEQUENTIAL EXTRACTION OF CARROT CELL-WALL MATERIAL

Amino acid	*Amino acid composition (μg/mg)*					
			M KOH-soluble fractions			*4M KOH-soluble Insoluble residue*
	Supernatant after ball-milling	*Purified CWM*	*Insoluble residue*	*KB*	*KB1*	
Ala	8.6	0.6	21.9	1.5	0.6	4.7
Gly	7.4	0.4	17.6	1.6	1.3	4.7
Val	5.4	0.6	10.7	0.5	0.1	2.7
Thr	6.6	0.5	10.5	0.7	0.3	2.8
Ser	9.5	0.7	18.8	2.3	1.6	5.7
Leu	10.6	0.9	28.6	1.3	0.4	6.4
Ile	3.4	0.6	7.9	1.0	0.2	2.0
Pro	6.7	0.4	11.9	0.8	0.4	4.0
Hyp	4.2	0.7	4.1	0.9	0.4	6.2
Asp	14.1	0.9	18.3	1.5	0.9	5.8
Phe	6.0	0.8	19.8	0.7	0.9	4.5
Glu	16.3	0.9	25.6	2.4	1.4	10.2
Lys	3.4	0.4	10.7	0.5	(7.5)[a]	2.5
Tyr	2.9	0.6	5.2	0.5	1.0	2.5
Arg	1.6	0.3	3.9	0.8	n.d.[b]	0.9
His	1.6	0.2	7.0	n.d.	n.d.	1.2
Total	108.3	9.5	222.5	17.0	(9.5)	66.8

[a]This value is probably too high, because of an interfering compound. [b]Not determined.

TABLE IV

SUGAR COMPOSITIONS OF FRACTIONS OBTAINED FROM THE HOT-WATER-SOLUBLE AND ALKALI-SOLUBLE EXTRACTS OF CARROT CELL-WALL MATERIAL

Fraction		*Sugar composition (mol/100 mol of monosaccharide[a])*							
	Yield (%)	*Deoxyhexose*	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*	*Carbohydrate recovered (%)*
Hot-water-soluble polymers[b]	(100)	7.2	19.7	1.5	0.2	18.6	2.4	50.3	78.9
DEAE-Sephacel, fractions W1	21	7.1	32.1	0.6	1.0	23.9	2.7	32.6	93.6
DEAE-Sephacel, fractions W2	49	4.6	14.0	2.7	—	17.5	0.8	60.4	92.9
M KOH-soluble polymers	(100)	6.4	26.2	1.8	0.8	38.9	2.1	23.8	82.1
Insoluble residue	3	3.2	25.9	2.3	4.9	16.0	40.4	7.3	34.7
DEAE-Sephacel fractions KA	40	6.8	28.9	1.1	1.6	42.8	3.3	15.5	86.2
DEAE-Sephacel fractions KA1	5	1.7	31.1	2.2	14.0	16.6	20.5	13.9	82.2
DEAE-Sephacel fractions KA2	23	6.0	29.5	1.2	—	37.7	2.8	22.8	99.6
DEAE-Sephacel fractions KB	28	8.7	16.9	2.2	0.8	43.2	1.0	27.1	77.2
DEAE-Sephacel fractions KB1	14	6.8	26.3	2.9	—	35.2	—	28.8	64.6
DEAE-Sephacel fractions KB2	5	7.2	24.0	3.1	—	38.4	—	27.3	74.6
DEAE-Sephacel fractions KB3	3	6.2	19.6	2.6	—	30.3	7.5	33.8	45.8
DEAE-Sephacel fractions KB4	3	7.4	18.7	2.2	0.2	21.2	9.4	40.9	68.8
4M KOH-soluble polymers	(100)	5.4	8.9	26.4	6.9	11.6	31.3	9.5	88.7
Insoluble residue	8	1.4	9.7	7.0	5.5	10.4	50.9	15.1	68.0
DEAE-Sephacel fraction 4KA	52	5.7	15.9	14.7	8.3	24.0	15.8	15.6	90.6
Cellulose fraction 4KA1	25	7.0	19.4	13.2	0.7	28.9	3.5	27.3	82.3
Cellulose fraction 4KA2	4	1.8	12.3	24.1	0.5	11.6	37.3	12.4	66.5
Cellulose fraction 4KA3	3	1.7	7.0	18.0	1.3	1.7	49.9	20.4	81.1
DEAE-Sephacel fraction 4KB	22	5.7	17.3	16.4	—	25.6	1.2	33.8	101.2

[a]After Saeman hydrolysis. [b]From first extraction with hot water.

α-cellulose. The proportions of the fractions obtained and the sugar composition (after hydrolysis) are given in Table II. Although most of the pectic polysaccharides were extracted by the hot-water and oxalate treatments, a significant amount, more strongly bound, remained to be extracted by M KOH. This increase in binding was probably caused by the procedure used in the preparation of the alcohol-insoluble residue; a similar effect has been observed in the fractionation of CWM from cabbage[3]. However, the association between the polymers, caused by the initial alcohol treatment during preparation of the alcohol-insoluble residue from fresh carrot, was largely (if not completely) nullified by fractionation of the polymers on anion-exchange columns. As with other plant tissues[3,4,10], the final cellulose residue contained some pectic material.

The hot-water-soluble polysaccharides were chromatographed on DEAE-Sephacel to yield two fractions, W1 and W2 (Table IV). WI contained less acid, but more arabinose and galactose, than W2 and may have been a product of degradation by β-elimination, because the cold-water-soluble material only yielded one fraction.

After removal of a water-insoluble residue, the M KOH-soluble material was chromatographed on DEAE-Sephacel to yield two fractions, KA (not retained on the column) and KB (eluted with NaCl) (Table IV). These fractions were re-chromatographed on a longer column, using acetate buffer, to yield further fractions KA1, KA2, and KB1–KB4 (Table IV). With the exception of fraction KA1, which contained mannose and less uronic acid, the fractions were comparable, with arabinose, galactose, and uronic acid as the main components. Fractionation of the water-soluble portion of the 4M KOH-soluble material on DEAE-Sephacel gave two fractions 4KA and 4KB. The component 4KA was resolved on a cellulose column into fractions 4KA1–3 (Table IV), containing mainly arabinose, galactose, glucose, and uronic acid. Interestingly, the water-insoluble residues from both M and 4M KOH-soluble fractions were rich in glucose, as were the fractions 4KA2 and 4KA3. The carbohydrate compositions of the latter fractions suggest that they may be xyloglucans associated with pectic polymers. Similar complexes have been isolated from cabbage cell-walls[5].

The water-insoluble residues from the M and 4M KOH-soluble material contained appreciable proportions of protein (Table III). That from the M KOH-soluble fraction had an amino acid composition comparable with that of a similar glycoprotein fraction from cabbage cell-walls[5], except that, in the fraction from carrot, the hydroxyproline content was low. The sugar composition of the carrot fraction was comparable to that of the soluble fraction KA1, which suggests that they both may have been derived from a more complex proteoglycan. The same may be true of the insoluble residue from the 4M KOH-soluble material and the fractions 4KA2 and 4KA3. The above fractions are rich in glucose and, like xyloglucans, may be strongly associated with cellulose since strong alkali is required to release them. It would appear that carrot cell-walls contain small but significant proportions of proteoglycans, which may serve to cross-link the cell-wall polymers. Proteoglycans

TABLE V

PARTIALLY METHYLATED ALDITOL ACETATES FROM POLYSACCHARIDES PRESENT IN FRACTIONS OBTAINED FROM THE HOT-WATER-SOLUBLE AND KOH-SOLUBLE FRACTIONS OF CARROT CELL-WALL MATERIAL

Alditol acetates	*T*[b]	*Relative mol %*						
		Water-soluble	*M KOH-soluble fractions*[a]				*4M KOH-soluble*	
		W2	*KA2*	*KB1*	*KB2*	*KB4*	*4KA1*	*4KA1 reduced*[c]
3,4-Me_2Rha[d]	0.87	6.1	4.7	1.0	1.5	4.0	2.7(1.9)[e]	4.1(3.2)
2-MeRha	1.37	0.5	—	—	—	—	—	
3-MeRha	1.67	4.2	6.9	2.2	3.3	5.1	3.6(2.6)	3.8(3.0)
2,3,5-Me_3Ara	0.41	14.7	15.5	13.4	11.0	9.6	8.8(6.4)	6.5(5.1)
2,3-Me_2Ara	1.07	13.5	11.4	11.2	7.5	10.4	6.4(4.7)	5.1(4.0)
3,5Me_2Ara	0.80	0.4	—	—	—	—	—	—
2-MeAra	1.93	8.3	7.1	6.4	6.3	5.3	5.3(3.9)	3.8(3.0)
Arabinitol	2.66	3.3	4.0	5.8	8.2	4.7	5.0(3.6)	3.7(2.9)
2,3,4-Me_3Xyl	0.54	1.4	0.5	—	0.9	1.5	—	—
2,3-Me_2Xyl	1.19	4.2	—	5.3	5.1	5.3	23.2(16.9)	27.9(22.1)
3,4-Me_2Xyl	1.19	0.5	—	0.5	0.9	—	2.0(1.4)	2.1(1.7)
2,3,4,6-Me_4Gal	1.19	7.5	8.9	5.1	3.3	7.7	4.3(3.1)	6.2(4.9)
2,3,4-Me_3Gal	2.89	1.1	0.7	0.6	1.6	0.9	0.8(0.6)	0.5(0.4)
2,3,6-Me_3Gal[f]	2.22	28.2	37.0	40.5	41.6	37.6	30.4(22.2)	19.1(15.1)
2,3-Me_2Gal	4.7	2.1	1.0	3.6	3.2	5.1	1.0(28.0)	8.9(28.0)
2,4-Me_2Gal	5.1	1.3	—	1.7	1.6	—	—	—
2,6-Me_2Gal	3.14	0.5	0.3	0.5	0.9	0.9	0.3(0.2)	—
Hexitol		2.2	2.0	2.1	3.1	1.9	3.8(2.8)	3.5(2.8)

[a]Values for fractions KA1 and KB3 are not included, because the results were difficult to quantify. Main types of glycosidic linkages were: *KA1* T-Ara*f*, (1→5)-Ara*f*, (1→3,5)-Ara*f*, (1→2)-Ara*f*, (1→4)-Gal*p*, (1→4)-Man*p*, (1→2)-Rha*p*; *KB3* T-Ara*f*, (1→5)-Ara*f*, (1→3,5)-Ara*f*, (1→4)-Xyl*p*, (1→4)-Gal*p*. [b]Retention time relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol on OV-225 at 170°. [c]Esterified, reduced with $LiBD_4$, hydrolysed, and converted into partially methylated alditol acetates; deuterium was detected only in the spectra of 2,3-Me_2Gal. [d]3,4-Me_2Rha = 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methylrhamnitol, *etc.* [e]Values in parentheses are scaled to correspond with a galacturonic acid content of 27.3 mol % (Table IV); the value of 28.0 takes into account the initial level of the 2,3-Me_2Gal. [f]May include some 2,3,6-Me_3Glc.

have been isolated from the aqueous extracts of a range of plant tissues[11] and, although some of them may be of intracellular origin, there is growing evidence to suggest that a large proportion of them are of cell-wall origin[2] and may have an important structural role.

The hot-water-soluble fraction W2 and fractions KA1–2 and KB1–4 of the M KOH-soluble material and 4M KOH-soluble fraction 4KA1 were subjected to methylation analysis, and the results are given in Table V. The results for fractions KA1 and KB3 were difficult to quantify because insufficient amounts of sample were available. The results for fraction W2 indicate that it contains the well-established pectic rhamnogalacturonan-backbone to which neutral sugar side-chains, of variously linked arabinosyl and galactosyl residues, are mostly attached at O-4 of the rhamnosyl residues. Evidence for this mode of attachment in the carrot pectin was confirmed by carboxyl-reduction and partial hydrolysis, as carried out in studies on cabbage pectins[4]. From the proportions of galactosyl and arabinosyl residues to (1→2,4)-linked rhamnosyl residues, it can be deduced that the side chains contain an average of ~10 arabinosyl residues, of which about one-third are branched, and 9 galactosyl residues. The galactosyl residues were mainly (1→4)-linked with only 10% of the total residues branched, mainly (1→4,6)-linked, which suggests that only a small proportion of an arabinogalactan moiety is present. It is possible that a galactan is present as a fairly long chain, as in some other pectic polysaccharides[12,13].

The small proportion of xylose in fraction W2 is present mainly as (1→4)-linked residues which, although rare in pectic polysaccharides from parenchymatous tissues, have recently been found in pectic fractions[14] from apple cell-wall.

The fractions from the M KOH-soluble material were comparable in composition and structural features to fraction W2, although fraction KA1 contained an appreciable proportion of (1→4)-linked mannose, less uronic acid, and an arabinose-to-galactose ratio of 0.5 compared with ratios of 1.1 to 1.6 for the other fractions, including 4KA1. All of the fractions contained small proportions of (1→4,6)-linked galactosyl residues, which are uncommon in dicotyledonous tissues but have been found in field-bean hulls[15].

The low recoveries of carbohydrate in fractions KA, KA1, KB1-4, and 4KA1–3, and in the M KOH and 4M KOH-insoluble residues (Table IV) suggested that the carbohydrate may have been complexed with polyphenolic material by a non-ester linkage (ester linkages would have largely been hydrolysed during the extraction with alkali). Arabinose– and galactose–ferulic acid complexes have recently been found in pectic polysaccharides of suspension-cultured spinach[16]. Monitoring of the elution sequence of fraction KA at 280 nm revealed a high u.v.-absorbance. Subsequently, the u.v. spectra of the portion of KA soluble in aqueous 10% methyl sulphoxide[17] were examined at pH 7 and 14, and then after adjustment[18] to 2.5, and compared with those of ferulic and *p*-coumaric acids in the same solvent and pH ranges. The spectral shifts in KA, although not strictly comparable

to those of the phenolic acids tested, clearly suggested the presence of phenolic compounds.

The 4M KOH-soluble fraction 4KA1 contained an appreciable proportion of (1→4)-linked xylosyl residues, in addition to arabinosyl, galactosyl, and rhamnosyl residues having the same linkages and comparable proportions as in the M KOH-soluble fractions. To ensure complete esterification of the carboxyl groups, methylated 4KA1 was treated with diazomethane and then reduced with lithium borodeuteride. After conversion into partially methylated alditol acetates, g.l.c.–m.s. revealed that only the 2,3-di-*O*-methylgalactitol derivative increased in amount and was deuterated at C-6, which showed that it was derived from (1→4)-linked galacturonic acid. Because of the losses of uronic acid which occur during methylation and reduction[4], and in order to facilitate comparison with the unreduced polysaccharide, the values in parentheses in Table V are scaled to correspond with 27.3 mol% of uronic acid (assumed to be all galacturonic acid) determined colorimetrically on the unmethylated fraction.

These results suggest that fraction 4KA1 contains a pectic polysaccharide similar to those in the water-soluble and M KOH-soluble fractions, but associated with a polysaccharide containing (1→4)-linked xylosyl residues terminated by other sugars, because terminal xylosyl groups were not detected. The higher proportion of xylosyl residues in 4KA1 suggests that a longer chain xylan may be present, instead of the shorter chains that are probably present in the water- and M KOH-soluble polymers. Although fraction 4KB was not methylated, the sugar composition (Table IV) indicates that it is similar to 4KA1.

The relatively low levels of xylose and glucose in fraction 4KA and the low yields of fractions 4KA2 and 4KA3 suggest that xyloglucans, which are the main hemicellulosic polysaccharides of most dicotyledonous primary cell-walls[1], are present only in small proportions in cell walls of carrot root. Again, the low recovery of carbohydrate and relatively high u.v.-absorption of these fractions suggest the presence of phenolic complexes.

The results of this study show that the preponderant polymers in cell walls of carrot root are pectic polysaccharides with associated arabinans and galactans, and that they are probably complexed with proteins and polyphenolic material. The numerous fractions obtained suggest that a range of pectic complexes is present, perhaps reflecting the composition of tissues in early stages of differentiation. Mature carrot contains both parenchymatous and lignified tissues, and some of the xylem (ray) parenchyma cells have thickened walls. Therefore, the cell walls from immature carrot would be expected to contain a heterogeneous collection of cell-wall complexes, as shown in this study. The occurrence of similar complexes in suspension-cultured sycamore cells, which have the potential to differentiate into a range of cell types, has been reported[19], and ranges of closely related hemicellulosic polymers have been found in cabbage[5] and runner bean[2,20] cell-walls which are also capable of varied differentiation.

EXPERIMENTAL

Chemicals. — Methyl sulphoxide was vacuum-distilled over CaH_2 and stored over molecular sieve 3A. Tetrahydrofuran was distilled over $LiAlH_4$ and stored under Ar. All other chemicals were of the highest purity available. DEAE-Sephadex and DEAE-Sephacel were purchased from Pharmacia (Uppsala, Sweden).

General methods. — Neutral sugars were released by Saeman or M H_2SO_4 hydrolysis, and analysed as their alditol acetates by g.l.c.[21]. Uronic acid was determined colorimetrically by a modification[21] of the method of Blumenkrantz and Asboe-Hansen[22]. The degree of esterification was calculated from the methanol content (determined colorimetrically[23]) as a molar proportion of the uronic acid content. Amino acids were determined by g.l.c. as their *n*-propyl heptafluorobutyryl derivatives[24]. Diazomethane was prepared by the method of Bjerke and Herman[25]. U.v. measurements were made with a Perkin–Elmer 550S spectrophotometer, and i.r. spectra were recorded with a Pye-Unicam SP200 G spectrophotometer, using KBr discs.

Preparation of CWM. — The alcohol-insoluble residue of carrot (20 g), as used by Cummings *et al.*[26], was passed through a grain mill (0.5-mm aperture sieve), ball-milled in water, and treated with Pronase, phenol–acetic acid–water (2:1:1), and methyl sulfoxide, as described previously[27], to yield 9 g of purified CWM.

Sequential extraction of CWM. — CWM was fractionated by sequential extraction with water at 80°, ammonium oxalate (pH 5.0) at 80°, and then M and 4M KOH containing 10mM $NaBH_4$, as described previously[28], to leave a residue of α-cellulose.

Ion-exchange chromatography. — *System* a. Cell-wall fractions were suspended in potassium phosphate buffer (10mM, pH 6.4), insoluble material was removed by centrifugation, and the soluble material was applied to columns of DEAE-Sephacel (Cl^- form). Elution was with the potassium phosphate buffer (30 to 140 mL) initially, and then with this buffer in a linear gradient of NaCl (0→M) (150 to 200 mL). Fractions (2–3 mL) were collected, and monitored for carbohydrate by the reaction with phenol–sulphuric acid[29]. Appropriate fractions were combined, dialysed, and freeze-dried.

System b. Cell-wall fractions were suspended in water, insoluble material was removed by centrifugation, and the soluble material was applied to columns of DEAE-Sephacel (acetate form). Elution was with water (75 mL) initially, and then with a linear gradient (0→M) of potassium acetate (pH 6, 200 mL), and finally with 5M potassium acetate (60 mL). Fractions were collected, monitored, and processed as in system *a*.

Fractionation of cold-water-soluble material. — The material (200 mg) precipitated by ethanol (90% v/v) from the supernatant solution after ball-milling was chromatographed (system *a*; 24 × 1.5 cm column), after removal of an insoluble re-

sidue (*A*, 32 mg), to yield one fraction, *B* (136 mg), which was eluted with NaCl up to 0.3M.

Fractionation of hot-water-soluble material. — Hot-water-soluble material (150 mg) was chromatographed (system *a*; 34 × 0.9 cm column), to yield fraction W1 (32 mg), which was not retained on the column, and W2 (73 mg), which was eluted with NaCl up to 0.15M.

Fractionation of M KOH-soluble material. — After removal an insoluble residue (5 mg), the soluble portion (145 mg) of the M KOH-soluble material was chromatographed (system *a*; 30 × 0.9 cm column), to yield fraction KA (60 mg), which was not retained on the column, and KB (42 mg), which was eluted with NaCl up to 0.1M. In addition to monitoring with phenol–sulphuric acid, fractions were also monitored for u.v. absorption at 280 nm. Fraction KA (30 mg) was re-chromatographed (system *b*; 33 × 1 cm column), to yield KA1 (4 mg, not retained on the column), a trace (<1 mg) eluted with 0.05M potassium acetate, and KA2 (17 mg) which was eluted with 0.5M potassium acetate. Fraction KB (29 mg) was re-chromatographed under the same conditions, to yield KB1 (14 mg), which was eluted with 0.1–0.25M potassium acetate, and KB2 (5 mg), which was eluted with 5M potassium acetate. Further elution with 0.2M NaOH (80 mL) yielded KB3 (3 mg) and KB4 (3 mg). In addition to monitoring with phenol–sulphuric acid, fractions were also monitored for u.v. absorption at 280 nm.

Fractionation of 4M KOH-soluble material. — The 4M KOH-soluble material (130 mg) was dispersed in phosphate buffer (pH 6.4), an insoluble residue (10 mg) removed, and the soluble portion chromatographed (system *a*; 12 ×1 cm column) to yield fractions 4KA (68 mg), which was not retained on the column, and 4 KB (28 mg), which was eluted with NaCl up to 0.15M. Fraction 4KA (50 mg) in water (10 mL) was re-chromatographed on a column (34 × 1 cm) of cellulose (Whatman CC31). After elution with water (100 mL), a linear gradient of NaOH (0→M, 50 mL) was used. Fractions (2 mL) were collected and, in addition to monitoring with phenol–sulphuric acid, they were monitored for absorption at 280 nm. Appropriate fractions were combined, adjusted to pH 5 with acetic acid, dialysed, and freeze-dried, to yield five fractions: 4KA1 (27 mg) not retained on the column, 4KA2 (4 mg), 4KA3 (3 mg), and two further broad peaks that were eluted with 0.7M to M NaOH.

Methylation analysis. — Polysaccharides were methylated by a modification of the Hakomori method and then converted into partially methylated alditol acetates, which were separated by g.l.c. on OV-225 and ECNSS-M columns, and examined by g.l.c.–m.s. with an OV-225 column, as described previously[30]. Methylated fractions showed negligible hydroxyl absorption in their i.r. spectra.

Carboxyl reduction. — The carboxylic acid groups in the methylated polysaccharide were esterified with diazomethane and then reduced with $LiBD_4$ as described previously[4].

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