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STRUCTURAL FEATURES OF CELL-WALL POLYMERS OF THE APPLE

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ABSTRACT

Cell-wall material was isolated from ripe-apple cortical tissues by sequential extraction with aqueous 1.5% sodium dodecyl sulphate and aqueous 90% methyl sulphoxide. The wall material, which contained ~1% of protein, with proline and hydroxyproline as the preponderant amino acids, was sequentially extracted with water at 80°, oxalate at 80°, M KOH at 1°, and M and 4M KOH at 20°, to leave a residue of α-cellulose, which was associated with an appreciable amount of arabinose-rich pectic material. The depectinated material was also extracted with 6M guanidinium thiocyanate at 20° to solubilise preferentially polysaccharides rich in mannose. The hot-water-soluble pectic substances were richer in arabinose compared with the oxalate-soluble ones and were resolved into five fractions by anion-exchange chromatography. The bulk of the hemicelluloses, which were xyloglucans, were solubilised by 4M KOH. The alkali-soluble hemicellulose polymers were resolved by anion-exchange chromatography into polysaccharides, mainly xyloglucans, arabinoxylan–pectic–xyloglucan, and arabinoxylan–pectic complexes. Small amounts of polysaccharide–protein–polyphenol complexes (where the polysaccharide moieties were arabinoxylans), pectic substances, and xyloglucans were also present. The glycosidic linkages of the above polymers were determined by methylation analysis. The general structural features of the cell-wall polymers are discussed.

INTRODUCTION

The composition, structure, and properties of cell-wall polymers from a range of edible plant organs[1–4] have been studied in order to obtain a better understanding of the chemistry of dietary fibre and its possible mode of action in the human alimentary tract.

The pectic polysaccharides of apples have been studied[5–13] in some detail, but the hemicelluloses have received little attention[2,6,14]. Ranges of polysaccharide complexes have been isolated from the cell walls of runner bean[15,16], cabbage[3], and carrot[4], possibly reflecting the heterogeneity of the tissues and the types of polymers deposited during growth and maturation. If a similar diversity is found in apple cortex, essentially a homogeneous tissue, then it would provide evidence that

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such ranges of polymers are an inherent feature of the primary cell-wall. We now report the results of a detailed study of the cell-wall polymers of the apple with special emphasis on the hemicellulosic polymers.

RESULTS AND DISCUSSION

Cell-wall material (CWM) was prepared from the cortex of ripe apples by extraction and ball-milling with aqueous 1.5% sodium dodecyl sulphate (SDS), to remove cytoplasmic compounds, followed by extraction with aqueous 90% methyl sulphoxide to remove starch. The residue after these treatments was defined as purified CWM. During the isolation of the walls, the polymeric material solubilised amounted to 6.6% of the final weight of the CWM. Most of this material was in the supernatant solution obtained after ball-milling and contained 40.6% of carbohydrate, mainly pectic polysaccharides with low levels of neutral sugars (Table I), and possibly derived from the middle lamella.

The CWM was extracted sequentially with hot water and hot aqueous ammonium oxalate, to solubilise the bulk of the pectic substances, and then with M KOH at 1°[17] and at 20–22° and, finally, with 4M KOH at 20–22°, to achieve a primary fractionation of the hemicellulosic polymers. The monosaccharide compositions of these fractions are given in Table I. The treatments with hot water and oxalate solubilised 37% of the total uronic acid, 6% was extracted with the alkali, and 57% remained with the α-cellulose residue. As with the cell walls of lupin hypocotyls[17], the polymers extracted with cold M KOH contained more xylose and glucose than those extracted at room temperature, which were richer in arabinose.

TABLE I

MONOSACCHARIDE COMPOSITION OF FRACTIONS OF APPLE CELL-WALL MATERIAL (CWM)

Fraction	*Yield (%)*[a]	*Monosaccharide composition*[b] *(μg/mg dry wt.)*						
		Deoxy-hexose	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*
Cold 1.5% SDS soluble[c]	(5.7)	8	46	4	16	34	33	299
Purified CWM	—	20	123	33	43	57	227	328
Sequential extraction of CWM								
H_2O at 80°, soluble	6.9	20	219	13	10	39	12	685
Oxalate at 80°, soluble	6.3	27	159	12	3	37	10	707
M KOH at 1°, soluble	2 9	69	67	273	25	103	322	140
M KOH, 20–22°, soluble	0.7	52	114	161	28	97	261	166
4M KOH, 20–22°, soluble	14.6	76	31	235	64	115	406	70
α-Cellulose residue	68.6	28	122	45	23	60	411	206
Guanidium thiocyanate extraction of CWM	(4.9)	19	47	79	134	54	146	78

[a]Percentage of the total material recovered; values in parenthesis are percentages of purified CWM. [b]After Saeman hydrolysis. [c]Solubilised during the ball-milling (6 h at 1°) stage of purification of the CWM.

However, unlike the lupin hypocotyls[18], alkali at 1° extracted more protein (including more hydroxyproline) than at 20–22°, but the level of hydroxyproline was higher in the fraction extracted at 20–22°. The bulk of the hemicellulosic polymers was extracted with 4M KOH and was rich in xylose and glucose.

Pectic polysaccharides. — As a prelude to looking for the occurrence of pectic hemicellulosic complexes in apple cell-walls, it was necessary to determine the monosaccharide composition of the main, pectin-rich fractions extracted with hot water and oxalate. The compositions of these fractions were similar, but the hot-water-soluble fraction contained more arabinose (Table I). The galactose content of these fractions was lower than for those of pectins extracted by other workers[19,20], probably as a result of commercial storage (~4 months at 10°) of the apples used in this study. During storage, there is a decrease in the galactose content of the pectic substances[7]. Chromatography of the hot-water-soluble material on DEAE-Sephadex A50 (Cl^- form) gave two fractions, one rich in arabinose and of low acid-content, which was not retained on the column, and a more acidic fraction that was eluted with NaCl and contained much less arabinose. Flow rates through the column were very slow, owing either to the high viscosity of the sample or to the occurrence of precipitation. Because of this and the relatively poor resolution, the fractionation of a diluted sample in acetate buffer at pH 6 was carried out on a larger column. This gave a better separation, which yielded three major components (P1–P3, Table II) and two minor ones (P4 and P5, Table II). An interesting feature was the presence of small but significant amounts of xylose, glucose, and mannose in each of the fractions. These sugars are usually present in the hemicelluloses, but they have also been found in pectic fractions of cabbage[21]. Knee[6] and De Vries *et al.*[11] have reported the occurrence of low levels of xylose and glucose, but not mannose, in apple pectic fractions. It would appear that some of the hot-water-soluble polysaccharides are pectic–hemicellulosic complexes containing very high proportions of the pectic components. This is the reverse of the

TABLE II

MONOSACCHARIDE COMPOSITION OF PARTIALLY PURIFIED PECTIN FRACTIONS

Fraction	*Yield (%)*[a]	*Monosaccharide composition*[b] *(μg/mg of dry wt.)*							
		Deoxy-hexose	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*	*D.e.*[c]
DEAE-Sephadex fractions									
P1	18.5	59	259	9	14	32	22	553	68
P2	27.0	11	245	14	7	24	21	598	67
P3	27.0	17	127	19	2	22	10	712	68
P4	2.5	15	129	34	33	38	44	587	n.d.[d]
P5	3.0	4	53	4	83	4	7	415	n.d.

[a]Percentage of weight of material applied to column. [b]After Saeman hydrolysis. [c]Degree of esterification. [d]Not determined.

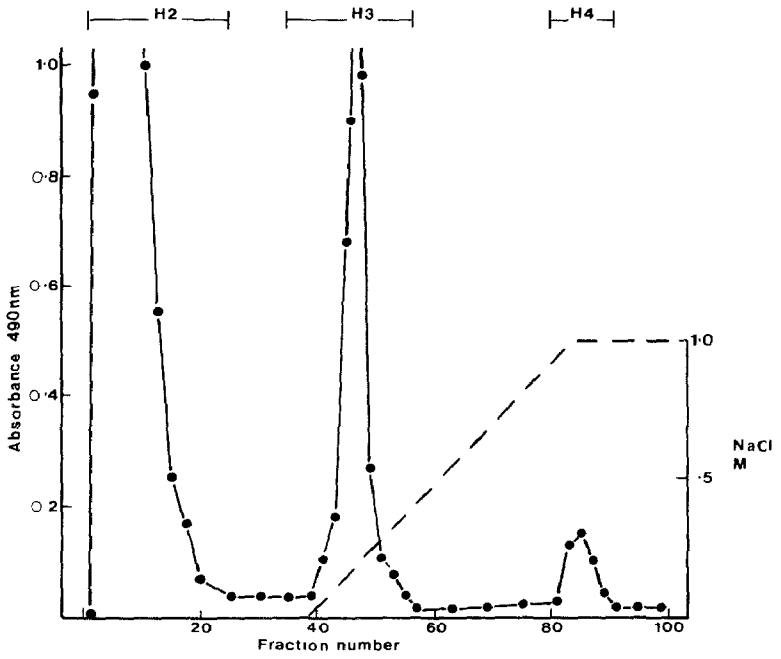

Fig 1. Fractionation of the M KOH (1°)-soluble fraction from apple cell-walls on DEAE-Sephadex: —●—, total carbohydrate; ---, solvent gradient. For details, see text

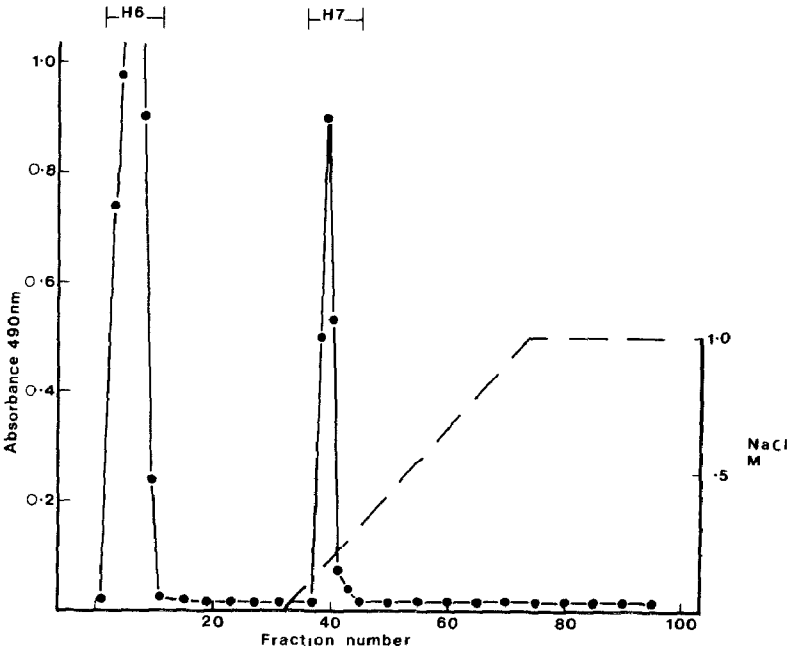

Fig. 2. Fractination of the M KOH (20–22°)-soluble fraction from apple cell-walls on DEAE-Sephadex· —●—, total carbohydrate; ---, solvent gradient. For details, see text

TABLE III

MONOSACCHARIDE COMPOSITION OF ALKALI- AND GUANIDIUM THIOCYANATE-SOLUBLE FRACTIONS OF APPLE CELL-WALL MATERIAL

Fraction	*Yield (%)*[a]	*Monosaccharide composition*[b] *(μg/mg dry wt.)*						
		Deoxy-hexose	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*
M KOH at 1°, DEAE-Sephadex fractions								
Insoluble residue H1	9.3	25	127	100	22	47	144	56
H2	77.7	67	39	228	24	96	330	19
H3	10.0	37	130	309	6	37	52	154
H4	2.0	2	21	10	3	5	17	0
M KOH, 20–22°, DEAE-Sephadex fractions								
Insoluble residue H5	23.8	23	174	58	14	46	83	124
H6	38.8	83	47	211	34	114	350	31
H7	10.8	31	202	83	4	85	37	104
4M KOH, 20–22°, DEAE-Sephadex fractions								
Insoluble residue H8	21.0	75	93	229	77	122	371	53
H9	61.0	83	19	243	49	115	401	18
H10	4.0	61	107	126	134	141	250	54
DEAE-Sephacel (borate) fraction								
H9A	88	84	15	269	35	118	459	n.d.[c]
Guanidium thiocyanate, 20–22°, DEAE-Sephadex fractions								
H11	40.0	47	33	121	305	83	264	56
H12	11.8	16	114	83	12	25	59	240

[a]Percentage of material taken for fractionation. [b]After Saeman hydrolysis. [c]Not determined.

situation encountered with the alkali-soluble fractions described below.

Fractionation of the hemicellulosic polymers. — After removal of the insoluble residues (H1 and H5), the alkali-soluble fractions were chromatographed on DEAE-Sephadex to yield slightly acidic fractions (H2 and H6) which were not retained on the columns, and more acidic fractions (H3 and H7) which were bound (Figs. 1 and 2). The monosaccharide compositions of these and of the insoluble residues are given in Table III. The fractions with relatively low levels of uronic acid generally contain mainly xylose and glucose, whereas those with higher levels of uronic acid have higher levels of arabinose and lower levels of xylose (except H3), deoxyhexose, galactose, and glucose.

Because of the relatively low recoveries of carbohydrate in the insoluble residues H1 and H5 and in the fractions H3, H6, and H7 of the M KOH-soluble

material, determinations of the contents of amino acids and phenols were carried out where possible. Of the fractions examined, only H1 and H5 had an appreciable content of amino acid (Table IV), and fractions H1 and H3 (the only ones examined) had phenolic contents of 4.2 and 3.4% (by an acetyl bromide method), respectively. In view of the very low recovery of carbohydrate, fraction H4 is probably complexed with appreciable amounts of polyphenolics; similar complexes have been isolated from cabbage[3]. Like similar insoluble-fractions from cabbage[3] and carrot[4], the insoluble residues H1 and H5 are probably proteoglycans containing less protein than the fractions from cabbage and carrot and possibly complexed with phenolics. The occurrence of comparable complexes has been discussed[13,22,23]. There were no large differences between H1 and H5 in the proportions of amino acids (Table IV), although H5, the insoluble residue from the M KOH extraction at 20–22°, had a higher level of hydroxyproline. Although only a small amount of protein was extracted with 4M KOH, the proportion of hydroxyproline was higher than in H1 and H5. The residue of α-cellulose contained only a low level of protein (Table IV) and, unlike the cellulose from runner-bean cell-walls, was not rich in hydroxyproline. As with other tissues[3,16,24], the bulk of the "free" xyloglucan was extracted only with 4M KOH (fraction H9, Table III). Further chromatography of H9 on DEAE-Sephacel (borate form) did not result in

TABLE IV

AMINO ACID COMPOSITION OF ALKALI-SOLUBLE FRACTIONS OF APPLE CELL-WALL MATERIAL

Amino acid	*Amino acid composition (μg/mg)*							
	Cell-wall material	*M KOH (1°)-soluble*			*M KOH (20–22°)-soluble*		*4M KOH-soluble*	*α-Cellulose residue*
		Unfractionated	*Fraction H1*	*Fraction H3*	*Unfractionated*	*Insoluble H5*		
Ala	0.2	1.7	3 5	0.1	1.4	1.5	0.2	0.3
Gly	0 3	2.2	0.8	0.6	1.4	0.6	0 3	0.2
Val	0.1	1.1	5.6	0.3	0.7	3.3	0.2	Tr[b]
Thr	0.2	1.5	1.2	0.3	0.8	1.4	0.2	0.1
Ser	0.6	3.6	5.8	0.4	2.7	6.3	0.6	0.1
Leu	0 4	2.6	12.6	1.2	2.1	11.9	0.2	0.4
Ile	0.1	0.9	3.2	0.8	0.5	4.2	0.1	0.2
Pro	1.8	2.0	2.8	n.d.[a]	1 5	2.9	0.3	0.1
Hyp	1.7	3.5	7 2	0.5	3.3	10.2	1.0	0.2
Asp	0.8	3.8	16.4	1.4	2 4	11.7	0.4	0.3
Phe	0.4	1 9	15 4	0.5	1.2	14 5	0.2	0.6
Glu	0.4	4.9	11.5	1.2	3.4	10.3	0.8	0.1
Lys	0.3	3.0	3.4	0.1	1.0	3.6	0.4	0.2
Tyr	0.4	1.6	0.9	Tr[b]	1.3	0 5	0.3	n.d.
Arg	0.2	0.7	0.6	0.1	0.2	0.6	0.2	n.d.
His	0.1	1.9	7 4	(0 6)[c]	0.5	0.4	Tr[b]	n.d.
Total	8.0	36 9	98.3	8.1	24.4	83 9	5.4	2.8

[a]Not detected. [b]Trace. [c]This value is probably too high because of an interfering compound

the separation of a fraction depleted in mannose as with a similar fraction from cabbage[3].

Extraction of de-pectinated lupin hypocotyls with guanidinium thiocyanate (GTC, a powerful chaotropic reagent) gave a fraction enriched in 6-deoxyhexose and mannose[25]. Extraction of the depectinated apple-CWM with GTC yielded a fraction rich in mannose (Table I) which was resolved into "neutral" (H11) and acidic (H12) components by chromatography on DEAE-Sephadex. The neutral component was rich in mannose and relatively rich in glucose and xylose, which suggested the presence of glucomannan(s) and xyloglucan(s).

Methylation analysis of hemicellulosic polymers. — With the exception of fractions H1, H4, H8, and H9A (which was very similar to H9), the hemicellulose fractions were subjected to methylation analysis, and the results are given in Table V. Fraction H9 contained relatively pure xyloglucans which have small, but significant proportions of (1→4)- and (1→4,6)-linked mannosyl residues associated with them. The characteristic features of "free" xyloglucans are the presence of appreciable proportions of (1→4)- and (1→4,6)-linked glucosyl residues, moderate proportions of terminal xylosyl, arabinosyl, galactosyl, and fucosyl groups, and relatively small proportions of (1→2)-linked xylosyl and galactosyl residues. In fractions that contain xyloglucans as the main components, there is a discrepancy between the proportions of terminal residues and branch points (Table V). The possible reasons for this have been discussed previously[3]. In g.l.c.–m.s. of the products of methylation of fraction H11, on both packed and capillary columns of OV-225, there were three major unidentifiable peaks, probably of carbohydrate origin, with retention times (relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol on OV-225 at 170°) of 1.06, 1.92, and 2.15. The last two peaks had very similar fragmentation patterns, with major ions of *m/z* 87 (100%), 129 (78), 141 (73), 74 (41), and 88 (39). The peak at 1.06 had a lower yield of ions of *m/z* 141 and 74. In contrast to fraction H9, all of the other fractions contained various proportions of xyloglucans in combination with pectic substances, arabinoxylans, and possibly glucomannans; the only exception was fraction H3, which is a complex of only pectic substances and arabinoxylans.

The presence of pectic substances in the above fractions is inferred from (*a*) the occurrence of small but significant proportions of uronic acid (most probably galactopyranosyluronic acid), and (*b*) the presence of small proportions of (1→2)- and (1→2,4)-linked rhamnopyranosyl residues and variously linked arabinofuranosyl residues, particularly those that are (1→5)-linked. The presence of (arabino)xylans is inferred from the occurrence of variously linked xylose (and arabinose) residues, particularly the (1→4)-linked xylosyl residues.

The GTC fraction, H11, from the depectinated cell-walls is unusual in that it contains a very high proportion of (1→4)-linked mannosyl residues, probably arising from a glucomannan (or mannan) in combination with small proportions of xyloglucan, pectic substances, and arabinoxylans. Xyloglucan fractions containing (1→4)- and (1→4,6)-linked mannosyl residues have been isolated from cabbage[3]

TABLE V

PARTIALLY METHYLATED ALDITOL ACETATES FROM ALKALI- AND GTC[a]-SOLUBLE FRACTIONS OF APPLE CELL-WALL MATERIAL

Alditol acetates	*Relative mol %*							
	1M KOH (1°)-soluble fractions		*1M KOH (20–22°)-soluble fractions*			*4M KOH-soluble fractions*		*GTC-soluble fraction*
	H2	*H3*	*H5*	*H6*	*H7*	*H9*	*H10*	*H11*
3,4-Me_2Rha[b]	0.2	0.8	2.0	—	1.2	—	0.5	0.1
3-MeRha	0.5	0.2	0.5	—	1.1	—	0.5	0.1
2,3,4-Me_3Fuc	Tr[c]	—	0.7	1.2	—	2.5	0.3	0.2
2,3,5-Me_3Ara	1.3	3.8	12.7	1.4	12.6	0.8	6.0	1.5
2,3-Me_2Ara	2.3	5.7	8.4	2.4	12.5	—	7.9	1.6
2,5-Me_2Ara	0.2	0.7	3.2	0.4	2.2	—	0.7	0.2
3,5-Me_2Ara	Tr	1.1	6.5	0.7	1.9	—	0.6	0.1
2-MeAra	0.9	2.7	5.9	0.1	3.2	—	3.8	—
Arabinitol	0.9	2.4	5.5	Tr	7.4	—	2.0	—
2,3,4-Me_3Xyl	15.0	8.6	6.7	13.8	5.7	12.3	4.5	4.6
2,3-Me_2Xyl	9.8	39.4	12.5	1.5	24.8	1.0	8.0	2.6
3,4-Me_2Xyl	9.7	4.2	3.5	8.2	3.2	10.7	3.0	4.9
3-MeXyl	—	16.2[d]	1.4	0.2	2.2	—	—	—
Xylitol	0.5	1.4	1.4	—	1.2	—	0.5	—
2,3,4,6-Me_4Gal	4.0	0.8	3.8	6.1	1.1	6.5	7.5	2.7
2,3,4-Me_3Gal	0.6	0.4	—	0.6	0.9	0.9	4.6	2.0
2,3,6-Me_3Gal	2.2	Tr	—	—	Tr	—	—	—
2,4,6-Me_3Gal	—	—	—	—	2.1	—	—	—
3,4,6-Me_3Gal	2.4	—	—	—	6.2	5.7	1.5	4.7
2,3-Me_2Gal	—	2.9	—	—	—	—	—	—
2,4-Me_2Gal	—	1.0	—	—	3.2	—	1.2	—
2,6-Me_2Gal	Tr	Tr	—	—	—	—	—	1.5
Galactitol	—	—	5.1	3.0	1.1	—	0.4	0.2
2,3,6-Me_3Man	5.4	—	0.5	3.8	—	7.5	11.3	40.8
2,3-Me_2Man	1.9	1.0	—	0.6	—	0.5	6.1	3.2
Mannitol	—	—	—	—	—	—	0.6	0.2
2,3,6-Me_3Glc	7.6	—	9.4	18.5	1.1	16.8	15.3	10.9
2,4,6-Me_3Glc	—	—	—	—	—	—	0.5	—
2,3-Me_2Glc	32.5	—	8.3	36.7	3.6	33.1	10.2	14.4
2,4-Me_2Glc	—	—	—	—	—	—	—	0.2
3,6-Me_2Glc	—	—	—	—	—	—	—	1.0
4,6-Me_2Glc	—	—	—	—	—	—	—	0.5
Glucitol	0.8	0.9	—	—	1.1	1.1	2.0	0.9
MeHex	1.3	5.8	2.0	0.8	0.4	0.6	0.5	0.7

[a]Guanidium thiocyanate. [b]3,4-Me_2Rha = 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methylrhamnitol, *etc.* [c]Trace. [d]3-MeXyl + 2-MeXyl.

and tobacco[26]. From both the extracellular polysaccharides and cell walls of suspension-cultured tobacco cells, galactoglucomannans have been isolated and characterised[27]. The 4M KOH-soluble acidic fraction H10 also contained appreciable amounts of glucomannans in combination with the other polysaccharides.

DISCUSSION

As with other vegetable and fruit parenchymatous tissues, the main hemicellulosic polysaccharides in apple fruit cortex are xyloglucans, extractable mainly with 4M KOH, but, as with cabbage, a range of complexes containing xyloglucans was extracted with M KOH.

Apparent covalent linkages between pectic polysaccharides and xyloglucans in suspension-cultured sycamore cells have been reported[28] and evidence has been found for the occurrence of small proportions of such complexes in the M KOH-soluble fraction of immature cabbage cell-walls[3]. The present study provides further evidence that these complexes, in which the hemicellulosic component preponderates, are also present in the M KOH-soluble fractions of apple cell-walls. In contrast, some of the hot-water-soluble polysaccharides appear to contain small proportions of hemicelluloses associated with the preponderant pectic components. In addition, small proportions of pectic–xyloglucan–arabinoxylan–protein complexes may also be present. Apple fruit cortex is much less heterogeneous than immature cabbage leaf, so it seems likely that these complexes are a fundamental feature of parenchymatous cell-walls and not confined to tissues capable of developing into specialised structures such as xylem. The complexes are present in small proportions and may serve as linking polymers in the cell-wall matrix.

EXPERIMENTAL

Chemicals. — Methyl sulphoxide was vacuum-distilled over CaH_2 and stored over molecular sieve 3A. DEAE-Sephadex and DEAE-Sephacel were purchased from Pharmacia. All other chemicals were of the highest purity available.

General methods. — Neutral sugars were released by Saeman or M H_2SO_4 hydrolysis[29] and analysed as their alditol acetates by g.l.c.[29]. Uronic acid was determined colorimetrically by a modification[29] of the method of Blumenkrantz and Asboe-Hansen[30]. The degree of esterification was calculated from the methanol content (determined by a g.l.c. method[31]) as a molar proportion of the uronic acid content. Amino acids were determined by g.l.c. of their propyl heptafluorobutyryl derivatives[32]. Phenolic content was determined by the acetyl bromide method[33]. U.v. measurements were made with a Perkin–Elmer 550S spectrophotometer, and i.r. spectra were obtained with a Pye–Unicam SP 200 G spectrophotometer, using KBr discs.

Preparation of CWM. — Ripe apples (var. Cox's Orange Pippin) were purchased locally and peeled, and the cores were removed. The cortex was cut into pieces (~1 cm^3), immediately frozen in liquid N_2, and stored in polythene bags at −30° until required (~1 week maximum). Apple pieces (1 kg) were blended with aqueous 1.5% sodium dodecyl sulphate (1.5 L, containing 5mM sodium metabisulphite and adjusted to pH 5) and filtered through cheesecloth and Miracloth (Evans, Adlard and Co. Ltd., Cheltenham, England). The residue was resus-

pended in fresh aqueous sodium dodecyl sulphate containing metabisulphite (1 L), ball-milled (Pascall, 1-L and 1.5-L pots at 60 r.p.m.) for 6 h at 1°, and centrifuged, and the residue was blended with aqueous Me_2SO (90% final concentration), sonicated (Bransonic) for 30 min at 30°, stirred overnight at room temperature, and then sonicated again for 30 min. The residue was separated by centrifugation, exhaustively dialysed at 1°, and then freeze-dried to yield 14.8 g of CWM.

Sequential extraction of CWM. — CWM was sequentially extracted at pH 5 with H_2O at 80° and then with ammonium oxalate at 80°, as previously described[34]. The largely depectinated CWM was extracted twice at 1° for 2 h, with 100 vol. of M KOH containing 10mM $NaBH_4$, and filtered in the cold, and the residue was extracted with M and 4M KOH, as described peviously[34], to leave a residue of α-cellulose.

GTC extraction. — Depectinated CWM, equivalent to 2 g dry-wt., was stirred in 6M GTC (200 mL) overnight at room temperature, the residue was removed by filtration, and the filtrate was dialysed and freeze-dried; yield, 108 mg.

Ion-exchange chromatography. — (*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 (16 × 1 cm) of DEAE-Sephadex (Cl^- form). Elution initially was with the potassium phosphate buffer (40 to 65 mL, depending on the sample size) and then with this buffer in a linear gradient of NaCl (0 → M, 75 to 100 mL), and finally with phosphate/M NaCl (20 to 40 mL). Fractions (2 mL) were collected, and monitored for carbohydrate by reaction with phenol–sulphuric acid[35]. Appropriate fractions were combined, dialysed, and freeze-dried.

(*b*) Cell-wall fractions were suspended in potassium acetate buffer (20mM, pH 6.0), insoluble material was removed by centrifugation, and the soluble material was applied to columns (30 × 1.5 cm) of DEAE-Sephadex (Cl^- form). Elution was with the acetate buffer initially (100 mL), then with this buffer in a linear gradient of NaCl (0 → M, 225 mL), and continuing with M NaCl (90 mL). Fractions (3 mL) were collected, monitored, and processed as in (*a*).

(*c*) Cell-wall fractions were suspended in sodium borate (5mM, pH 8.0), insoluble material was removed by centrifugation, and the soluble material was applied to columns (23 × 1 cm) of DEAE-Sephacel (borate form). Elution was with the sodium borate buffer initially and then with a linear gradient of sodium borate buffer (0.05 → M, 150 mL). Fractions (2 mL) were collected and processed as for (*a*), except that the combined fractions were adjusted to pH 4.5 with acetic acid before dialysis.

Fractionation of the hot-water-soluble material. — Hot-water-soluble material (200 mg) was chromatographed, using system *b*, to yield fractions P1 (37 mg) and P2 (54 mg), as partially resolved peaks that were not retained on the column, and P3 (54 mg), P4 (15 mg), and P5 (6 mg) which were eluted with the NaCl gradient.

Fractionation of the material extracted with M KOH at 1°. — After removal of an insoluble residue (fraction H1, 14 mg), chromatography of the M KOH (1°)-

soluble material (136 mg) by system (*a*) yielded fraction H2 (116 mg) which was not retained on the column, and H3 (16 mg) which was eluted with NaCl up to 0.3M.

Fractionation of material extracted with M *KOH at 20–22°.* — An insoluble residue (fraction H5, 9.5 mg) was removed, and the soluble material (30.5 mg) was chromatographed (system *a*) to yield fraction H5 (15.5 mg) which was not retained on the column, and H7 (4.3 mg) which was eluted with NaCl up to 0.2M.

Fractionation of the 4M *KOH-soluble material.* — After removal of an insoluble residue (fraction H8, 21 mg), the soluble material (79 mg) was chromatographed (system *a*) to yield fraction H9 (61 mg) which was not retained on the column, and H10 (4 mg) which was eluted with NaCl up to 0.4M. Rechromatography (system *c*) of fraction H9 (40 mg) yielded one fraction (H9A, 35 mg) that was eluted with sodium borate up to 0.3M.

Fractionation of material with GTC. — GTC-soluble material (55 mg) was chromatographed (system *a*) to yield fraction H11 (22 mg) which was not retained on the column, and H12 (6.5 mg) which was eluted with NaCl up to 0.15M.

Methylation analysis. — Polysaccharides were methylated by a modification of the Hakomori method, and converted into partially methylated alditol acetates that were subjected to g.l.c. (OV-225 and ECNSS-M columns) and g.l.c.–m.s. OV-225 column), as described previously[36]. G.l.c.–m.s. was also performed, using a wide-bore (25 m × 0.55 mm), wall-coated, OV-225 capillary column, as described by O'Neill and Selvendran[16]. For capillary-g.l.c. of partially methylated alditol acetates, the same column was used in a Carlo-Erba Fractovap 4160 gas chromatograph, using on-column injection with an initial oven temperature of 55° for 1 min, after which the temperature was rapidly (50°/min initially) raised to 170°, held at that temperature for 80 min, and then raised at 3°/min to 220°. Peaks were recorded and integrated with a Spectra-Physics SP4100 computing integrator.

ADDENDUM

After the preparation of this manuscript, Aspinall and Fanous[37] published the general structural features for an arabinan and the major xyloglucan from apples. They found xylose and glucose in the pectic fractions and uronic acid-containing material in some of the hemicellulosic fractions, but the possibility of complexes, such as those reported here, was not discussed.

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