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STRUCTURAL FEATURES OF CELL-WALL POLYSACCHARIDES OF ONION *Allium cepa*

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ABSTRACT

Cell-wall material has been isolated from immature onion tissues and extracted in sequence with cyclohexane-*trans*-1,2-diaminetetra-acetate (CDTA) at 20°, 0.05M Na_2CO_3 at 1°, 0.05M Na_2CO_3 at 20°, and 0.5, 1, and 4M KOH at 20° to leave the α-cellulose residue, which contained a significant amount of pectic material. The polymers isolated from the extracts were fractionated by anion-exchange chromatography and subjected to methylation analysis. This study helped to distinguish between the pectic polysaccharides of the middle lamellae (solubilised by CDTA) and those of primary cell walls (solubilised by dilute alkali); the latter contained more highly branched rhamnogalacturonan backbones. All the rhamnogalacturonans were substituted to various degrees with side chains comprising galactans or arabinogalactans which contained mainly (1→4)-linked galactose, lesser amounts of (1→4,1→6)- and (1→2,1→6)-linked galactose, and (1→5)-linked arabinose, and small proportions of (1→2)-linked galactose. Most of the branched residues were terminated by galactopyranosyl and arabinofuranosyl groups. The major hemicellulose was a xyloglucan which showed structural features in common with the xyloglucans of dicotyledonous plants. Small amounts of hemicellulose-pectic complexes were also isolated.

INTRODUCTION

In order to obtain a better understanding of the chemistry of dietary fibre, the composition and structure of the cell-wall polymers of the organs of various edible plants have been studied[1–5]. The cell-wall polymers of onion bulbs have not been studied in detail, although there is evidence for the presence of large proportions of pectic substances, rich in (1→4)-linked galacturonic acid and galactose

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TABLE I

SUGAR COMPOSITION OF PURIFIED CELL-WALL MATERIAL OF ONION AND OF MATERIAL SOLUBILISED DURING PURIFICATION

Fraction	*Yield (g/kg f. wt. onion)*	*Sugar composition (μg/mg)*[a]							
		Deoxy-hexose	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*	*Total sugars*
1.5% SDS-soluble	1.52	2.8	15.2	1.9	32.9	31.1	95.6	36.1	215
1.5% SDS-ppt.[b]	1.03	—	—	—	4.8	14.8	12.5	56.6	89
0.5% SDS-soluble	1.45	6.7	17.2	3.3	5.8	131.3	22.4	234	420
Me_2SO	0.18	15.2	8.4	74.9	9.9	40.6	137.8	44.7	331
Purified CWM	9.15	14.5	20	23.2	11.9	267.4	241.9	278.6	857.5

[a]"Anhydro sugar" values after Saeman hydrolysis. [b]Material precipitated during dialysis of 1.5% SDS-soluble fraction.

TABLE II

SUGAR COMPOSITION OF FRACTIONS OF CELL-WALL MATERIAL OF ONION OBTAINED BY SEQUENTIAL EXTRACTION WITH AQUEOUS SOLVENTS

Fraction	*Recovery (%)*	*D.e.*[a]	*Sugar composition (μg/mg)*[b]							
			Deoxy hexose	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*	*Total sugars*
Water	0.3	—	2	12	19	—	145	33	391	602
CDTA-1	8.1	65	9	16	—	—	134	5.0	720	884
CDTA-2	2.9	83	22	26	trace	—	273	8	475	804
Na_2CO_3 (1°)	12.6	4.0	20	19	—	—	275	—	553	867
Na_2CO_3 (20°)	11.3	0.0	26	24	—	—	451	—	350	851
KOH 0.5M	9.5		30	29	2	—	563	6	290	920
KOH 1.0M	3.2		33	33	60	—	464	84	233	907
KOH 4.0M	7.7		43	19	220	34	154	300	71	841
Residue	44.4		7	11	5	18	186	498	105	830

[a]Degree of esterification. [b]"Anhydro sugar" values after Saeman hydrolysis.

residues, and a significant amount of xyloglucan in the cell walls[6–8]. In this respect, the cell walls of immature tissues of onion, a monocotyledon, differ from that of grass mesophyll and resemble those of parenchymatous tissues of dicotyledons. We now report on the composition and structural features of the cell-wall polymers of onion, extracted and fractionated under conditions which should cause minimum degradation of the polymers.

RESULTS AND DISCUSSION

Isolation of cell-wall material (CWM). — The tissues were first extracted with aqueous 1.5% sodium dodecyl sulphate (SDS), and the triturated material was filtered through nylon cloth and washed with aqueous 0.5% SDS; the filtrate contained mainly intracellular compounds. The residue was ball-milled in aqueous 0.5% SDS for 15 h at 2° in order to disrupt the tissue structure, and the polymers solubilised during this treatment were mainly cold water-soluble pectic substances. The residue was extracted with aqueous 90% methyl sulphoxide to remove small proportions of starch and give the purified CWM, which was stored as a frozen suspension. The amounts and composition of the polymers solubilised during the preparation of the CWM, and of the purified CWM, are given in Table I. As expected, the polymers solubilised by aqueous 0.5% SDS and Me_2SO contained significantly larger amounts of carbohydrate than those solubilised by aqueous 1.5% SDS. The high contents of uronic acid, galactose, and glucose in the CWM indicated the presence of large proportions of pectic galactans and cellulose.

Fractionation of CWM. — The cell walls stored in the frozen state were allowed to thaw at room temperature and the small proportion of pectic material solubilised by distilled water at 20° was removed by centrifugation. The CWM was then sequentially extracted with 0.05M cyclohexane-*cis*-1,2-diaminetetra-acetate (CDTA, Na salt) twice, 0.05M Na_2CO_3 at 1° and then at 20°, and 0.5M, 1, and 4M KOH at 20°, to leave a residue of α-cellulose (see Experimental). The relative amounts of polymers solubilised and their sugar composition are given in Table II. Most of the pectic substances held in the walls by Ca^{2+} only were solubilised by CDTA at 20° as recommended[9,10], except that 0.05M sodium acetate was not incorporated. Extraction with hot aqueous solutions of chelating agents (*e.g.*, ammonium oxalate) was avoided in order to minimise degradation of the pectins. The bulk of the CDTA-insoluble pectic substances was solubilised by 0.05M Na_2CO_3 at 1° and then at room temperature; these pectic substances were presumably held in the wall matrix by Ca^{2+} and by ester linkages. The extraction at 1° was carried out in order to ensure de-esterification of the pectins and thus avoid degradation on subsequent exposure of the (remaining) pectic polymers to alkaline conditions at 20°.

The results indicated that most of the pectic substances were extracted by the CDTA, Na_2CO_3, and 0.5M KOH, and accounted for 44.7% of the CWM. The first CDTA extraction (CDTA-1) solubilised a very viscous material having a high ratio

TABLE III

RECOVERY OF CDTA-1 PECTIC FRACTION FROM A RANGE OF ION-EXCHANGE SYSTEMS[a]

Ion exchanger	*Buffer*	*Recovery (%)*
DEAE Sepharose	Phosphate (pH 6.3)	55.8
DEAE Sepharose	Acetate (pH 4.5)	60.0
DEAE Trisacryl	Phosphate (pH 6.3)	87.8
DEAE Trisacryl	Acetate (pH 4.5)	51.0
DEAE Sephacel	Acetate (pH 4.5)	44.0
DEAE Sephadex	Phosphate (pH 6.3)	59.0

[a]The ion exchangers were converted into the acetate or phosphate form before use. The CDTA-1-soluble fraction (50 mg) was dissolved in water, diluted with the appropriate buffer to give a final buffer concentration of 0.05M, and added to the column (12 × 1.8 cm). Following elution with 200 mL of 0.05M buffer, the retained polymers were recovered in 200 mL of the buffer containing 0.5M NaCl. The neutral and retained fractions were combined, dialysed, and freeze-dried to give the total recovery.

of uronic acid to galactose. The pectic fractions subsequently extracted were less viscous and had lower ratios of uronic acid to galactose. Thus, the CDTA-1-, Na_2CO_3 (20°)-, and 0.5M KOH-soluble fractions had ratios of uronic acid to galactose of 5:1, 2:1, and 0.5:1, respectively. The second CDTA extraction ensured the efficient removal of the chelator-soluble polymers before commencing the extractions with Na_2CO_3. However, compared with CDTA-1, CDTA-2 was much less viscous and had a quite different sugar composition. Unless otherwise stated, CDTA-soluble polymers connotes the CDTA-1 fraction. The sugar composition of the 1M KOH-soluble fraction suggested that it contained a mixture of pectic and hemicellulosic polymers. The bulk of the hemicellulose (7.7% of CWM) was extracted with 4M KOH and was rich in xylose and glucose, indicating the presence of a significant amount of xyloglucan.

Anion-exchange chromatography. — Attempts to purify CDTA-1 on various commonly used anion exchangers usually gave unacceptably low recoveries of the pectic polysaccharides (see Table III). In our opinion, the poor recoveries are due to the irreversible adsorption of the pectic polysaccharides on the anion-exchangers, a phenomenon previously observed with other pectic polysaccharides[3–5]. Of the systems tested, only DEAE-Trisacryl M, eluted with phosphate buffer–NaCl, gave a satisfactory recovery (88%). The use of DEAE-Trisacryl consistently gave recoveries in the range 70–90%.

Fractionation of the CDTA- and Na_2CO_3-soluble fractions. — The polymers extracted with CDTA-1, CDTA-2, Na_2CO_3 (1°), and Na_2CO_3 (20°) were fractionated on DEAE-Trisacryl M by sequential elution with 0.05M phosphate buffer and buffer containing 0.125, 0.25, and 0.5M NaCl. The elution profiles are shown in Fig. 1, and the amounts of polymer recovered from the columns and the sugar composition of each fraction are given in Table IV. Uronic acid and galactose accounted for >92% of each fraction, but their relative amounts varied considerably. The fractions from the CDTA-extracts contained uronic acid as the major

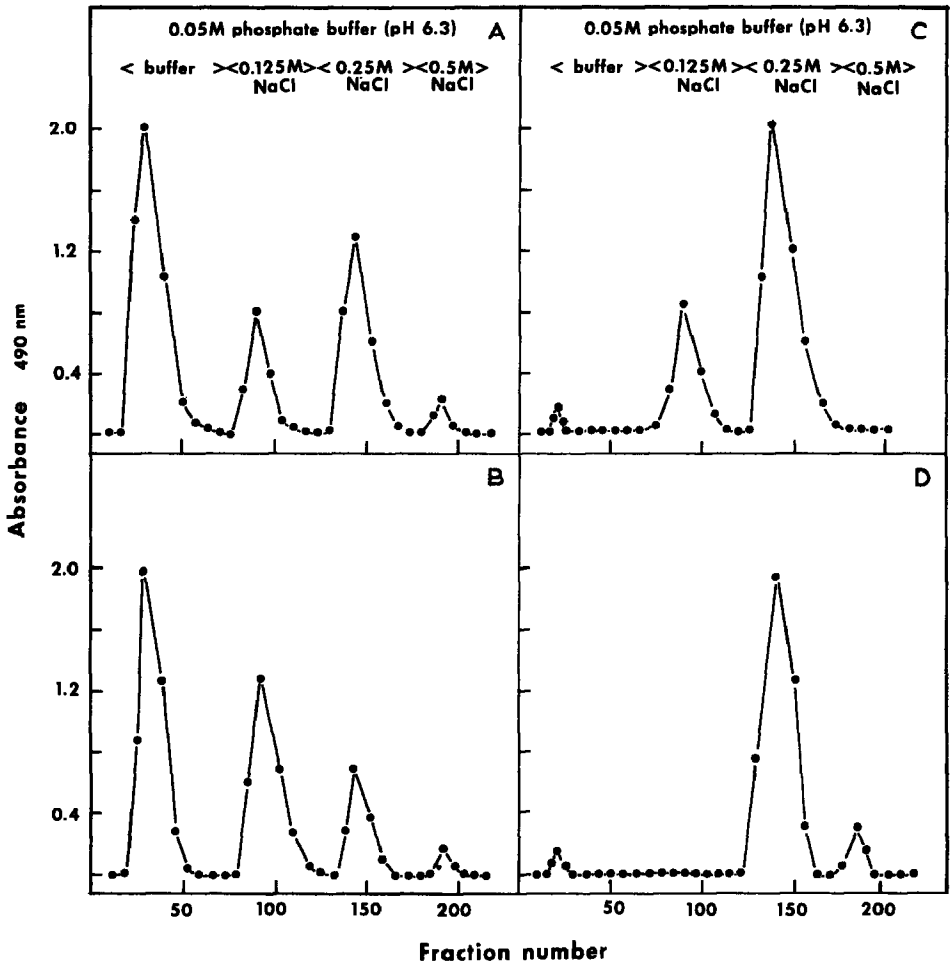

Fig. 1. Chromatography on DEAE-Trisacryl of the polymers extracted with CDTA and Na_2CO_3. Fractions (8 mL) were analysed for total sugars: A, CDTA-1; B, CDTA-2; C, Na_2CO_3 (1°); D, Na_2CO_3 (20°).

sugar, whereas, in most of the Na_2CO_3-soluble polymers, galactose preponderated. The most marked difference in the ratio of uronic acid to galactose was shown by fractions C1D and N2B. The degrees of methyl esterification of the components of the CDTA-soluble polymers varied in the range 36–76%. The Na_2CO_3 (1°)-soluble fractions had low levels of methyl esterification, and none could be detected in the Na_2CO_3 (20°)-soluble fractions. As the conditions of extraction and fractionation used would have caused minimum degradation of the pectic polymers, the above results clearly showed the heterogeneity of the pectic polymers of immature onion tissues. The heterogeneity of apple xyloglucans has been reported[11].

The major components (C1A and C2A) of the CDTA-soluble fractions were not retained on the column despite the high levels of uronic acid. This anomalous behaviour could be due to the aggregation of the pectin molecules *via* inter-chain

TABLE IV

SUGAR COMPOSITION OF COMPONENTS OF CDTA- AND Na_2CO_3-SOLUBLE FRACTIONS OF ONION CELL-WALL MATERIAL SEPARATED ON DEAE TRISACRYL

Fraction		*Amount (mg)*	*D.e.*[a]	*Sugar composition (μg/mg)*[b]							
				Deoxy-hexose	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*	*Total sugars*
CDTA-1											
Buffer	C1A	78.2	66	16	21	—	—	226	4	557	824
0.125M NaCl	C1B	33.4	76	20	27	—	—	272	5	506	830
0.25M NaCl	C1C	55.5	36	13	16	—	—	151	2	633	815
0.5M NaCl	C1D	8.5	39	8	12	—	—	129	7	683	839
CDTA-2											
Buffer	C2A	65	56	19	29	—	—	330	—	437	815
0.125M NaCl	C2B	44.5	88	23	34	—	—	396	—	374	827
0.25M NaCl	C2C	27.4	36	23	32	—	—	268	—	582	905
0.5M NaCl	C2D	3.3	—	8	10	—	—	143	—	485	646
Na_2CO_3 (1°)											
Buffer	N1A	3.6	—	2	9	—	—	498	—	—	509
0.125M NaCl	N1B	49.5	—	31	32	—	—	547	—	242	852
0.25M NaCl	N1C	121.6	—	19	17	—	—	247	—	622	905
Na_2CO_3 (20°)											
Buffer	N2A	3.7	—	5	7	—	—	426	33	—	471
0.25M NaCl	N2B	137.5	—	29	27	—	—	635	—	223	914
0.5M NaCl	N2C	19.1	—	18	18	—	—	234	—	508	778

[a]Degree of esterification. [b]"Anhydro sugar" values after Saeman hydrolysis.

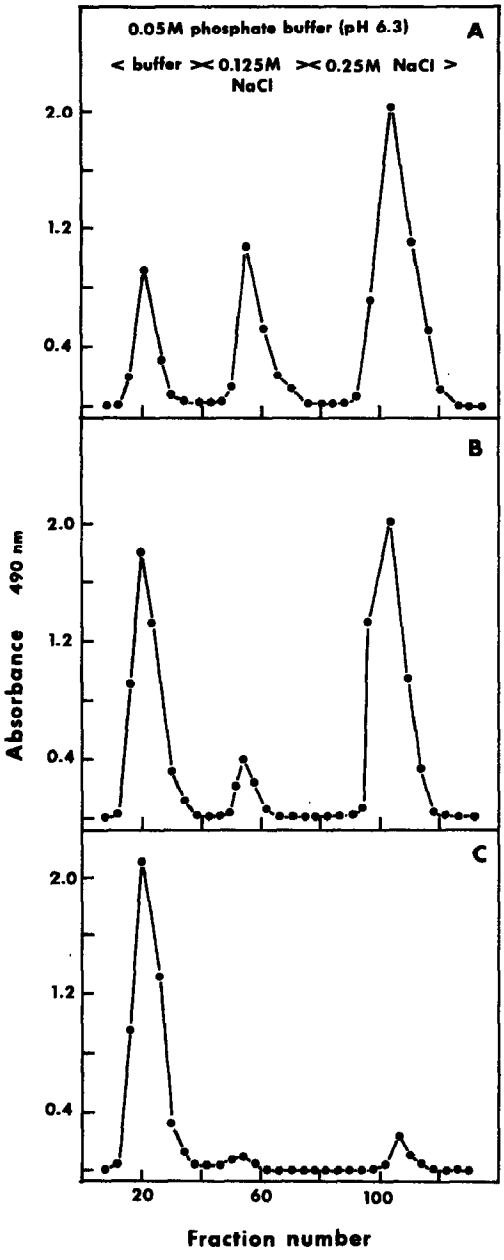

Fig. 2. Chromatography on DEAE-Trisacryl of the polymers extracted with KOH. Fractions (8 mL) were analysed for total sugars: A, 0.5; B, 1; C, 4M KOH.

TABLE V

SUGAR COMPOSITION OF COMPONENTS OF KOH-SOLUBLE FRACTIONS OF ONION CELL-WALL MATERIAL SEPARATED ON DEAE TRISACRYL

Fraction		*Amount (mg)*	*Sugar composition (μg/mg)*[a]							
			Deoxy-hexose	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*	*Total sugars*
0.5M KOH										
Buffer	K1A	29.2	11	20	6	—	487	9	16	548
0.125M NaCl	K1B	38.2	30	32	1	2	544	8	202	825
0.25M NaCl	K1C	62.4	32	30	—	13	448	5	365	893
1.0M KOH										
Buffer	K2A	41.2	30	32	141	4	465	167	59	897
0.125M NaCl	K2B	9.5	31	53	80	2	470	15	177	827
0.25M NaCl	K2C	48.6	24	25	8	[b]	444	13	360	873
4.0M KOH										
Buffer	K3A	208.5	60	15	270	40	140	424	27	976
0.125M NaCl	K3B	6.8	25	58	278	11	157	75	126	731
0.25M NaCl	K3C	26.1	27	30	23	5	408	43	184	721

[a] "Anhydro sugar" values after Saeman hydrolysis. [b] Trace.

TABLE VI

PARTIALLY METHYLATED ALDITOL ACETATES FROM POLYSACCHARIDES PRESENT IN FRACTIONS OBTAINED FROM THE CDTA- AND Na_2CO_3-SOLUBLE FRACTIONS OF ONION CELL-WALL MATERIAL

Alditol	T[a]	*Relative mol %*[b]									
		CDTA-soluble fractions[c]				*Na_2CO_3 (1°)-soluble fractions*				*Na_2CO_3 (20°)-soluble fractions*	
		C1A		*C1C*		*N1B*		*N1C*		*N2C*	
		A	B	A	B	A	B	A	B	A	B
2,3,5-Me_3-Ara	0.45	1.2	1.3	1.9	1.5	1.5	1.1	2.6	1.3	1.4	1.0
2,3-Me_2-Ara	1.08	6.5	4.0	8.3	4.4	5.9	3.8	4.3	3.0	4.9	1.1
2,3,4-Me_3-Xyl	0.56	[e]	[e]	0.9	1.1	0.7	0.6	1.2	0.9	0.7	0.2
3,4-Me_2-Rha	0.89	2.5	3.0	3.0	3.7	2.2	2.9	3.2	2.5	2.5	3.0
3-Me-Rha	1.68	1.0	1.3	0.9	1.0	1.1	1.9	0.9	0.9	1.5	2.2
2,3,4,6-Me_4-Gal	1.2	5.9	4.2	5.7	3.6	8.9	6.1	7.6	5.2	8.6	7.1
3,4,6-Me_3-Gal	2.15	0.7	0.7	1.2	0.6	[e]	[e]	0.8	0.4	1.0	0.5
2,3,6-Me_3-Gal	2.22	72.9	50.4	67.3	26.8	69.1	63.7	66.6	29.2	70.4	48.9
2,3,4-Me_3-Gal	2.93	—	—	—	1.0[d]	—	[d,e]	—	0.6[d]	—	0.2[d]
3,6-Me_2-Gal	3.71	2.2	1.2	3.8	1.7	1.6	1.2	5.8	1.4	1.4	0.7
2,3-Me_2-Gal	4.71	6.1	27.1[d]	5.6	44.4[d]	7.8	15.5[d]	5.4	46.0[d]	6.3	25.7[d]
2-Me-Gal	6.27	—	3.0[d]	—	4.5[d]	—	1.3	—	3.7[d]	—	2.5[d]
3-Me-Gal	8.19	—	4.1[d]	—	4.8[d]	—	1.2[d]	—	4.0[d]	—	2.5[d]
Unknown	3.18	0.9	0.9	1.4	0.9	0.9	0.8	1.4	0.9	0.6	0.6

[a]Retention time relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol on OV-225 at 170°. [b]Values corrected using the molar response factors of Sweet *et al.*[27]. [c]*A*, Methylated polysaccharide; *B*, methylated and reduced ($LiAl^2H_4$) polysaccharide. [d]Deuterium labelled. [e]Trace.

hydrogen bonding of the non-esterified carboxyl groups, as occurs in partially esterified pectins[12,13]. The Na_2CO_3-soluble fractions did not follow this pattern; each gave a small proportion of a neutral fraction (N1A and N2A), and the acidic components were retained on the column. There would be less tendency for aggregation in the Na_2CO_3-soluble acidic fractions because of the mutual repulsion of the more numerous carboxylate groups. N1A and N2A had high contents of galactose (>90% of total carbohydrate), suggesting that they were mainly galactans. Only 50% of these fractions could be accounted for as carbohydrate; the non-carbohydrate material was not investigated.

Fractionation of the 0.5, 1, and 4M KOH-soluble fractions. — The elution profiles of the KOH-soluble fractions on DEAE-Trisacryl M are shown in Fig. 2, and the amounts and sugar composition of each fraction are given in Table V. The 0.5M KOH-soluble fraction contained mainly an acidic fraction (K1C) which was retained on the column and resembled fraction C2B in sugar composition. K1A, which was not retained on the column, contained only 50% of carbohydrate, of which 90% was galactose. Thus, the composition of K1A was similar to those of N1A and N2A.

The M KOH-soluble fraction contained similar amounts of a slightly acidic fraction (K2A), which was not retained on the column, and consisted mainly of xylose, glucose, and galactose in the ratios 1:1:3, and a more acidic fraction (K2C) which contained mainly uronic acid and galactose together with small proportions of other neutral sugars. The major component (86%) of the 4M KOH-soluble fraction was a neutral fraction (K3A) which was not retained on the column and contained xylose, glucose, and galactose in the ratios 2:3:1.

Methylation analysis of the CDTA- and Na_2CO_3-soluble fractions. — Selected fractions were de-esterified with cold dilute alkali, methylated (Hakomori), and reduced with $LiAl^2H_4$. The unreduced (*A*) and reduced (*B*) fractions were analysed by g.l.c.–m.s. after conventional conversion into the partially methylated alditol acetates. Fractions N1A and N2A were not subjected to methylation analysis as insufficient material was available. The results are given in Table VI. The efficiency of carboxyl-reduction was low and variable. Fractions C1A, C1C, N1B, N1C, and N2C gave 42, 70, 40, 72, and 100%, respectively, of the expected value based on the uronic acid content of the original polysaccharide. The reduction procedure had previously given quantitative assays of glucuronic acid in methylated, carboxyl-reduced, Kiwi-fruit gum[14]. The lower efficiency here could reflect the greater tendency of pectic polymers to undergo β-elimination during the Hakomori methylation despite a prior low-temperature de-esterification.

The results of methylation analysis of the pectic fractions indicated that (*a*) reduction of the galacturonic acid residues resulted in increased values for (1→2)- and (1→2,1→4)-linked rhamnose residues, owing to the greater acid lability of the reduced-galacturonic acid–rhamnose linkage, (*b*) the Na_2CO_3- and alkali-soluble pectic polymers generally had a more highly branched rhamnogalacturonan backbone, compared with the CDTA-soluble pectic polymers, an inference based

TABLE VII

PARTIALLY METHYLATED ALDITOL ACETATES FROM POLYSACCHARIDES PRESENT IN FRACTIONS OBTAINED FROM THE KOH-SOLUBLE FRACTIONS OF ONION CELL-WALL MATERIAL

Alditol	T[a]	*Relative mol %*[b]									
		0.5M KOH-soluble fractions[c]			*1M KOH-soluble fractions*			*4MKOH-soluble fractions*			
		K1A	*K1B*		*K2A*	*K2C*		*K3A*	*K3B*	*K3C*	
		A	A	B	A	A	B	A	A	A	B
2,3,5-Me_3-Ara	0.45	[d]	1.2	0.9	0.2	1.3	1.2	1.4	1.1	1.4	2.0
2,3,4-Me_3-Ara	0.56	[d]	0.7	0.9	—	1.3	1.4	—	—	1.5	2.6
2,3-Me_2-Ara	1.08	1.5	4.3	3.2	1.6	4.7	3.0	—	1.9	4.4	4.6
2,3,4-Me_3-Xyl	0.56	—	—	—	9.0	—	—	16.1	10.4	—	—
3,4-Me_2-Xyl	1.2	—	—	—	—	—	—	9.6	—	—	—
2,3-Me_2-Xyl	1.2	—	—	—	12.0	—	—	—	39.7	—	—
3-Me-Xyl	2.15	—	—	—	1.5	—	—	—	6.2	—	—
2,3,4-Me_3-Fuc	0.58	—	—	—	1.6	—	—	3.0	1.9	1.3	2.2
3,4-Me_2-Rha	0.89	[d]	1.4	3.1	—	1.7	2.7	—	0.3	1.1	3.2
3-Me-Rha	1.68	0.5	1.3	3.0	—	1.4	2.7	—	0.3	1.1	2.9
2,3,4,6-Me_4-Gal	1.2	8.9	10.6	6.9	7.4	11.6	7.8	6.4	9.7	10.8	9.0
3,4,6-Me_3-Gal	2.15	—	0.2	0.3	—	[d]	[d]	4.7	—	—	—
2,3,6-Me_3-Gal	2.22	78.9	71.8	56.5	47.1	70.4	47.2	—	13.9	65.3	33.9
2,3,4-Me_3-Gal	2.93	—	—	0.3[e]	—	—	0.4[e]	—	—	—	0.5[e]
3,6-Me_2-Gal	3.71	0.8	0.7	0.9	—	0.8	0.5	—	1.0	0.7	0.8
2,3-Me_2-Gal	4.71	9.1	7.4	21.3[e]	4.8	6.1	28.6[e]	—	—	6.2	26.3[e]
2-Me-Gal	6.27	—	—	1.0[e]	—	—	1.8[e]	—	—	—	2.4[e]
3-Me-Gal	8.19	—	—	1.2[e]	—	—	2.0[e]	—	—	—	2.4[e]
2,3,6-Me_3-Glc	2.34	—	—	—	2.0	—	—	18.5	4.2	—	—
2,3-Me_2-Glc	4.48	—	—	—	12.8	—	—	33.5	8.0	3.5	3.8
3-Me-Glc	7.17	—	—	—	—	—	—	1.9	—	—	—
2,3,6-Me_3-Man	2.03	—	—	—	—	—	—	4.8	1.4	1.7	2.3
Unknown	3.18	0.3	0.6	0.5	—	0.7	0.6	—	—	0.9	0.7

[a]Retention time relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol on OV-225 at 170°. [b]Values corrected using the molar response factors of Sweet *et al.*[27]. [c]*A*, Methylated polysaccharide; *B*, methylated and reduced ($LiAl^2H_4$) polysaccharide. [d]Trace. [e]Deuterium labelled.

on the ratio of (1→2,1→4)- and (1→2)-linked rhamnose residues, (*c*) in addition to the (1→4)-linked galacturonic acid residues, the rhamnogalacturonan backbone probably contained (1→2,1→4)- and (1→3,1→4)-linked galacturonic acid residues (an inference based on dideuteration at C-6 of the 3-OMe and 2-OMe galactitol derivatives), (*d*) in some of the pectic fractions, the side chains (or the rhamnogalacturonan backbone) were terminated by Gal*p*A residues (an inference based on the dideuteration of C-6 of the 2,3,4-tri-*O*-methylgalactitol derivative), and (*e*) the major carbohydrate moieties linked to the rhamnogalacturonan backbone were galactans or arabinogalactans, which contained mainly (1→4)-linked galactose residues, lesser amounts of (1→4,1→6)- and (1→2,1→6)-linked galactose residues and (1→5)-linked arabinose residues, and small proportions of (1→2)-linked galactose residues, and most of the branched residues were terminated by galactopyranosyl, arabinofuranosyl, or xylopyranosyl groups.

*Methylation analysis of the 0.5, 1, and 4*M *KOH-soluble fractions.* — The results of methylation analysis are given in Table VII. Fraction K1A, which was comparable to N1A and N2A, was essentially a galactan containing mainly (1→4)-linked galactose residues (~10% of which were branched through C-6) and most of the branches were terminated by galactopyranosyl groups. The detection of small proportions of (1→2,1→4)-linked rhamnose residues suggested that the galactan moiety was probably linked to C-4 of the rhamnose residues, as in the pectic polymers. This galactan may have been a breakdown product of a more complex pectic polymer. However, in view of the mild conditions of extraction, it is believed that the small amounts of the galactans K1A, N1A, and N2A were native to the walls and not artefacts. These neutral pectic polymers must have been held in the wall matrix by ester cross-links, since alkaline conditions were required to release them, and may have been metabolic breakdown products of more complex pectic polymers, as encountered with xyloglucans[15]. Significantly larger amounts of "neutral" galactans can be extracted from cell walls of potato and onion with hot water, but the bulk of these are artefacts[16].

The types and amounts of glycosidic and galactosiduronic linkages present in K1B and K2C clearly showed that they were comparable to the fractions C1C, N1C, and N2C, and indicated that the former were pectic rather than hemicellulosic in nature. The relative difficulty in solubilising the fractions K1B and K2C compared with the CDTA- and Na_2CO_3-soluble pectic polymers suggested that a wide variation in the degree of binding and cross-linking of chemically related polymers existed within the cell-wall matrix.

Fractions K2A and K3B are comparable in that, in addition to the linkages found in pectic polymers, they contained significant amounts of linkages found in hemicellulosic polymers, including (1→4)- and (1→2,1→4)-linked xylose, terminal xylose, and (1→4)- and (1→4,1→6)-linked glucose residues. These results suggest that both K2A and K3B were complexes containing the polysaccharides xyloglucan, xylan, and pectic arabinogalactan. Likewise, it was inferred that K3C was a complex containing a xyloglucomannan and a pectic arabinogalactan. The

occurrence of small proportions of comparable complexes in the cell walls of cabbage[3], apples[5], and runner beans[17] has been reported[16].

The neutral fraction (K3A) from the 4M KOH-soluble extract contained the glycosidic linkages usually associated with xyloglucans from parenchymatous tissues of dicotyledons, including, in decreasing order of amount, (1→4,1→6)-linked glucose, (1→4)-linked glucose, terminal xylose, (1→2)-linked xylose, terminal galactose, (1→2)-linked galactose, terminal fucose, and terminal arabinose residues. The mode of occurrence of the small proportions of (1→4)-linked mannose residues in K3A is not known, but similar amounts of mannose residues have been detected in other xyloglucans[3,5,11,18].

The chemical and physical procedures used in this study were designed to preserve, as much as possible, the *in vivo* state of the cell-wall polymers. The CWM was kept fully hydrated and stored as a frozen suspension rather than as a freeze-dried powder. This facilitated the rapid solubilisation of the cell-wall polymers. Despite this, the α-cellulose residue contained significant amounts of non-cellulosic polysaccharides, mostly pectic in origin. This component of the α-cellulose fraction was either involved in covalent interpolymer linkage or held by strong non-covalent forces, or both. Similar observations were made with α-cellulose residues from a range of soft tissues[5,18–20]. For cell walls of tissues rich in phenolic substances, *e.g.*, apples, the pectic fraction of the α-cellulose may be linked to the cellulose by phenolic cross-linkages. This is not likely in onions, as measurements of u.v. absorption, at 300 nm, of CWM and α-cellulose dispersed in aqueous 72% H_2SO_4 and then diluted to M acid showed negligible levels of phenolic compounds.

The pectic substances of onion cell walls consisted of a range of structurally related polymers which differed widely in their ease of extraction from the cell-wall complex. The CDTA-soluble pectic polymers gave viscous solutions and were highly esterified. It is suggested that most of these polymers were derived from the middle lamellae region, because the ratio of galacturonic acid to rhamnose was high (~35:1, *cf.* 39:1 for the middle lamellae pectins of potatoes[16]). The remaining, larger part of the pectic polymers was extracted with cold dilute alkali and then with alkaline solvents of increasing strength at room temperature. The alkali-soluble pectic polymers gave solutions that were much less viscous than those of the substances extracted with CDTA, and generally had ratios of galacturonic acid to rhamnose of ~10:1; most of them were probably derived from the primary cell walls. In order to solubilise these polymers, it appears that, in addition to removing the "bridging" Ca^{2+}, the ester cross-links between the galacturonic acid residues and hydroxyl groups of sugar residues elsewhere in the wall matrix had to be hydrolysed. The structural variations between the two groups of pectic polymers were largely reflected in the different degrees of branching of the rhamnogalacturonan backbone and in the relative sizes of the arabinogalactan side-chains.

The hemicellulosic polymers of onion accounted for ~10% of the cell walls. Most of these polymers can be attributed to a xyloglucan that had structural features similar to those of the xyloglucan from parenchymatous tissues of runner

bean[18]. This finding indicates that the xyloglucan of onion, a monocotyledon, has the composition and structural features of the xyloglucans found in a variety of dicotyledonous plants. Evidence for "covalent linkages" between the pectic polymers and a xyloglucan in the cell walls of suspension-cultured sycamore cells[21] and in the M KOH-soluble fractions of cabbage[3], apple[5], and runner-bean[17] cell walls has been reported. In this study also, the M and 4M KOH extracts yielded small proportions of fractions which contained both pectic and hemicellulosic polymers. This is additional evidence that such complexes are native to the walls and probably serve to cross-link the matrix polymers.

EXPERIMENTAL

General methods. — Neutral sugars were released by Saeman or M H_2SO_4 hydrolysis and analysed[22] as their alditol acetates by g.l.c. Uronic acid was determined colorimetrically, after dispersing the polymers in M H_2SO_4, by a modification[22] of the method of Blumenkrantz and Asboe-Hansen[23]. The degree of esterification was calculated from the methanol content[24] as a molar proportion of the uronic acid content.

Preparation of CWM. — Mature onions (*Allium cepa* var. Jumbo) were used. The outer third of each bulb was discarded and the inner tissue zone (1000 g) was cut into pieces (~2 cm^3) which were immediately frozen in liquid nitrogen. The tissue pieces were fragmented with a pestle, powdered in a Waring Blendor, and homogenised with aqueous 1.5% SDS (1400 mL) containing 5mM sodium metabisulphite and 4 drops of octanol[5]. The homogenate was filtered through muslin and Mira cloth, and centrifuged, and the combined residues were suspended in fresh aqueous 0.5% SDS containing 5mM sodium metabisulphite. More octanol was added and the tissue suspension was ball-milled (Pascall 1-L pots for 8 h, 1°) and then centrifuged. The residue was suspended in aqueous Me_2SO (final concentration, 90%), ultrasonicated for 30 min at 30°, stirred overnight at ambient temperature, and ultrasonicated for 30 min. The residue was recovered, exhaustively dialysed at 1°, and stored as a frozen suspension (~450 mL) in water at −20°. A freeze-dried aliquot indicated the yield to be ~9.0 g.

Sequential extraction of CWM. — To an aqueous suspension of CWM (~9 g in 450 mL) was added 0.1M CDTA (pH 6.5, 450 mL), the solution was stirred at 25° for 6 h and then centrifuged, and the residue was extracted with 0.05M CDTA (pH 6.5, 450 mL) for a further 2 h. The extracts (CDTA-1 and CDTA-2) were filtered and dialysed. The residue was suspended in 0.05M Na_2CO_3 (800 mL) containing 20mM $NaBH_4$ and stirred at 1° for 20 h. After centrifugation, the residue was extracted with a fresh volume of the same solution for 2 h at 20°. The extracts [Na_2CO_3 (1°) and Na_2CO_3 (20°)] were filtered, neutralised, and dialysed. The largely depectinated CWM was sequentially extracted under argon for 2 h at 20° with successive amounts (600 mL) of 0.5, 1, and 4M KOH containing 20mM $NaBH_4$. The extracts were neutralised with acetic acid and dialysed. All of the dialysed extracts were concentrated and freeze-dried, as was the α-cellulose.

Ion-exchange chromatography. — The CDTA-1, CDTA-2, Na_2CO_3 (1°), and Na_2CO_3 (20°) extracted polymers (205, 204, 210, and 214 mg, respectively) were suspended in distilled water (100 mL) at 1° overnight and then stirred for 2 h at 20°. 0.1M Phosphate buffer (100 mL, pH 6.5) was added and the solution passed through a column (3 × 35 cm) of DEAE-Trisacryl M in the (phosphate form) at 40 mL/h. The fractions were eluted sequentially with 400 mL of 0.05M buffer and 400 mL of 0.05M buffer containing 0.125, 0.25, and 0.5M NaCl. Further elution with stronger salt solutions or NaOH did not remove significant amounts of additional material. Fractions (8.0 mL) were collected and 0.1-mL portions were assayed for carbohydrate by the phenol–sulphuric acid method[25].

The fractions (200, 130, and 282 mg, respectively) extracted with 0.5, 1, and 4M KOH were each dissolved in 0.05M phosphate buffer (100 mL) and fractionated as for the pectic fractions.

Methylation analysis. — The CDTA-extracted fractions were first de-esterified in 0.1M NaOH for 2 h at 1°, neutralised, and recovered by dialysis and freeze-drying.

Polysaccharides (pre-reduced with $NaBH_4$ if necessary) were methylated by a modification of the Hakomori method[26] and then converted into partially methylated alditol acetates, which were separated by g.l.c. on an OV-225 column and examined[26] by g.l.c.–m.s., using the molar response factors of Sweet *et al.*[27].

Carboxyl-reduction with $LiAl^2H_4$. — This was effected by treatment[28] for 4 h with a boiling solution of $LiAl^2H_4$ in dichloromethane and ether (1:4). When tetrahydrofuran was used as the solvent, the recovery of the reduced material was poor. In order to ensure reasonably good reduction and recovery of carboxyl-reduced material, the following modification was used. The methylated material and apparatus were dried beforehand as for the methylation procedure. A large excess of fresh reagent (40–50 mg for 5 mg of methylated material) was used. The solvents were peroxide-free. A suspension of the reagent in the solvent (5 mL) was added to the solution (~2 mL) of the methylated sample. Agitation for a few seconds in an ultrasonic bath helped to disperse the reagent. At the end of the reaction, after the excess of reagent had been destroyed, the copious precipitate of hydroxide was collected and washed thoroughly with $CHCl_3$–MeOH to remove adsorbed polymer. This was achieved by alternate filtration and resuspension on a sintered filter (Porosity 3; diameter ~25 mm). The combined solutions of polymer and washings were concentrated to dryness, and a solution of the residue in $CHCl_3$–MeOH was filtered through glass-fibre paper (Whatman GF/C) and concentrated to dryness prior to hydrolysis.

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