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Structures of the pectic polysaccharides from the cell walls of kiwifruit

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ABSTRACT

The structure and distribution of the neutral side chains of three pectic polysaccharide fractions of kiwifruit, possessing widely differing degrees of branching, were studied by chemical methods, gel-permeation chromatography, and ^{13}C-n.m.r. spectroscopy, following degradation with a purified endo-polygalacturonase. Three categories of side chain were identified: (*a*) (1→4)-linked β-D-galactosyl residues, which occurred in regions where the galacturonan backbone contained little rhamnose and small proportions of other neutral sugars; (*b*) side chains containing proportionately less galactose and more arabinose, xylose, and fucose, which occurred in regions where the galacturonan backbone contained increased proportions of rhamnose; and (*c*) large (1→4)-β-D-galactans, which were attached to the rhamnogalacturonan backbone. The possible contribution of these side chains to cell-wall breakdown during kiwifruit ripening is discussed.

INTRODUCTION

During the ripening of kiwifruit [*Actinidia deliciosa* (A. Chev.) C. F. Liang and A. R. Ferguson var. *deliciosa* "Hayward"], considerable solubilisation of the cell-wall pectic polysaccharides occurs[1,2]. The process appears to occur[3] without modification of the structure of the polysaccharides, despite a considerable reduction in the content of galactose of the cell wall during ripening. It is probable that the loss of galactose occurs along with, or soon after, solubilisation of the pectic polysaccharides and contributes to the swelling changes and texture of the kiwifruit during ripening[3]. Knowledge of the structure and distribution of the galactose side chains within the pectic molecule may assist in understanding how the loss of galactose residues can change the structure of the cell wall during ripening. We now report on the fragmentation, by a highly purified endo-polygalacturonase, of three pectic fractions possessing widely differing degrees of branching. Two of these fractions were extracted from cell-wall material (CWM) by 0.05M sodium carbonate and 6M guanidinium thiocyanate (GTC), respectively, and the

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third was the CWM residue remaining after further extraction with 4M potassium hydroxide.

TABLE I

Composition of Na_2CO_3-, GTC-soluble, and CWM-residue pectic fractions used as substrates for endo-polygalacturonase

Fraction	*Composition (mol.%)*[a]							*Total (μg/mg)*
	Rha	*Fuc*	*Ara*	*Xyl*	*Gal*	*Glc*	*Uronic acid*	
Na_2CO_3	1.4	0.10	1.0		4.0		93.6	800
GTC	6.1	0.3	4.4	0.8	15.2	0.6	72.5	880
CWM-Res[b]	6.4	0.10	5.8	1.1	45.6	4.9	36.1	270

[a] "Anhydro-values" after CF_3CO_2H and Saeman hydrolysis. [b] Composition does not include cellulosic glucose.

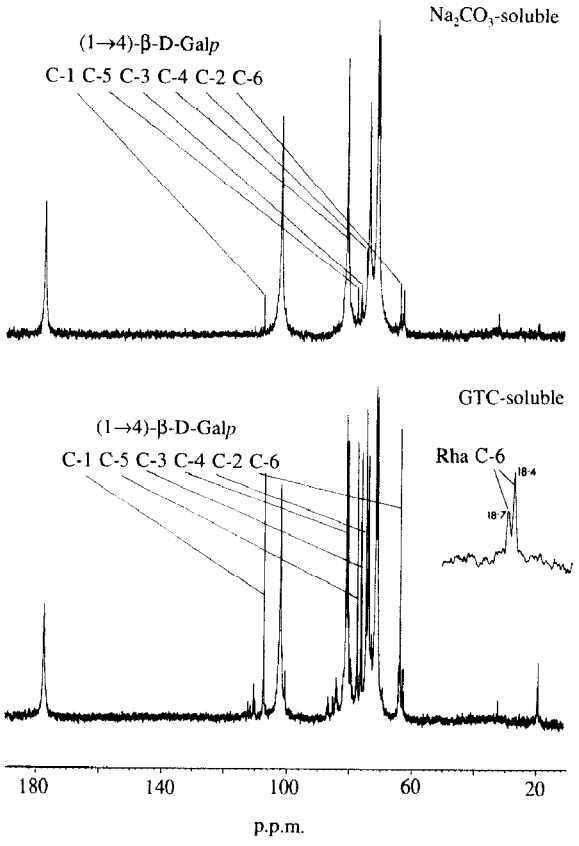

Fig. 1. ^{13}C-N.m.r. spectra of the Na_2CO_3- and GTC-soluble pectic fractions isolated from the CWM of kiwifruit.

RESULTS AND DISCUSSION

Composition of the pectic fractions before degradation. — The degree of galactosylation of the GTC-soluble fraction was 4 times that of the Na_2CO_3-soluble fraction, and that of the CWM residue was 3 times that of the GTC-soluble fraction (Table I).

The ^{13}C-n.m.r. spectra of the Na_2CO_3- and GTC-soluble fractions (Fig. 1) showed that the higher content of galactose of the latter involved (1→4)-linked β-D-galactopyranosyl residues. Assignments were based on the published spectra of pectic polysaccharides from tomato[4], onion[5], flax[6], grape[7], and sugar beet[8]. The intensity of the signals at 106.2, 73.8, 75.2, 79.5, 76.4, and 62.6 p.p.m., corresponding to C-1/6 of the (1→4)-linked β-D-galactopyranosyl residues in the GTC-soluble fraction, was ~ 9 fold that in the Na_2CO_3-soluble fraction, as was a signal at ~ 18.0 p.p.m., attributed to C-6 of the rhamnopyranosyl residues. The latter signal contained components at 18.4 and 18.7 p.p.m. (see inset in Fig. 1) that were assigned tentatively to the C-6 of 2- and 2,4-linked rhamnosyl residues, respectively, based on the expected downfield shifts induced by an alkyl substituent[9].

Fragmentation of the Na_2CO_3- and GTC-soluble polysaccharides. — The amount of endo-polygalacturonase added to each pectic fraction was ~ 100-fold in excess of that theoretically required to degrade the polysaccharides completely if it was 100% polygalacturonic acid. Therefore, it is likely that the substrate degradation was exhaustive.

The distribution of the degraded fragments among the NH_4HCO_3 fractions F-1/10 is shown in Fig. 2 and the monosaccharide composition of each fraction is given in Table II. These fractions accounted for 90% of the Na_2CO_3- and 88% of the GTC-soluble material. F-1, the neutral fraction, accounted for 1% of the Na_2CO_3- and 14% of the GTC-soluble fraction. The relative proportions of the remaining fractions were similar in each pectic fraction, despite the fact that the GTC-soluble polysaccharides contained 4-fold more galactose, arabinose, and rhamnose than the Na_2CO_3-soluble polysaccharides, and therefore appeared to be more highly branched. F-2, which was composed almost totally of galacturonic acid (Table II), accounted for 20.7 and 21.5% of the Na_2CO_3- and GTC-soluble polymers, respectively. F-3 was a negligible component and was not analysed. Therefore, the relative proportions of "smooth" and "hairy" (rhamified) regions were of the same order, suggesting that the extra side-chain sugars of the more-branched backbone of the GTC-soluble polymers were accommodated in the existing "hairy" regions. This inference was supported by the composition of F-4/9 (Table II). Although their relative proportions were similar, the contents of galactose, arabinose, and rhamnose were greater in the GTC-soluble polymers than in the Na_2CO_3-soluble polymers.

Based on monosaccharide composition, the degraded fractions retained on the QAE column could be grouped broadly into three categories: (*a*) F-2, for which the content of neutral sugars was negligible and most of the galacturonic acid was in the form of monomer (t.l.c.), appeared to be derived from "smooth" homogalacturonan regions of the polysaccharides where the action of endo-polygalacturonase would be

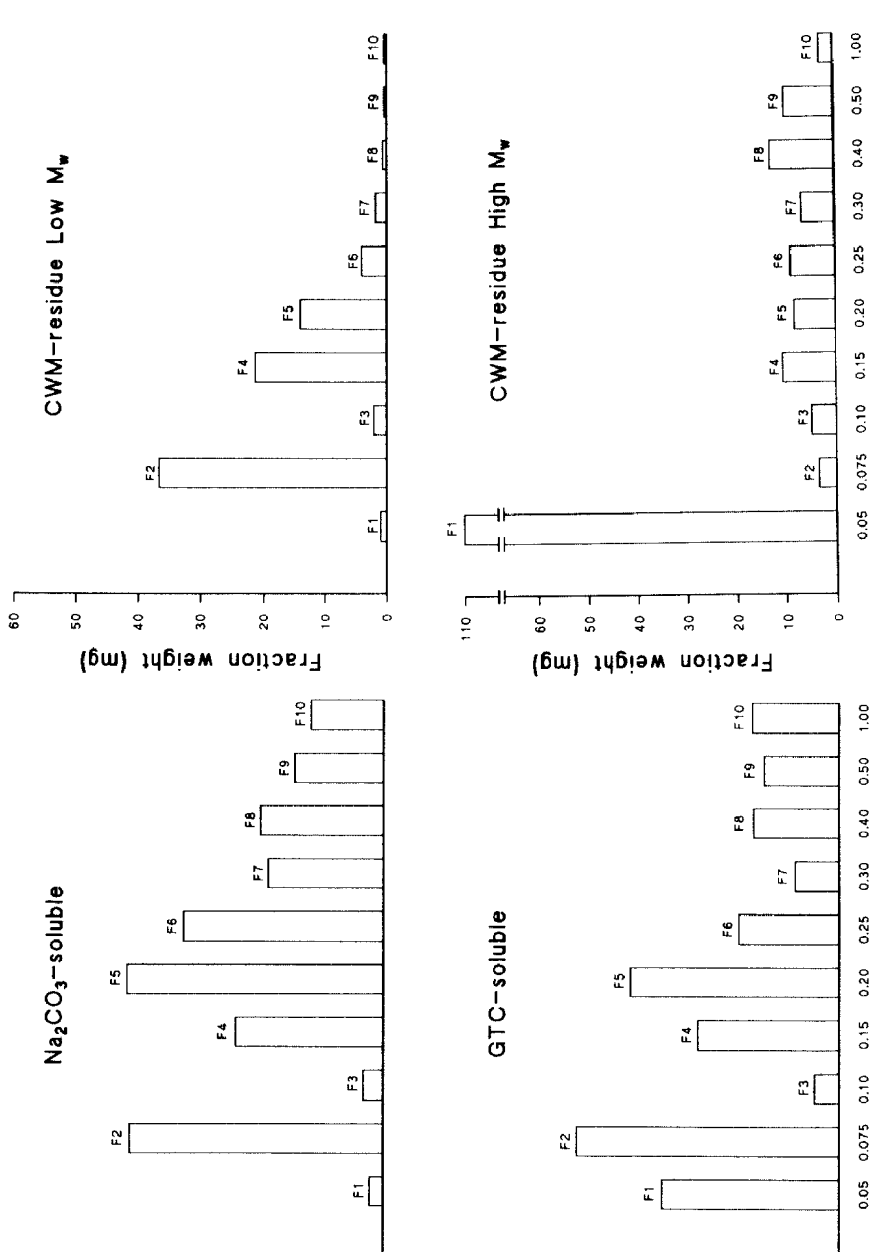

Fig. 2. Yield of fractions eluted from QAE-Sephadex with increasing concentrations of NH_4HCO_3 following treatment of the Na_2CO_3- and GTC-soluble fractions and the CWM-residue with endo-polygalacturonase.

TABLE II

Composition of fractions recovered from QAE-Sephadex following treatment of Na_2CO_3- and GTC-soluble polymers with endo-polygalacturonase

Fraction	*Composition (mol.%)*[a]								*Total (μg/mg)*
	Rha	*Fuc*	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*	
F-1									
Na_2CO_3	1.2	2.2	5.5	6.6	13.3	41.8	14.1	15.2	140
GTC	11.6	0.3	9.5	2.7	0.4	51.8	2.9	20.9	939
F-2									
Na_2CO_3	0.1			0.1		0.2		99.6	896
GTC	0.6		0.2			0.7	0.1	98.3	876
F-4									
Na_2CO_3	0.4		0.1	0.2		8.0	0.3	91.1	952
GTC	1.2		0.9	0.4		14.1	0.2	83.2	900
F-5									
Na_2CO_3	0.2		0.2	0.1		2.4		97.2	998
GTC	0.7		0.5			3.5		95.3	986
F-6									
Na_2CO_3	0.5		0.4	0.1		6.1		92.8	898
GTC	1.5		1.2	0.2		13.1	0.1	83.8	972
F-7									
Na_2CO_3	1.0		0.8	0.2		7.6		90.4	862
GTC	4.7	0.1	4.5	0.8		24.5	0.4	64.9	875
F-8									
Na_2CO_3	5.2	0.4	3.7	0.4		9.8	0.1	80.3	734
GTC	15.8	1.2	12.6	1.8		18.0	0.9	49.7	801
F-9									
Na_2CO_3	9.8	0.8	5.2	0.7		7.4	0.2	75.9	708
GTC	22.6	0.9	12.3	2.8		13.4	0.41	47.5	693
F-10									
Na_2CO_3	5.1	0.2	2.7	0.4		5.3	0.1	86.2	800
GTC	4.8	0.1	2.9	0.7		4.9	1.1	85.5	853

[a] "Anhydro-values" after CF_3CO_2H and Saeman hydrolysis.

optimised; (*b*) F-4/7, which were mainly low molecular weight oligosaccharides of galacturonic acid together with some galactose, which was considerably in excess of the arabinose and rhamnose; (*c*) F-8/9, which contained high proportions of neutral sugars that, in some instances, exceeded that of the galacturonic acid; the proportions of rhamnose and arabinose were up to 3-fold higher than that of galactose, and those of fucose and xylose were up to 10-fold higher than those in F-4/7.

The relative distributions of the monosaccharides amongst the fractions of the Na_2CO_3- and GTC-soluble pectic polysaccharides, shown in Table III, were similar, particularly with regard to the distribution of galactose compared to the other neutral sugars. Thus, for the Na_2CO_3-soluble fraction, most of the galactose occurred as side chains that were located in regions where the proportions of other neutral sugars were low. The remaining galactose occurred in the heavily branched region of the molecule, which accounted for 20% of the pectic polysaccharides, almost all of the fucose, and 79.7, 84.3, and 67.9% of the rhamnose, arabinose, and xylose, respectively.

TABLE III

Percentage distribution of monosaccharides among degraded fragments of Na_2CO_3- and GTC-soluble fractions

Fractions	*Amount (mg)*	*Monosaccharide (%)*					
		Rha	*Fuc*	*Ara*	*Xyl*	*Gal*	*Uronic acid*
Na_2CO_3-soluble							
F-2–F-7	200	17.0	[a]	15.1	32.1	64.3	85.0
F-8–F-10	50	79.7	99.8	84.3	67.9	34.6	14.8
GTC-soluble							
F-2–F-7	170	23.2	2.7	26.4	27.8	69.9	86.1
F-8–F-10	50	76.7	96.3	73.6	72.2	30.1	13.8

[a] Trace.

The results of methylation analysis of selected fractions are shown in Table IV. F-4 of the Na_2CO_3-soluble polysaccharides contained 93.7% of 4-linked galactosyl residues. The ratio (~ 25:1) of terminal to 4-linked residues and the small proportions of 2,4-linked rhamnosyl residues indicated a d.p. of ~ 25 for the side chains. F-4 of the GTC-soluble polysaccharides gave a similar pattern, but with slightly increased proportions of terminal and 5-linked arabinosyl residues.

In contrast, F-8 and F-9 contained lower proportions of 4-linked galactosyl residues and had ratios of terminal to 4-linked residues as low as 1:3. There was a marked increase in the proportions of 2- and 2,4-linked rhamnosyl residues and of the other types of rhamnosyl linkage. The proportions of terminal and 5-linked arabinosyl residues also increased. The proportion of terminal residues was considerably in excess of that of branch-point residues. Either other branch points existed on the galacturono-

TABLE IV

Glycosyl-linkage composition of fractions derived from the degradation of Na_2CO_3-, GTC-soluble, and CWM-residue pectic polymers with endo-polygalacturonase

Linkage	*Composition (mol.%)*										
	Na_2CO_3-soluble			*GTC-soluble*			*CWM-residue*				
	F-4	*F-8*	*F-9*	*F-4*	*F-8*	*F-9*	*F-1*	*F-4[a]*	*F-4[b]*	*F-8*	*F-9*
T-Ara	[c]	7.2	11.4	2.5	12.1	16.6	1.7	4.1	3.9	18.2	16.7
5-Ara		4.7	3.9	1.9	4.6	6.4	0.9		2.1	5.9	8.0
T-Rha		2.2	6.4		5.6	4.9			0.6	3.3	1.6
2-Rha	[c]	2.8	13.0	2.9	8.2	23.2	0.7	8.2	1.9	16.7	24.3
3-Rha		1.7	8.4		5.0	2.5	0.4		0.4	1.9	0.9
2,4-Rha	1.7	1.7	4.2	2.2	3.7	6.8	0.9	[c]	2.0	7.7	10.9
2,3,4-Rha		1.6	3.3	[c]	2.8	3.3			[c]	2.4	2.0
T-Fuc		1.2	3.5		3.1	3.2	0.5		0.4	1.7	1.3
T-Xyl		2.2	3.6		3.8	4.4	0.6		0.9	3.0	2.0
T-Gal	3.7	5.7	6.9	5.2	8.9	5.2	1.0	5.8	3.9	6.8	5.2
4-Gal	93.7	60.1	19.3	82.8	29.1	13.3	90.8	76.9	83.8	24.2	19.1
3-Gal		1.1	[c]		1.2	0.6	0.2			1.0	1.3
3,4-Gal	[c]	1.9	2.5	[c]	1.6	0.8	1.2	[c]	[c]	2.2	1.9
2,4-Gal	[c]	1.3	3.3	[c]	2.4	2.4	0.5	[c]		1.5	1.4
4,6-Gal	0.9	1.9	3.8	2.5	1.2	3.5	0.5	4.9	[c]	0.4	2.3
Unknown		2.6	6.4		6.6	3.0				3.2	1.2

[a] Low M_w fraction. [b] High M_w fraction. [c] Trace.

syl residues (not detected in unreduced polysaccharides), or β-elimination of the methylated galacturonosyl backbone occurred and created extra terminal residues, particularly terminal rhamnosyl residues. However, β-elimination is unlikely to account for the 3- and 2,3,4-linked rhamnosyl residues. Undermethylation was unlikely, as the fractions dissolved readily in methyl sulphoxide, and a double methylation did not diminish significantly the proportion of these types of linkage.

The distribution of molecular weights of F-4/8 of the GTC-degraded polysaccharides was examined by gel-permeation chromatography on Sephacryl S-200. The elution profiles and sugar ratios are given in Fig. 3. Each fraction contained a high and a low molecular weight component. Based on dextran standards, the M_w of the components of F-4 were >80k and ~1k, and those of the components of F-8 were >80k and ~8k. The ratio of the high to low molecular weight components in F-4 was 1:6.5, whereas it was 1:2 in F-8. Most of the galactose and rhamnose in F-4 was contained in the small proportion of high molecular weight component.

Fragmentation of the CWM residue. — Following incubation of the CWM residue (2 g) with endo-polygalacturonase, two solubilised fractions were obtained, namely the

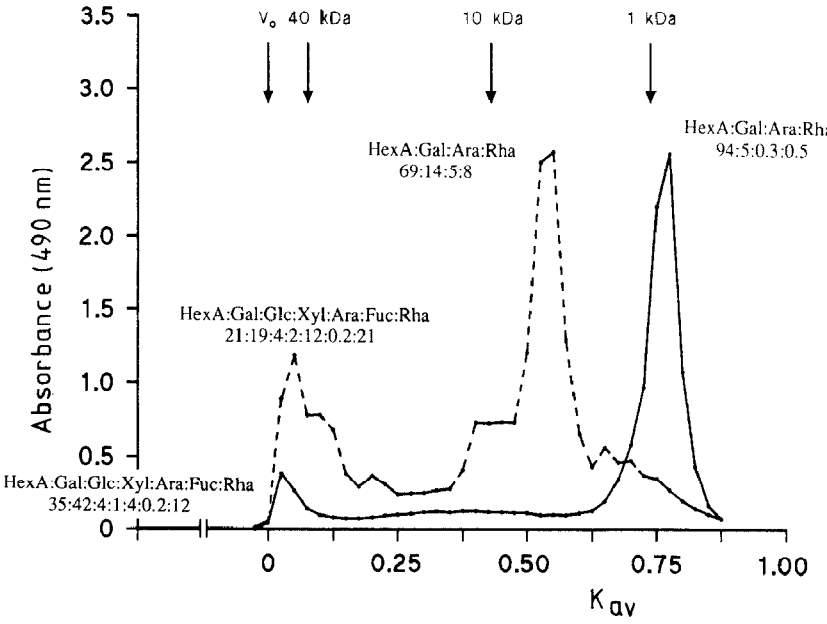

Fig. 3. Gel-permeation profiles on Sephacryl S-200 of F-4 (——) and F-8 (- - -) following treatment of the GTC-soluble fraction with endo-polygalacturonase.

dialysable (<14 k, which yielded 93.8 mg) and the non-dialysable fraction (>14 k, 221 mg). Equivalent fractions for the control treatment yielded 5.8 and 186 mg, respectively. Thus, solubilisation of some pectic polysaccharides occurred independently of the action of the endo-polygalacturonase.

The fractions with high and low M_w from the samples treated with endo-polygalacturonase were each fractionated on QAE-Sephadex. Based on their compositions (Table V), the fraction (F-2/7) with low M_w fell into the same categories as the Na_2CO_3 and GTC fragments. F-2 was the main fraction (Fig. 2), accounting for 44.1% of the fragments of low M_w, and consisted almost entirely of galacturonic acid. F-4/7 contained >90% of galacturonic acid and up to 7.4% of galactose as the major neutral sugar. The relative amounts in F-2/4 followed the patterns of the Na_2CO_3- and GTC-soluble polysaccharides. Linkage analysis (Table IV) showed F-4 to contain mainly 4-linked galactosyl residues with a high ratio of mid-chain to terminal residues, similar to the equivalent fractions in the Na_2CO_3- and GTC-soluble fractions.

F-1, which was not retained on the QAE-Sephadex, represented 62% of the fractions of high M_w, and 44% of the total degraded CWM residue (Fig. 2), and contained 73.8% of galactose and 7.0% of uronic acid. F-1 is considered in detail below. The high galactose to uronic acid ratio was maintained in the early fractions of high M_w (6.6:1 and 4.7:1 in F-2 and F-4, respectively) but decreased in each subsequent fraction, until, in F-8/10, the uronic acid preponderated. In F-4, the polysaccharides were characterised by high levels of 4-linked galactosyl residues and ratios of terminal to inner residues of ~ 1:20 (Table IV). The contents of rhamnose and arabinose were low.

TABLE V

Composition of fractions recovered from QAE-Sephadex following treatment of CWM-residue with endo-polygalacturonase

Fraction	*Composition (mol.%)*[a]							*Total (μg/mg)*
	Rha	*Fuc*	*Ara*	*Xyl*	*Gal*	*Glc*	*Uronic acid*	
F-1								
High M_w	4.9		5.3		73.8	9.1	7.0	873
F-2								
High M_w	5.2		4.0	0.2	77.0	1.7	11.8	860
Low M_w	0.3				0.1		99.6	943
F-4								
High M_w	6.9		7.3	0.2	70.3	0.3	15.1	862
Low M_w	0.3				3.6		96.1	980
F-5								
High M_w	8.1		9.5	0.3	57.5	2.6	22.1	844
Low M_w	0.2				0.9		98.9	973
F-6								
High M_w	9.4	0.1	10.7	0.9	50.6		28.2	808
Low M_w	0.4		1.1	7.4			91.2	836
F-7								
High M_w	13.2	0.2	15.1	1.4	39.9	0.3	29.9	777
Low M_w	1.0		1.0	1.6	4.9		91.4	685
F-8								
High M_w	20.2	0.7	18.5	1.2	23.1		36.2	922
F-9								
High M_w	27.0	1.1	14.7	1.9	12.5	0.1	42.6	829
F-10								
High M_w	16.1	0.4	9.9	0.7	14.5	0.7	57.6	845

[a] "Anhydro-values" after CF_3CO_2H and Saeman hydrolysis.

In contrast, in F-8 and F-9, the proportions of 4-linked galactosyl residues were considerably decreased, the ratios of terminal to mid-chain residues were lower (~ 4:1), and the proportions of rhamnosyl and arabinosyl residues were increased. Therefore, these fractions were similar to the equivalent fractions in the Na_2CO_3- and GTC-soluble fractions.

2-O-Methylfucose and 2-O-methylxylose in the degraded polysaccharides. — Capillary g.l.c. of the alditol acetates revealed traces of peaks that could not be attributed to known sugar derivatives. In the CWM fractions, these were not present in significant proportions, but as the polysaccharides were purified and the degraded fractions were concentrated, the proportions of these peaks increased, and two became significant in F-8 and F-9 in the Na_2CO_3- and GTC-soluble fractions and the CWM residue. The relative retention times (compared to that of acetylated *myo*-inositol) of the alditol acetates, 0.32 and 0.41, were almost identical with those published by Darvill *et al.*[10] for the derivatives of 2-*O*-methylfucose and 2-*O*-methylxylose in RG II. The e.i.-mass spectra for the alditol acetates of the 2 peaks in F-8 and F-9 were characteristic for derivatives of 2-*O*-methylfucose (*m/z* 117, 275, and 215) and 2-*O*-methylxylose (*m/z* 117 and 261). The composition of F-8, taking into account these two extra components, is given in Table VI. Similar relative proportions were found in F-9 and their occurrence is further evidence of the presence of RG II in the polysaccharides of kiwifruit. The presence of RG II in F-8 and F-9 would also explain the moderate proportions of the terminal, 2-, 3-, and 2,3,4-linked rhamnosyl residues (Table IV).

F-1: a high-molecular-weight galactan. — F-1, as solubilised from the CWM residue, had M_w ~ 500k, as determined by gel-permeation chromatography on Sepharose CL-6B. The ^{13}C-n.m.r. spectrum (Fig. 4) contained six sharp signals, corresponding to C-1/6 of the residues of a (1→4)-linked β-D-galactan, which dominated the spectrum. The sharpness of these signals indicated chains that were long and flexible. No signal

TABLE VI

Adjustment of glycosyl-linkage composition of F-8 polymers to include 2-*O*-methylfucose and 2-*O*-methylxylose

Glycosyl residue	*Composition (mol.%)*[a]		
	Na_2CO_3-soluble	*GTC-soluble*	*CWM-residue*
Rha	5.2	15.4	20.2
Fuc	0.4	1.2	0.7
2-*O*-Methylfucose	0.4	1.2	0.7
2-*O*-Methylxylose	0.7	1.6	0.8
Ara	3.7	12.2	18.3
Xyl	0.42	1.7	1.2
Gal	9.7	17.5	22.8
Glc	0.1	0.83	[b]
Uronic acids	79.5	48.3	35.3

[a] "Anhydro-values" after CF_3CO_2H and Saeman hydrolysis. [b] Trace.

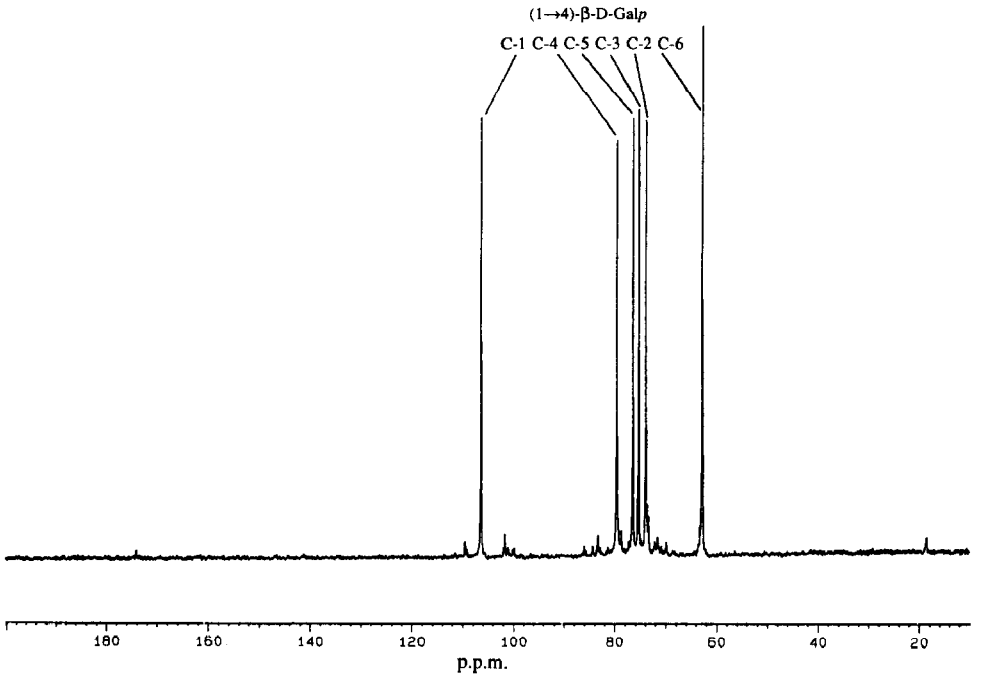

Fig. 4. ^{13}C-N.m.r. spectrum of F-1 from the CWM-residue.

corresponding to C-1 of a terminal galactosyl residue could be detected, which indicated that the ratio of terminal to inner residues was low. This inference was supported by linkage analysis, which gave a ratio of 90:1 for inner to terminal residues (F-1, Table IV). There was a signal at 174.0 p.p.m. corresponding to C-6 of a galacturonosyl residue, and signals at 18.4 and 18.6 p.p.m. assigned to C-6 for 2- and 2,4-rhamnosyl residues. However, it is not known whether these components were attached to the galactan or were constituents of another pectic molecule.

F-1 was not retained on QAE-Sephadex A-25, but was eluted with the neutral fraction. This fact did not preclude the possibility that the galactan was linked covalently to the pectic backbone. The acidic moiety in the polysaccharide backbone could be shielded from the exchange sites of the Sephadex by the numerous galactose side chains. In addition, QAE A-25, normally used to retain low molecular weight compounds, has a low exclusion limit, which limits its capacity to retain large molecules. Two experiments were performed in order to determine whether the galactose residues of F-1 were part of an acidic molecule: (*a*) a solution of F-1 was treated with cetyltrimethylammonium bromide (CTAB) to precipitate the acidic polysaccharides and (*b*) a solution of F-1 in 0.05M phosphate buffer (pH 6.4) was eluted from a column (1 × 10 cm) of DEAE-Sepharose. In each of these experiments, the acidic fractions [the precipitate in (*a*) and the 0.25M NaCl fraction in (*b*)] contained mostly galactose (Table VII), suggesting that the galactan and rhamnogalacturonan moieties were linked covalently. The most noticeable feature of the "non-acidic" fractions was a marked increase in the content of

TABLE VII

Composition of CWM-residue F-1 fractions following ion-exchange chromatography on DEAE-Sepharose and CTAB precipitation, and of the control CWM-residue fractions following gel-permeation chromatography on Sepharose Cl-2B

Fraction	*Composition (mol.%)*[a]								*Total (μg/mg)*
	Rha	*Fuc*	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*	
F-1 of CWM-residue									
DEAE acidic	6.0		6.9	0.1	0.2	73.5	1.3	12.1	775
DEAE neutral	1.5		1.4	0.1	0.4	79.5	13.2	3.8	650
CTAB acidic	5.6		5.6	0.2		80.4	1.2	7.0	820
CTAB neutral	2.2		4.7	0.3	0.9	39.1	46.9	5.8	614
Control CWM-residue									
Fraction A	6.9	0.1	6.4	1.5	0.5	59.2	3.6	21.9	811
Fraction B	3.2	0.1	3.6	0.4	0.3	22.4	2.0	67.9	679

[a] "Anhydro-values" after CF_3CO_2H and Saeman hydrolysis.

glucose. The latter fractions also contained large proportions of galactose, but small proportions of rhamnose and uronic acid were also detected. Therefore, F-1, like all the pectic polysaccharides of kiwifruit, appeared to be heterogeneous with regard to its degree of galactosylation.

Although F-1 was separated from the pectic polysaccharides of the CWM residue following treatment with endo-polygalacturonase, this did not prove that the enzyme was necessary to effect solubilisation. The CWM residue itself also released a highly galactosylated fraction. A gel-permeation profile on Sepharose CL-2B of this fraction compared to that of F-1 is shown in Fig. 5. The pectic polysaccharides released from the CWM residue contained components with M_w similar to, and larger than, that of F-1. The monosaccharide compositions of fractions 20–59 and 60–75 from the CWM residue (A and B in Table VII) indicated that F-1 may belong to a separate class of highly galactosylated pectic polysaccharides, which were not linked covalently to the less galactosylated pectic polysaccharides that are more susceptible to degradation by endo-polygalacturonase.

The F-1 type polysaccharides appeared to be concentrated in the CWM residue, but there was evidence that lesser proportions were solubilised in the Na_2CO_3- and GTC-soluble fractions. F-1 in the latter fractions accounted for 1 and 14%, respectively, of the original polymers, and were highly galactosylated compared to other fractions. The ^{13}C-n.m.r. spectrum of the GTC F-1 fraction was similar to that for F-1 from the CWM residue.

There have been several reports of the occurrence in higher plants of high molecular weight galactans[11–15]. However, in most instances, the galactan contained small proportions of galacturonic acid, suggesting that the galactan was linked covalently to the pectic backbone and had been released during the extraction. A galactan,

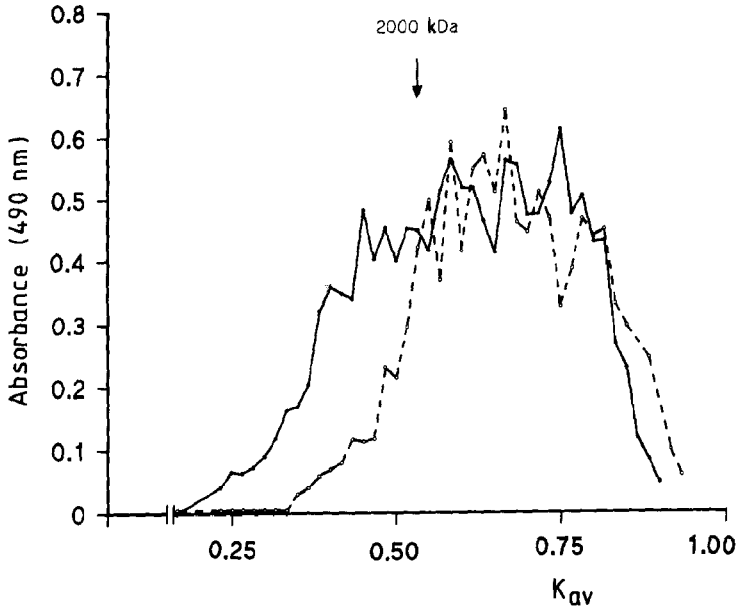

Fig. 5. Gel-permeation profiles on Sepharose 2B of F-1 from the CWM-residue (- - -) and the soluble fraction from the control CWM-residue (——).

homogeneous by free-boundary electrophoresis, has been isolated from the bark of white willow[11]. The conditions of extraction and purification were mild enough to suggest that the polysaccharide existed *in vivo* as a free galactan.

Role of the F-1 type polysaccharides in the cell wall. — The structural features of the F-1 type polysaccharides have important implications for the overall architecture of the cell wall. F-1 could possess a tertiary structure, stabilised by co-operative interactions at the intra- or inter-molecular level, which could affect the immediate physicochemical environment. The non-bonded interaction of such extremely long galactose side chains with other polysaccharides in the matrix, and perhaps the cellulose fibrils, could play a role in maintaining the integrity of the cell wall. The implications for cell-wall breakdown during ripening are no less significant.

It has been shown that, during the ripening of kiwifruit, most of the galactose was lost from the highly branched pectic polysaccharides of the KOH-soluble fraction and the CWM residue[16]. It seems logical to infer that most of this galactose was lost from F-1-type polysaccharides. The extended chains of the (1→4)-β-D-galactan would be ideal substrates for an exo-β-D-galactosidase. The single galactose residues thus released could be phosphorylated by a hexokinase and metabolised into other products[17], which would account for the rapid loss of galactose from kiwifruit during ripening. It has been postulated[3] that solubilisation of pectin may occur as the cell wall becomes swollen or "hydrated" during ripening. A decrease in the molecular weight of the xyloglucan accompanies this phenomenon, and this was suggested as a possible contributing factor

to a loosening of the cell-wall polysaccharides. If most of the F-1 polysaccharides were associated intimately with the cellulose/hemicellulose polysaccharides, then a sudden loss of the extended chains of the (1→4)-β-D-galactan could contribute to the loosening and "hydration" of the CWM residue. It may be significant that most of the galactose was lost from the cell wall during the period when the CWM swelled most[16].

EXPERIMENTAL

Preparation of pectic fractions. — CWM (23 g) was prepared[18] from the outer pericarp tissue of kiwifruit (2 kg; 8.0° Brix, firmness 7.0 kg). The CWM (20 g) was fractionated[18] into CDTA- (0.85 g), Na_2CO_3- (4.2 g), 6M GTC- (1.5 g), and 4M KOH-soluble (2.2 g) fractions, and the CWM residue (9.0 g).

Samples (1.5 g) of the Na_2CO_3- and GTC-soluble polysaccharides were fractionated[18] on a column (2.5 × 30 cm) of DEAE-Trisacryl by sequential elution with 0.05M phosphate buffer (pH 6.5) and buffer containing 0.125, 0.25, and 0.5M NaCl. Each fraction was recovered by dialysis and freeze-drying. The amounts of polysaccharide recovered were as follows. Na_2CO_3-soluble fraction: buffer 161 mg, 0.125M 83.6 mg, 0.25M 791.2 mg, 0.5M NaCl 89.1 mg. GTC-soluble fraction: buffer 794 mg, 0.125M 63.4 mg, 0.25M 300 mg, 0.5M NaCl 18.7 mg.

Purification of endo-polygalacturonase. — Endo-polygalacturonase [poly(1→4)-α-D-galacturonide) glycanohydrolase, EC 3.2.1.15] was purified from ripening tomato fruits (*Lycopersicon esculentum*) by the method of Ali and Brady[19], with the modifications described by Dellapenna *et al.*[20]. Tomatoes (~9 kg) were harvested and processed the same day. Following elution from a column of Concanavalin-A–Sepharose, purification of the enzyme was verified by SDS-PAGE. The purified enzyme (dissolved in the Concanavalin-A–Sepharose buffer) was concentrated to ~1 mL by suspending a dialysis bag containing the enzyme solution in solid carboxymethylcellulose for 12 h. Enzyme activity was assayed as the increase in the number of reducing groups during incubation of the enzyme with aqueous 1% polygalacturonic at pH 4.2 and 37°. Reducing sugars were measured by reaction with 3,5-dinitrosalicylate, using α-D-galacturonate as the standard[20]. Protein was measured by the method of Bradford[21]. The unit of activity was the μkat; 1 μkat is the amount of enzyme which produces 1 μmol of reducing groups per s. The concentrated enzyme was stored as 1-mL aliquots (3.7 μkat/mL) in liquid nitrogen until required.

Enzymic degradation of polysaccharides (a) Na_2CO_3- and GTC-soluble fractions. — A 1-mL aliquot of endo-polygalacturonase was dialysed against 0.02M acetate buffer (pH 4.2) overnight at 4°, then diluted to 15 mL with acetate buffer, and added to 15 mL of an aqueous solution of the 0.25M-NaCl Na_2CO_3- or GTC-soluble fractions containing 360 and 300 mg of pectic material, respectively. Each solution was incubated at 37° for 3 h, then diluted to 50 mL with H_2O, and solid NH_4HCO_3 was added to give a 0.05M solution.

Each solution was applied to a column (10 × 2.5 cm) of QAE-Sephadex (HCO_3^- form) followed by 100 mL each of 0.05, 0.075. 0.1, 0.15, 0.2, 0.25, 0.3, 0.4, 0.5, and 1.0M

NH_4HCO_3. Each of the fractions (F-1/10) was concentrated to dryness on a rotary evaporator. Small amounts of H_2O were added, and the process was repeated several times in order to remove the residual NH_4HCO_3. The residue, dissolved in 1 mL of H_2O, was passed through a column (2-mL bed volume) of Dowex 50W-X4 (H^+) resin (200–400 mesh). Each eluate was concentrated and freeze-dried.

(b) CWM residue. A suspension of the CWM residue (~ 2 g) in H_2O was dialysed overnight against 0.02M acetate buffer. Endo-polygalacturonase (12 μkat), treated as for the Na_2CO_3- and GTC-soluble samples, was added, and the suspension was incubated for 8 h at 37°, then dialysed against water (2 × 1000 mL) overnight. Each dialysate and external solution was concentrated and freeze-dried. Each fraction was then subjected to ion-exchange chromatography on QAE-Sephadex, as described for the Na_2CO_3- and GTC-soluble fractions. The control treatment contained the CWM residue and boiled enzyme.

Chemical analysis. — The monosaccharide composition of polysaccharides was determined by capillary g.l.c. of their alditol acetates following acid hydrolysis[18] in 2M CF_3CO_2H. The column, SP-2330 fused silica (30 m × 0.32 mm), was maintained at 120° for 2 min and then raised to 220° at 25°/min. Uronic acid was determined by the method of Blumenkrantz and Asboe-Hansen[22]. Prior to methylation analysis, selected fractions (~ 2 mg) were reduced with $NaBH_4$ and the resulting oligosaccharide-alditols were methylated by the Hakomori procedure as described by Jansson *et al.*[23]. Methylated products were purified on Sep-pak cartridges, using the procedure of Waeghe *et al.*[24]. The partially methylated alditol acetates were identified[3] by g.l.c.–m.s. as previously described.

^{13}C-N.m.r. analysis. — Spectra (100.62 MHz) were recorded under conditions of broad-band proton decoupling on a Bruker AM 400 spectrometer. Native pectic polysaccharides were examined as solutions in D_2O (50 mg in 3 mL) in 10-mm spinning tubes at 55°. Spectra were obtained using 90° pulses with a pulse repetition time of 1.3 s; 64K data points and 50 000 transients were acquired. Spectra were processed with exponential multiplication, typically 5 Hz. Solutions of the fragments (30–40 mg in 0.5 mL of D_2O) were examined in 5-mm tubes at 30°. Between 1000 and 6000 transients were acquired. All spectra were referenced to external sodium 4,4-dimethyl-4-silapentane sulphonate.

Gel-filtration chromatography. — A column (2.0 × 90 cm) of Sephacryl S-200 was used for the enzyme-degraded fractions F-4 and F-8, and Sepharose 2B (2.5 × 70 cm) for F-1. The fractions (~ 5 mg) were dissolved in 1.0 mL of 0.05M acetate buffer (pH 6) containing 125mM NaCl, and eluted through the column at 10 mL/h. Fractions (1.8 mL) were collected and assayed for carbohydrate by the phenol–sulphuric acid method[25].

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