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STRUCTURAL FEATURES OF CELL-WALL POLYSACCHARIDES OF POTATO *(Solanum tuberosum)*

PETER RYDEN AND ROBERT R. SELVENDRAN*
AFRC Institute of Food Research, Norwich Laboratory, Colney Lane, Norwich NR4 7UA (Great Britain)

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ABSTRACT

Cell-wall material from the parenchyma of mature potato tubers was fractionated by successive extractions with cyclohexane-*trans*-1,2-diaminetetra-acetate (CDTA) at 20°, 0.05M Na_2CO_3 at 1° and 20°, 0.5M and M KOH at 1°, M and 4M KOH at 20°, and 4M KOH + borate at 20° to leave the α-cellulose residue, which contained a significant amount of pectic material. The isolated polymers were fractionated by anion-exchange chromatography. The CDTA-soluble pectic polysaccharides were less branched than those solubilised by Na_2CO_3. The Na_2CO_3 (20°) extract contained appreciably more neutral side-chains. The residual cell wall contained cellulose and a highly branched pectic polysaccharide with a significant content of (1→4)-linked galactose residues. There were two main types of xyloglucan that had different degrees of branching. The less branched, solubilised by stronger alkali, were more strongly associated with the cellulose microfibrils. The cell wall contained 1.7% of protein and the content of hydroxyproline was low. Several of the fractions contained protein (0.2–18.0%), but only the fraction solubilised by 4M KOH + borate contained a high level of hydroxyproline. The neutral component of this fraction was also rich in hydroxyproline and contained (1→2)- and (1→3)-linked arabinose.

INTRODUCTION

The composition, structure, and properties of cell-wall polymers of edible plant organs have been studied in order to obtain a better understanding of the chemistry and properties of dietary fibre[1,2], and the determinants of texture in fresh, cooked, or processed vegetables. We have reported a method[3] for preparing gram-quantities of cell-wall material (CWM) from potato tubers, which are very rich in starch, and the isolation and partial characterisation of the major xyloglucan[4]. The sequential extraction procedure used in the above study caused significant β-eliminative degradation of the pectic polysaccharide, and improved methods

*Author for correspondence.

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were developed that minimised degradation[5,6]. We now report on the large-scale extraction of potato CWM, and on the composition and structural features of the major cell-wall polymers.

RESULTS AND DISCUSSION

Isolation of CWM. — When the potato was blended in aqueous 1.5% sodium dodecyl sulphate (SDS), the soluble material comprised mainly intracellular compounds, including a small proportion of the starch. When the residue was ball-milled in aqueous 0.5% SDS for 15 h at 2°, mainly cold-water-soluble pectic substances and a small amount of the total starch were solubilised. Extraction of the residue with aqueous 90% methyl sulphoxide removed the starch and left the purified CWM which was free of starch, as shown by negative reaction with I_2/KI and sugar analysis after hydrolysis with M H_2SO_4. Of the cell-wall polymeric material, ~6% was solubilised and the carbohydrate composition of these polymers has been reported[3]. Treatment of the SDS-extracted material with phenol–acetic acid–water (2:1:1) was omitted because the relatively small amounts of intracellular proteins were solubilised by the treatments with SDS.

Extraction of CWM. — The CWM was extracted in sequence with 0.05M cyclohexane-*trans*-1,2-diaminetetra-acetate (CDTA, Na salt) twice, 0.05M Na_2CO_3 at 1° and 20°, 0.5M and M KOH at 1°, M and 4M KOH at 20°, and 4M KOH containing boric acid. The residue was α-cellulose (see Experimental). The merits of this extraction sequence have been discussed[6]. Table I contains data on the various fractions. Because of precipitation during acid hydrolysis, the recovery of sugars from the CDTA-1, CDTA-2, and Na_2CO_3 (20°) fractions may be low. Similar discrepancies have been noted in earlier studies[6]. Most of the pectic substances were extracted by CDTA and Na_2CO_3, but small amounts were extracted by 0.5–4M KOH together with the xyloglucan, and a significant amount of pectic material that was rich in galactose and galacturonic acid remained in the α-cellulose residue. This result contrasts with that[4] on the sequential extraction of CWM under conditions where significant β-eliminative degradation of the pectic polysaccharide occurred. The pectic polysaccharides solubilised by hot water, hot oxalate, and KOH were relatively rich in galactose. The pectic material associated with the final α-cellulose residue had a much lower proportion of galactose than galacturonic acid, which suggests that the pectic galactans associated with the α-cellulose residue readily undergo β-eliminative degradation on exposure to hot water and hot oxalate and that the fragments are soluble. Methylation analysis[7] of the pectic polysaccharides solubilised by hot water showed that the ratio of (1→4)-linked galactose to terminal galactose was 25, which suggests that the pectic galactan isolated[8] by aqueous extraction at 85° was probably a degradation product. This and related aspects of the degradation of pectic polysaccharides during extraction have been discussed[9,10].

TABLE I

CARBOHYDRATE COMPOSITION OF POTATO CELL-WALL MATERIAL AND FRACTIONS THEREOF

Fraction[a]	*Recovery (%)*	*D.e.*[b]	*"Anhydro sugar" (μg/mg dry wt.)*								
			Rha	*Fuc*	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*	*Total*
CWM			14[c]	—	75	17	7	288	339	240	980
CDTA-1	13.1	51	3	10	70	4	5	55	45	436	629
CDTA-2	9.1	42	1	5	37	14	4	85	47	193	387
Na_2CO_3 (1°) (N1)	14.0	—	5	15	252	22	15	152	119	426	1000
Na_2CO_3 (20°) (N2)	5.1	—	5	10	156	7	8	147	121	189	643
Wall residue-1		—	12[c]	—	79	15	8	281	377	114	906
0.5M KOH	0.9	—	11	17	205	49	20	156	376	128	962
M KOH (1°)	3.6	—	2	4	114	150	12	60	515	42	899
M KOH (20°)	2.0	—	—	10	190	135	32	112	384	37	900
4M KOH	2.4	—	2	7	171	143	56	142	440	39	998
4M KOH + H_3BO_3	0.6	—	19	—	265	59	33	175	216	155	922
Wall residue-2	49.2	—	12	3	48	3	8	348	420	117	976

[a]See Experimental for the conditions of extraction. [b]Degree of esterification. [c]Deoxyhexose: mostly rhamnose, but contained some unresolved fucose.

The extractions with CDTA at 20° removed the chelator-soluble polymers, the bulk of which probably originate from the middle lamella. The first Na_2CO_3 (1°)-extract (N1) contained a significant proportion of pectic polysaccharides that were rich in galacturonic acid, whereas the pectic polymers present in the second Na_2CO_3 (20°)-extract (N2) contained much less galacturonic acid than neutral sugars, and were probably derived from the primary cell wall.

Each of the KOH extracts contained large proportions of xylose and glucose. In the previous study[4], the major xyloglucan was extracted with 4M KOH and only a small amount of xyloglucan was extracted with M KOH at 20°. However, in this study, some xyloglucan was extracted with 0.5M KOH at 1° and the major xyloglucan fraction was extracted with M KOH at 1°. It may be that the long exposure to dilute alkali during the extraction with Na_2CO_3 caused the hemicelluloses to be more extractable. Furthermore, the differential extractability of the hemicellulose fractions indicated the xyloglucans to be heterogeneous either in structure or mode of cross-linking. The M (20°) and 4M KOH extracts contained more mannose than the 0.5M and M KOH (1°) extracts. The small amount of polymers extracted with 4M KOH + borate contained less xylose and glucose but much more arabinose and some mannose.

The composition of the final cell-wall residue shows the extraction of xylose-containing polymers to be almost complete, but that cross-linked pectic polysaccharides were associated with cellulose.

Fractionation of the soluble extracts. — The above soluble fractions were not completely soluble in water, and only 3 and 10%, respectively, of the Na_2CO_3 extracts were soluble. This finding contrasts with that for the corresponding pectic fractions from onion CWM, which were very soluble. The proportions of the extracts which redissolved and the recoveries of the major fractions on anion-exchange chromatography, together with their carbohydrate composition, are given in Tables II and III. The relatively low recovery of sugars in the pectic fractions, *e.g.*, C1D, N1B, N1C, N2D, K2C, K3C, and K4B, may be due to incomplete hydrolysis.

The CDTA-1 soluble polymers gave pectic fractions with different degrees of esterification (d.e.); the less esterified pectins were bound more strongly to the column. The d.e. of the fractions from CDTA-2 was not determined, because there was insufficient material. Each fraction contained uronic acid as the major component and there was a large proportion of arabinose. The neutral fraction from CDTA-2 was rich in galactose and arabinose but had a small proportion of uronic acid. Similar neutral or weakly-acidic arabinogalactan fractions have been isolated from the Na_2CO_3- and 0.5M KOH-soluble polymers of onion CWM[6] and from oxalate-soluble polymers of cabbage and radish cell walls[11]. The acidic fractions of CDTA-2 were comparable to the corresponding fractions of CDTA-1 and contained a large proportion of uronic acid; the ratio of rhamnose to galacturonic acid ranged from 1:40 to 1:45, which suggests that they probably originate from the middle lamella[6,10].

TABLE II

CARBOHYDRATE COMPOSITION OF FRACTIONS FROM CDTA-SOLUBLE AND Na_2CO_3-SOLUBLE EXTRACTS AFTER ANION-EXCHANGE CHROMATOGRAPHY

Soluble fraction (Proportion of extract %)		*Recovery from column (%)*	*D.e.*[a]	*"Anhydro sugar" (μg/mg dry wt.)*								
				Rha	*Fuc*	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*	*Total*
CDTA-1	(72)											
Buffer	C1A	32.8	42	16	16	200	30	30	114	113	259	798
0.125M NaCl	C1B	27.0	57	19	6	110	21	18	96	87	643	1000
0.25M NaCl	C1C	7.8	35	16	13	216	37	21	48	112	532	1000
0.5M NaCl	C1D	7.2	29	38	—	130	25	21	17	104	251	585
CDTA-2	(62)											
Buffer	C2A	18.7	—	22	18	246	23	27	458	166	10	970
0.125M NaCl	C2B	38.1	—	19	7	80	9	12	170	57	646	1000
0.25M NaCl	C2C	20.3	—	12	—	68	24	18	39	57	497	715
0.5M NaCl	C2D	22.9	—	14	8	127	11	13	95	84	649	1000
Na_2CO_3 (1°)	(3)											
0.125M NaCl	N1B	44.4	—	22[b]	—	58	5	10	88	44	357	584
0.25M NaCl	N1C	30.6	—	35[b]	—	115	9	20	107	87	305	679
Na_2CO_3 (20°)	(10)											
Buffer	N2A	38.0	—	30[b]	—	124	11	10	161	33	339	709
0.125M NaCl	N2B	31.6	—	30[b]	—	147	9	13	200	52	268	718
0.25M NaCl	N2C	21.5	—	23[b]	—	69	12	9	65	28	517	725
0.5M NaCl	N2D	8.9	—	10[b]	—	24	4	4	31	28	421	522

[a]Degree of esterification. [b]Deoxyhexoses were not resolved.

TABLE III

CARBOHYDRATE COMPOSITION OF FRACTIONS FROM KOH-SOLUBLE EXTRACTS AFTER ANION-EXCHANGE CHROMATOGRAPHY

Soluble fraction (Proportion of extract %)		*Recovery from column (%)*	*"Anhydro sugar" (μg/mg dry wt.)*							
			Deoxyhexose	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Uronic acid*	*Total*
0.5M KOH	(39)									
Buffer	K1A	69.9	5	97	205	25	133	462	38	871
0.125M NaCl	K1B	8.0	95	129	13	20	402	133	194	986
0.25M NaCl	K1C	15.7	33	110	10	13	161	46	493	866
0.5M NaCl	K1D	6.4	30	113	36	36	97	97	578	986
M KOH (1°)	81									
Buffer	K2A	94.5	4	83	203	12	80	481	81	944
0.25M NaCl	K2C	3.3	14	85	15	11	128	35	311	598
M KOH (20°)	(90)									
Buffer	K3A	70.4	7	88	267	36	104	426	41	969
0.125M NaCl	K3B	10.3	27	232	15	16	211	102	81	660
0.25M NaCl	K3C	8.4	23	115	21	11	147	117	193	626
4M KOH	(91)									
Buffer	K4A	88.3	9	102	170	69	127	459	34	968
0.125M NaCl	K4B	3.7	28	130	8	9	234	67	109	585
0.25M NaCl	K4C	6.4	42	90	30	12	287	65	234	768
0.5M NaCl	K4D	1.3	13	156	28	32	100	126	197	652
4M KOH + H_3BO_3	(80)									
Buffer	K5A	67.6	6	113	48	66	111	167	52	562
0.125M NaCl	K5B	9.7	16	82	29	10	24	74	111	567
0.25M NaCl	K5C	16.6	23	134	16	16	464	103	244	1000
0.5M NaCl	K5D	5.2	25	140	23	30	337	179	203	938

The ratio of rhamnose to uronic acid for the major Na_2CO_3-soluble pectic fractions ranged from 1:20 to 1:10, which suggests that they are more highly branched compared with the CDTA-soluble pectic polysaccharides and are probably derived from the primary wall region[6]. Since, up to this stage, relatively mild treatments were used to solubilise the polysaccharides, the range of pectic polysaccharides obtained indicates their heterogeneity within the wall.

The KOH-soluble polymers were fractionated to give 69–95% of neutral components, which contained the bulk of the xyloglucans, and one or more acidic components. The presence of small proportions of uronic acids in the "neutral" fractions may be an artifact of colorimetric assay, although the small amounts of galacturonic acid in "neutral" pectic fractions might have arisen from degradation of pectin. The acidic components consisted of pectic material in which the ratio of rhamnose to uronic acid ranged from 1:20 to 1:5, and they were rich in arabinose and galactose. The relatively low recovery of arabinose from the M KOH (20°)-fraction may be due to the presence of arabinose-rich material in the insoluble fraction (of which only 90% was soluble), and also incomplete recovery of arabinose-rich pectic material on anion-exchange chromatography. This result may be due to association of phenolic material with the pectic fractions. Unlike the neutral fractions from the 0.5, 1, and 4M KOH-soluble polymers, the neutral fraction (K5A) from the 4M + borate-soluble polymers contained a significantly higher proportion of arabinose, suggesting the presence of an arabinose-rich polymer in addition to xyloglucan.

When the neutral fraction from the 4M KOH-soluble polymer was complexed with borate and chromatographed on DEAE Trisacryl (borate form), ~90% of the material was recovered, but there was little fractionation, indicating that the potato xyloglucans are not as heterogeneous as the apple xyloglucans[12].

Amino acid analysis. — The potato cell walls contained ~2% of protein that was not rich in hydroxyproline (Hyp). The extent of enrichment of cell-wall polymers in protein can help in assessing the association of proteins and polysaccharides[13,14]. Table IV shows the amino acid composition of some of the fractions from potato CWM. The "neutral" fraction (CIA) from CDTA-1 contained 18% of protein which was not rich in Hyp but contained high proportions of Glu, Asp, and Lys. Likewise, the acidic fraction (N2D) from Na_2CO_3 (20°)-soluble polymers was not rich in Hyp, but had high levels of Glu, Ser, and Asp. These findings contrast with those for the proportion of Hyp in pectic–protein complexes of *Vicia faba*[9] (field bean), but are comparable with those for the proteoglycans of runner bean[15]. In contrast, the neutral fraction (K5A) from the 4M KOH + borate-soluble polymers was rich in Hyp and relatively rich in Ser, and its composition is comparable with the Hyp-rich glycoprotein of runner bean CWM[16] and potato lectin[17], except that, unlike the latter, it does not contain Cys. This is the first report of a Hyp-rich (glyco)protein in the cell wall of free-growing potato tissues. The Hyp-rich glycoprotein content of the α-cellulose residue is low, which shows that the bulk of the glycoprotein does not require chlorite–acetic acid oxidation for its

TABLE IV

AMINO ACID COMPOSITION OF FRACTIONS OF POTATO CELL-WALL MATERIAL

Amino acid	*Amino acid composition (μg/mg)*										
	Cell-wall material	*CDTA-1-soluble*		*Na_2CO_3(20°)-soluble*			*M KOH (1°)-soluble*		*4M KOH + H_3BO_3-soluble*		*Residue-3*
		Unfractionated	*CIA*	*Unfractionated*	*N2A*	*N2D*	*Unfractionated*	*K1A*	*Unfractionated*	*K5A*	
Ala	0.5	0.7	8.1	0.2	1.0	7.2	1.0	0.8	4.4	7.4	Tr[a]
Gly	0.7	1.2	2.3	0.3	1.7	11.1	1.7	0.8	3.2	2.3	0.1
Val	1.2	1.5	4.0	0.2	2.1	11.3	2.4	1.6	4.4	1.7	Tr
Thr	0.8	1.2	6.5	0.1	2.7	6.3	1.8	0.7	2.7	5.3	0.1
Ser	1.0	1.6	10.1	0.5	5.0	20.8	2.6	1.3	6.2	10.2	0.3
Leu	1.3	1.7	13.1	0.3	1.5	8.1	2.7	0.7	3.7	1.8	0.1
Ile	1.1	1.1	4.5	0.2	1.4	2.8	1.9	0.2	1.6	—	0.1
Pro	0.8	1.2	7.7	0.2	1.4	6.4	1.7	0.6	2.1	2.3	Tr
Hyp	1.0	0.9	2.1	0.4	1.4	3.3	1.0	1.4	18.2	28.7	0.1
Asp	3.0	4.8	25.7	0.9	5.0	18.0	4.8	2.2	6.3	5.0	0.1
Phe	0.8	1.6	11.7	0.4	1.8	7.5	1.8	0.8	2.6	4.6	0.1
Glu	1.6	3.0	35.1	0.7	7.2	43.6	3.5	2.8	8.6	6.3	0.4
Lys	1.2	2.4	18.5	0.4	2.0	9.7	2.8	8.6	5.9	7.6	Tr
Tyr	0.7	1.5	4.3	0.3	1.0	3.4	1.4	0.4	2.2	2.0	0.1
Arg	0.5	1.4	12.3	0.4	1.3	6.3	2.1	0.7	2.6	—	—
His	0.3	0.3	8.5	0.2	0.6	2.6	1.2	—	2.9	—	—
Trp	0.6	—	0.7	—	—	—	0.6	—	0.2	—	—
Total	17.0	26.2	179.0	5.7	36.4	168.4	34.8	23.8	77.7	85.2	1.5

[a]Trace.

release. This finding is in contrast with our earlier observations on the firm association of Hyp-rich proteins with the α-cellulose residue of mature runner-bean pods[18,19]. The 4M KOH + borate extract of the CWM of runner bean parenchyma also contains a Hyp-rich glycoprotein fraction[20].

Methylation analysis of the CDTA and Na_2CO_3 fractions. — The major CDTA fractions, after de-esterification, were methylated and carboxyl-reduced. The C2A fraction was methylated, but not reduced because the proportion of uronic acid was small. The Na_2CO_3-soluble polymers were only slightly soluble in

TABLE V

PARTIALLY METHYLATED ALDITOL ACETATES DERIVED FROM FRACTIONS OF THE CDTA- AND Na_2CO_3-SOLUBLE EXTRACTS

Alditol acetate	*Relative mol %*[a]					
	C1A	*C1B*	*C2C*	*C2D*	*N1*	*N2*
3,4-Me_2-Rha[b]	1.9	0.9	6.8	4.0	2.1	Tr[c]
2,4-Me_2-Rha	1.8	—	—	—	0.5	—
4-Me-Rha	0.2	—	—	—	—	—
3-Me-Rha	1.2	1.1	1.7	2.2	Tr	5.4
Rhamnitol	1.7	—	—	—	—	—
2,3,5-Me_3-Fuc	1.0	—	—	—	—	—
2,3,5-Me_3-Ara	5.1	4.3	—	—	3.3	Tr
2,3,4-Me_3-Pent[d]	5.6	—	—	—	—	—
3,5-Me_2-Ara	1.1	—	—	—	—	—
2,5-Me_2-Ara	Tr	—	—	—	—	—
2,3-Me_2-Ara	3.8	6.6	38.4	46.0	13.1	29.4
Arabinitol	—	1.6	—	—	—	—
2,3-Me_2-Xyl	1.0	4.9	—	—	—	—
Xylitol	—	0.8	—	—	—	—
2,3,4,6-Me_4-Hex[e]	1.8	—	—	—	—	—
3,4,6-Me_3-Hex	—	—	3.2	—	—	—
2,3,6-Me_3-Man	3.7	—	—	—	—	—
2,3,4,6-Me_4-Gal	4.1	1.5	3.2	5.9	4.1	Tr
2,3,6-Me_3-Gal	3.2	13.9	—	—	7.7	18.6
2,3,4-Me_3-Gal[f]	2.0	2.1	—	—	—	—
2,3-Me_2-Gal[f]	45.6	40.5	44.0	36.6	64.5	39.4
2-Me-Gal[f]	1.7	3.1	2.7	5.3	1.3	3.7
3-Me-Gal[f]	2.1	3.7	—	—	—	2.1
Galactitol	1.0	2.5	—	—	—	—
2,3,6-Me_3-Glc	5.9	5.8	—	—	—	—
Glucitol	2.2	3.1	—	—	—	1.4

[a]Values corrected using the molar response factors of Sweet *et al.*[21]. [b]3,4-Me_2-Rha = 1,2,5-tri-*O*-acetyl-di-*O*-methylrhamnitol, *etc.* [c]Trace. [d]Ara or Xyl. [e]Man or Glc. [f]Deuterium labelled.

the buffer, so that only small amounts of the fractions from the Na_2CO_3-soluble polymers were available; therefore, the unfractionated polymers (N1 and N2) were subjected to methylation analysis (see Table V). The methylation analyses were not quantitative; there were lower recoveries of galacturonic acid, arabinose, and

TABLE VI

PARTIALLY METHYLATED ALDITOL ACETATES DERIVED FROM FRACTIONS OF THE KOH-SOLUBLE EXTRACTS AND FROM WALL RESIDUE-3

Alditol acetate	*Relative mol %*[a]							
	K1A	*K2A*	*K3A*	*K4A*	*K4B*[b]	*K5A*	*K5C*[b]	*Residue-2*[b]
3,4-Me_2-Rha[c]	—	—	—	—	—	—	3.4	1.3
2,4-Me_2-Rha	—	—	—	—	—	—	2.0	—
3-Me-Rha	—	—	—	—	8.5	—	3.2	3.1
Rhamnitol	—	—	—	—	—	—	2.6	—
2,3,5-Me_3-Fuc	—	—	—	—	4.3	—	3.6	—
2,3,5-Me_3-Ara	9.6	10.1	5.9	8.6	9.3	8.9	6.3	0.5
3,5-Me_2-Ara	—	—	0.8	0.9	—	8.1	—	—
2,5-Me_2-Ara	0.5	1.7	1.2	0.7	—	4.2	—	—
3,4-Me_2-Ara	0.2	—	0.2	—	—	—	—	—
2,3-Me_2-Ara	0.6	—	0.4	—	42.4	—	8.8	9.6
4-Me-Ara	—	—	—	—	—	—	1.0	—
2,3,4,-Me_3-Xyl	6.7	6.3	6.8	6.0	—	4.0	—	1.9
3,4-Me_2-Xyl	12.2	13.8	10.1	9.3	—	6.8	2.6	—
2,3-Me_2-Xyl	2.0	1.6	1.1	1.4	6.9	—	16.4	—
3-Me-Xyl	—	—	—	—	—	—	1.8	—
2,3,4,6-Me_4-Hex[d]	0.7	—	0.5	—	—	1.0	—	1.3
2,3,6-Me_3-Man	0.7	0.8	2.9	9.7	2.8	11.8	1.7	2.4
2,3-Me_2-Man	—	—	0.3	4.7	—	3.5	—	—
Mannitol	—	—	—	0.5	—	—	—	—
2,3,4,6-Me_4-Gal	7.5	8.0	7.5	9.9	11.1	7.8	3.4	4.4
3,4,6-Me_3-Gal	2.0	0.3	1.6	1.6	—	3.7	—	—
2,3,6-Me_3-Gal	—	—	—	—	4.1	—	5.6	10.9
2,3,4-Me_3-Gal[e]	—	—	—	—	—	—	3.6	0.6
2,3-Me_2-Gal[e]	—	—	—	—	7.2	—	19.6	7.0
2-Me-Gal[e]	—	—	—	—	—	—	2.7	—
3-Me-Gal[e]	—	—	—	—	—	—	1.6	0.1
Galactitol	—	—	—	—	—	—	—	0.1
2,3,6-Me_3-Glc	32.7	31.7	35.8	28.7	3.3	28.2	6.4	53.6
2,6-Me_2-Glc	—	—	—	—	—	—	—	0.2
3,6-Me_2-Glc	—	—	—	—	—	—	1.9	0.5
3,4-Me_2-Glc[e]	—	—	—	—	—	—	1.2	
2,3-Me_2-Glc	23.9	25.4	24.9	15.0	Tr[f]	11.7	0.6	0.8
Glucitol	0.6	0.2	0.5	2.8	—	0.5	—	—

[a]Values corrected using the molar response factors of Sweet *et al.*[21]. [b]Reduced with $LiAl^2H_4$. [c]3,4-Me_2-Rha = 1,2,5-tri-*O*-acetyl-di-*O*-methylrhamnitol, *etc.* [d]Man or Glc. [e]Deuterium labelled. [f]Trace.

galactose in some of the fractions. It is possible that segments of pectins carrying relatively short, neutral side-chains, released by β-eliminative degradation during methylation, were lost during dialysis of the methylated samples. Thus, there were losses of arabinose from C1A and C1B and of galactose (and glucose) from C2C and C2D.

Despite the poor recovery of galacturonic acid in one of the fractions (C2D), it is clear that the carboxyl reduction had occurred, as shown by the deuterium labelling of the product. From the labelling patterns of the reduced galacturonic acid residues in C2B, C2C, C2D, and N1, it was inferred that some of the galacturonic acid residues were 3-substituted. Aspinall[22] reported that some pectins carry terminal xylopyranose residues on C-3. Furthermore, assuming that C1A and N2 were not undermethylated, the results suggest that some of the galacturonic acid residues in these pectic polysaccharides were 3- and 2-substituted. It is possible, of course, that these branch points on the galacturonan backbone carry oligosaccharide substituents. This would mean that positions 4 of the (1→2)-linked rhamnose residues are not the only points of attachment of the neutral side chains in rhamnogalacturonans.

The results of methylation analysis of the pectic fractions indicated the following. (*a*) The presence of slightly and highly branched pectic polysaccharides. The Na_2CO_3 (20°)-soluble pectic polysaccharides had more branched rhamnogalacturonan backbones compared with those of the CDTA- and Na_2CO_3 (1°)-soluble pectic polysaccharides, an inference based on the ratio of (1→2,4)- and (1→2)-linked rhamnose residues and branched galacturonic acid residues. From the ratio

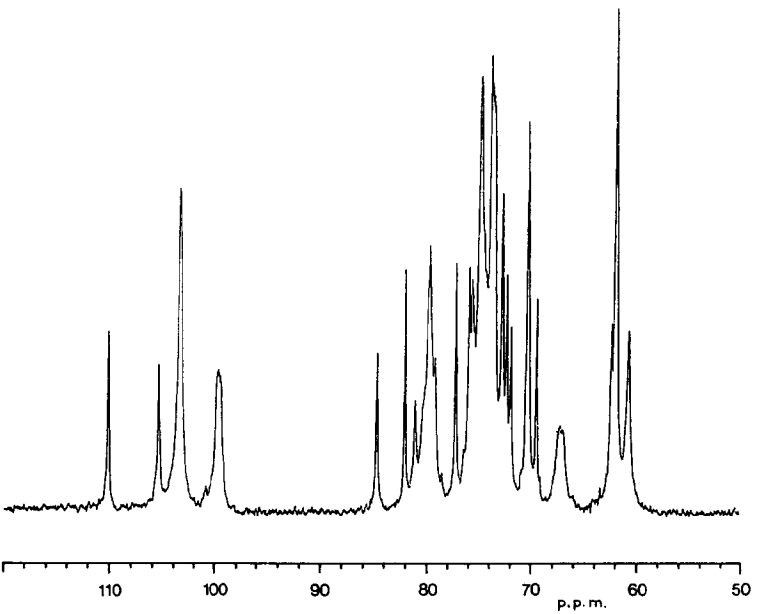

Fig. 1. The ^{13}C-n.m.r. spectrum of potato xyloglucan K2A.

of rhamnose to uronic acid, and by analogy with earlier work on onion CWM[6], it was inferred that the bulk of the pectic polysaccharides solubilized by KOH were also highly branched. The highly branched pectic polysaccharides probably originate from the primary wall. (*b*) The slightly branched pectic polysaccharides (C1A, C1B, C2C) had short oligosaccharide chains containing (1→5)-linked Ara*f* and (1→4)-linked Gal*p* residues. The highly branched pectic polysaccharides solubilised by Na_2CO_3 at 20° had much longer arabinan and galactan side-chains. (*c*) There was a small proportion of galacturonic acid in C1A and C2A. The pectic fractions C1A and C1B, particularly the latter, also contained (1→4)-linked Xyl*p*, terminal Xyl*p*, and (1→4)-linked Glc*p* residues. The presence of (1→4)-linked Xyl*p* in C1B is of particular interest, and a comparable fraction has been isolated from the CDTA extract of separated, intact potato cells[20]. Lignification is known to start from the middle lamellae region and involves the deposition of phenolics with xylan[10]. Although this tissue does not lignify, the carbohydrate necessary for lignification is present.

Methylation analysis of KOH-soluble neutral fractions. — The neutral fractions were methylated easily and there was a good correspondence between terminal and branched residues (see Table VI). Two main types of xyloglucan were detected. The xyloglucans solubilised by 0.5M and M KOH were very similar, were more branched than those solubilised by 4M KOH and 4M KOH + borate, and were probably less strongly associated with cellulose. These xyloglucans are comparable with the potato xyloglucans previously reported[4]. The less-branched xyloglucan required stronger alkali (4M KOH) for solubilisation and, presumably, was more strongly bound to cellulose microfibrils. Of particular interest is the absence of fucose in the xyloglucan. Fucose has been detected as a terminal residue in the xyloglucans of pea stems[23], sycamore callus[24], onion[6], and the parenchyma of runner bean pods[25]. The presence of significant amounts of (1→4)- and (1→4,6)-linked Man*p* proportions in K4A and K5A suggests the presence of mannans, possibly xyloglucomannans.

K5A, in addition to xyloglucan and mannan components, also contained significant proportions of 2- and 3-linked arabinose, the diagnostic linkages of hydroxyproline arabinosides. The molar proportions of Hyp and 3-linked arabinose were equal (0.25 μmol/mg), suggesting that all the Hyp residues may be substituted. The ratio of 2- to 3-linked arabinose in K5A was ~2:1, which implies a tetrasaccharide structure as in potato lectin[17] (terminal:3-linked:2-linked arabinose, 1:1:2). This situation is the most common form among dicotyledons, although mono-, di-, tri-[26], and penta-arabinosides[27] are also found. A proportion (4.7 mol%) of the terminal arabinose residues in K5A were, presumably, part of the xyloglucan component, which is similar in composition to that of K4A except that there is a lower proportion of branched glucose. The existence of cross-links between the polysaccharide and glycoprotein components was not established.

^{13}C-N.m.r. spectroscopy. — The chemical shifts of the resonances for anomeric carbons for K2A (see Fig. 1) confirmed the expected configurations,

namely, α-L-Ara*f* (110.1 p.p.m.), β-D-Gal*p* (105.3), α-D-Xyl*p* (99.6), and β-D-Glc*p* (103.3), and the ratios of their relative intensities were 1.00:0.97:2.57:4.50. Glucose was underestimated by 20%; being in the main chain, it is less mobile than the side chains and so experiences[28] a lower n.O.e. Peaks were assigned from published spectra[29] of xyloglucans and by comparisons with the spectra of a simpler xyloglucan from tamarind and other potato xyloglucans with different proportions of arabinose and galactose. The proportion of the glucose that is branched was estimated to be 40% by comparing the intensities of the C-6 resonances of the (1→4,6)-linked glucose (67.2 p.p.m.) with that of C-1 of total glucose (103.3 p.p.m.). This result is close to that (42%) obtained by methylation analysis. For xylose, the peaks at 71.9 and 81.0 p.p.m. are due to C-2 of unsubstituted and (1→2)-linked residues, respectively. The peak at 80 p.p.m., which was inadequately resolved, was due also to C-2 of (1→2)-linked xylose. The assignment of the signals at 80 and 81 p.p.m. to substitution by arabinose or galactose awaits more detailed analysis.

Methylation analysis of the KOH-soluble acidic fractions and residue-2. — The acidic fractions, K4B and K5C, from the KOH-soluble polymers were methylated and carboxyl-reduced, as was the residue-2. The recoveries of galactose were poor, and, presumably, the pectic galactan was degraded during methylation and lost on dialysis (see Table VI).

K4B is a highly branched pectic polysaccharide with a large proportion of (1→5)-linked arabinose. It also contains (1→4)-linked xylose residues. K5C contains pectic, xylan, and xyloglucan components. The pectic polysaccharide exhibits a diversity of rhamnose and galacturonic acid linkages, resembling the low-molecular-weight pectic fraction released by exhaustive treatment of potato cell walls with polygalacturonase[30]. A small proportion of 1,2,5,6-tetra-*O*-acetyl-3,4-di-*O*-methylglucitol-*6,6-d*$_2$ was detected (Table VI, column 7), which was derived from glucuronic acid and was probably part of an acidic polysaccharide. The retention on an anion-exchange column of the xylan and xyloglucan components with pectic polysaccharides suggests that K4B and K5C may be pectic–xylan and pectic–xylan–xyloglucan complexes, respectively, although a more thorough fractionation would be necessary for confirmation of this[15,31]; K4B and K5C each comprise 0.08% of the CWM. Such complexes have been isolated in larger amounts from runner bean CWM[15]. It is likely that many of the other KOH-soluble acidic fractions, which have not been analysed, are also pectic–hemicellulose complexes.

Residue-2 contained 53% of cellulose and was otherwise mainly a highly branched pectic polysaccharide with mostly unbranched galactose and arabinose chains. It is unlikely that any non-covalently bound polysaccharides would have remained with the α-cellulose residue, so that the pectic material must be covalently bound, if not to the cellulose then to other components such as phenolic material and proteins, so as to be enmeshed with the cellulose fibres.

EXPERIMENTAL

General methods. — Neutral sugars were released by Saeman hydrolysis and analysed[32] as their alditol acetates by g.l.c. Uronic acids were determined colorimetrically by a modification[32,33] of the method of Blumenkrantz and Asboe-Hansen[34]. Amino acids were released by hydrolysis with 6M HCl at 110° for 24 h and analysed by g.l.c. of their *N*-heptafluorobutyryl propyl ester derivatives[35]. The measurement of the degree of esterification was based on the method of Bociek and Welti[36]. Pectins were dissolved (20 mg/mL) in 84mM sodium hydrogenphosphate buffer (pH 8) in D_2O. An aliquot (15 μL) of the solution was dried (P_2O_5, 40°) to a film on a zinc cyanide plate. The absorbances at 1607 (carboxylate) and at 1743 cm^{-1} (ester) were compared, using an F.t.-i.r. Digilab FTS60.

Preparation of CWM. — Mature tubers (*Solanum tuberosum* var. Désirée) (180–350 g) were peeled and the stem and bud ends were discarded, leaving ~75% of the length of the tuber. Transverse 4-mm slices were placed in 5mM $Na_2S_2O_5$. The parenchyma was excised from each slice with a cork borer (7-mm diameter), and the tissue was blotted dry and frozen in liquid nitrogen. The parenchyma pieces (350 g) were homogenised with aq. 1.5% SDS (700 mL) containing 5mM $Na_2S_2O_5$ for 2 min. The homogenate was filtered through 2 layers of muslin and a nylon cloth (Postlip paper 150). The residue was washed (2 × 90 mL) with aq. 0.5% SDS containing 3mM $Na_2S_2O_5$, suspended in 700 mL of the same solution and ball-milled (Pascall 1-L pots for 15 h at 60 r.p.m. and 1°), and then centrifuged. The residue was washed twice with distilled water and centrifuged. The residue was suspended in aq. 90% Me_2SO (~1500 mL), sonicated for 20 min at 20°, stirred for 16 h at 20°, centrifuged, resuspended in aq. 90% Me_2SO (525 mL), and sonicated for 1 h at 20°. The residue was collected, washed with distilled water (6 × 500 mL), dispersed in distilled water (200 mL), exhaustively dialysed at 1°, and stored as a frozen suspension in water at −20°. Four batches of CWM were prepared using a total of 1400 g fresh weight of tissue; 10.06 g of CWM was obtained (0.072 g/100 g of fresh tissue).

Sequential extraction of CWM. — M CDTA (pH 6.8, 56 mL) was added to a suspension of CWM (9.9 g in 1066 mL) to a final concentration of 50mM. The mixture was stirred for 6 h at 20°, mixed with water (500 mL), and centrifuged, and the sediment was re-extracted with 50mM CDTA (pH 6.8, 500 mL) for 2 h. The extracts with their respective washings (CDTA-1 and CDTA-2) were filtered and dialysed. A suspension of the residue in 50mM Na_2CO_3 (890 mL) containing 20mM $NaBH_4$ at 1° was stirred for 20 h. The extraction was repeated for 2 h at 20°. The residue was extracted under argon for 2 h with successive amounts (660 mL) of 0.5M and M KOH at 1°, M and 4M KOH at 20°, and 4M KOH + 4% H_3BO_3 at 20°. Each solution also contained 20mM $NaBH_4$. The extracts were acidified to pH 5 with acetic acid (no precipitates formed) and dialysed, concentrated, and freeze-dried. The α-cellulose residue was acidified to pH 5 and frozen. Samples of the residue taken during extraction were dialysed and freeze-dried.

Ion-exchange chromatography. — The polymers (200, 50, 200, and 200 mg, respectively) extracted with CDTA-1, CDTA-2, Na_2CO_3 (1°), and Na_2CO_3 (20°) were each suspended in distilled water at 1° overnight, stirred for 12 h at 20°, and centrifuged, and each precipitate was frozen. Phosphate buffer (pH 6.5) was added to each supernatant solution to 50mM phosphate and 1 mg/mL of pectin. Each solution was passed through a column (1 × 15 cm) of DEAE-Trisacryl M (phosphate form) in batches of 50 mL. The fractions were eluted sequentially with 100 mL of 50mM buffer and 100 mL of 50mM buffer containing 0.125, 0.25, 0.5, and 1.0M NaCl. Fractions (6 mL) were collected and portions (0.05 mL) were assayed for carbohydrate by the phenol–sulphuric acid method[37].

Samples of the KOH extracts (50, 200, 25, 200 and 50 mg, respectively) were dissolved in 50mM phosphate buffer, and the solutions were centrifuged and fractionated as for the pectic extracts.

A sample (40 mg) of the 4M KOH-neutral fraction in 30mM sodium borate (pH 8.0, 15 mL) was eluted from a column (27 × 1 cm) of DEAE-Trisacryl M (borate form) with 30mM sodium borate (40 mL), then with a linear gradient of sodium borate (30mM→1.2M, 112 mL)[12]. Fractions (8 mL) were collected and analysed.

Methylation analysis. — The CDTA-extracted fractions were first de-esterified in 50mM Na_2CO_3 for 16 h at 1°, neutralised, dialysed, and freeze-dried. Polysaccharides were methylated by a modified[3] Hakomori method, using sodium methylsulphinylmethanide, 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. (OV-225 column).

Carboxyl reduction with $LiAl^2H_4$. — Methylated material (5 mg) was reduced[38] in a boiling solution of $LiAl^2H_4$ (40 mg) in 1:4 dichloromethane–ether for 4 h. The excess of reagent was then destroyed with ethyl acetate, water (10 mL) was added, then M phosphoric acid to pH 6. The reduced polymer was collected and was washed thoroughly with $CHCl_3$–MeOH.

^{13}C-N.m.r. spectroscopy. — Spectra (100.4 MHz) were recorded under conditions of broad-band proton decoupling with a JEOL GX 400 spectrometer on solution in D_2O (13 mg/mL) in 10-mm tubes at 55°. Spectra were obtained using 90° pulses with a pulse repetition time of 0.76 s, and 68 000 transients were acquired. Acetone was the internal reference, and the chemical shift of the resonances of the acetone methyl group was taken to be 31.07 p.p.m. with respect to that of Me_4Si.

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