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PECTIC POLYSACCHARIDES OF CABBAGE (*BRASSICA OLERACEA*)

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Key Word Index—*Brassica oleracea*, Cruciferae, cabbage, cell wall material, pectic substances, polysaccharides, arabinan, oligosaccharides, methylation analysis

Abstract—Pectic substances extracted from cabbage cell walls with water, at 80°, and $(NH_4)_2C_2O_4$, at 80°, accounted for 45%(w/w) of the purified cell wall material Only a small amount of neutral arabinan was isolated Partial acid hydrolysis and methylation analysis revealed that the major pectic polysaccharide had a rhamnogalacturonan backbone to which a highly branched arabinan was linked, at C-4 of the rhamnose units, mainly through short chains of (1 → 4)-linked galactopyranose residues The bulk of the soluble pectic substances had only small amounts of proteins associated with them After further extraction of the depectinated material with 1 M and 4 M KOH, to remove the hemicelluloses, the cellulose residue was found to contain a pectic polysaccharide which was solubilized by treatment with cellulase The general structural features of the pectic polymers are discussed in the light of these results

INTRODUCTION

The cell walls of parenchymatous tissues of dicotyledonous plants contain a range of 'neutral' and acidic pectic polysaccharides [1] Earlier work on the cell wall material of cabbage [2], used in clinical trials [3], led us to suspect that the 'neutral' pectic arabinan may be a breakdown product Further, the fractionation studies suggested that an appreciable proportion of the pectic substances were modified Since very little definitive information is available on the composition, structural features and mode of occurrence of pectic substances and hemicelluloses in cabbage, some of these aspects were investigated using cell walls isolated from fresh cabbage This paper reports studies on the pectic substances, and in the following paper the structural features of the hemicellulosic polymers are reported

RESULTS AND DISCUSSION

Isolation of cell wall material (CWM)

The CWM was prepared by blending and then ball-milling the wet tissue in aqueous 1% sodium deoxycholate (SDC) followed by extraction with phenol–acetic acid–water (2 1 1 w/v/v) These solvents effectively removed cytoplasmic compounds with minimal co-precipitation with the CWM [4] No starch removal stage was included because the starch content of the tissues was very low Three hundred grams of fresh tissue yielded 4 9 g (dry) of CWM As reported previously [5], about 10% of non-dialysable polymeric material was solubilized by the SDC (Table 1) The precipitate obtained on dialysis of the SDC soluble material contained 68% protein (Table 2), mainly of intracellular origin Low MW cytoplasmic compounds accounted for most of the SDC and ethanol soluble material though some non-dialysable uronic acid containing polymers were present (Table 1) The ethanol-insoluble portion contained mainly arabinose, galactose and protein (Tables 1 and 2) and was probably an intracellular proteoglycan The sugar composition of the purified CWM (Table 1) was similar to that from cabbage AIR [2], with a relatively large content of arabinose and uronic acid indicating the presence of appreciable amounts of pectic polysaccharides rich in arabinose The protein content was 2%, with a relatively high amount of hydroxyproline In this respect cabbage cell wall proteins are similar to those of most dicotyledonous plants [6, 7]

Hot water soluble polymers (WSP)

The CWM was extracted with hot water at pH 5 0 to yield a fraction (23% of the CWM) rich in uronic acid and arabinose(Table 3), with a degree of esterification (DE) of 25% and an acetyl content of 31 μg/mg The protein content of this fraction was low (Table 2) A neutral arabinan has been isolated from cabbage AIR [8] but this could have been released during the preparation of the AIR An attempt was therefore made to isolate the arabinan from fresh tissue used in this present study Chromatography of WSP on DEAE Sephadex yielded a slightly acidic fraction (A, Table 3), and a more acidic fraction (B, Table 3) Attempts to re-fractionate A (19 mg) on the same ion exchanger yielded only 3 mg of an unbound arabinose rich fraction C (Table 3) Further elution with sodium chloride failed to produce further fractions but when the total eluate was concentrated, dialysed and freeze dried 9 mg of carbohydrate containing material was obtained (D, Table 3) OR measurements on fraction C before freeze drying gave $[\alpha]_D^{21} - 150°$ (H_2O, *c* 0 293), showing that the bulk of the Ara*f* glycosidic linkages are in the α-anomeric configuration Methylation analysis revealed that arabinose accounted for 86 mol% of the partially methylated alditol acetates (PMAA), with the same types of glycosidic linkages as in the arabinan

Table 1 Sugar composition of material solubilized during stages of purification of cell wall material from cabbage

Stages of purification	Proportion solubilised (% dry wt)	Sugar composition (μg/mg dry wt)*							
		Deoxy hexose	Ara	Xyl	Man	Gal	Glc	Uronic acid	Total sugars
Ppt from SDC soluble material†	24	7	54	6	9	15	33	33	157
Supernatant from dialysed SDC soluble									
EtOH soluble	41	1	18	1	4	3	20	160	207
EtOH insoluble	25	19	147	21	9	149	28	16	389
Cell wall material	204	34	139	42	7	81	23(355)	279	605

*Sugars released on 1 M H_2SO_4 hydrolysis for 2 5 hr at 100°, values in parentheses are from Saeman hydrolysis
†Precipitate obtained on dialysis of material soluble in 1% Na deoxycholate

Table 2 Amino acid composition of cell wall material of cabbage and of material solubilized during purification and on subsequent sequential extraction with aqueous inorganic solvents and on further fractionation of these by ion exchange chromatography

Amino acid	Amino acid composition (μg/mg)								
	Ppt from SDC soluble	SDC soluble EtOH insoluble	Purified CWM	Soluble after cellulase treatment of CWM	Hot water soluble	Oxalate soluble	Oxalate soluble fractions		
							Insoluble residue	OA1	OA2
Ala	38 8	6 6	0 8	5 4	1 0	1 2	0 7	1 7	0 3
Gly	35 9	7 4	1 1	4 9	0 9	1 2	2 2	1 7	0 5
Val	42 6	6.2	0 9	1 6	1 1	1 5	1 8	1 3	0 4
Thr	32 5	6 0	0 8	4 0	0 9	1 2	5 4	1 3	0 4
Ser	31 2	7 0	1 2	5 7	1 6	1 8	5 6	2 5	0 8
Leu	54 8	7 9	1 2	3 9	1 5	1 6	5 9	1 9	0 6
Ile	29 5	3 8	0 7	1 0	0 9	1 0	1 9	6 8	0 1
Pro	28 7	6 1	0 9	3 8	1 0	1 2	7 2	1 5	0 4
Hyp	11 4	3 4	1 4	0 2	1 1	2 3	12 7	3 4	1 1
Asp	64 4	15 3	1 7	7 8	2 0	2 4	12 2	3 0	0 9
Phe	37 2	5 8	1 0	2 9	1 0	1 1	6 4	1 4	0 5
Glu	72 7	20 0	2 1	7 3	2 7	2 7	16 0	4 3	1 7
Lys	59 5	8 3	2 2	2 0	2 4	3 2	15 7	2 8	1 2
Tyr	25 0	3 1	0 9	2 3	0 9	1 4	2 5	1 3	0 1
Arg	49 5	4 2	0 9	n d	2 6	2 6	3 6	0 6	0 8
His	14 9	1 4	0 7	2 2	0 8	1 0	4 9	0 5	0 4
Total	628 6	112 5	18 5	55 0	22 4	27 4	104 7	30 0	10 2

from the AIR but in slightly different proportions (Table 4)

A further attempt was made to separate the arabinan from both unsaponified and saponified WSP by precipitation with cupric acetate [9] On addition of cupric acetate (7% w/v) to unsaponified WSP a precipitate (1, Table 3) was obtained and from the supernatant, further precipitates were obtained, on the addition of ethanol and then acetone (2, Table 3), and on addition of cupric acetate to de-esterified WSP (3, Table 3) Methylation analysis of precipitate 2 revealed that arabinose residues accounted for 69 mol % of the PMAA, with glycosidic linkages in the same proportion as in c (Table 4) A small, but significant, amount of (1 → 2,4)-linked xylose was also present All the precipitates from the cupric acetate treatment contained substantial amounts of uronic acid, deoxyhexose and galactose in addition to arabinose From the inability to isolate a neutral arabinan by Cu precipitation and only a trace (0 1% of the CWM) by ion-exchange chromatography it appears that cabbage arabinan, like that of beet [10], is present as part of the pectic complex The small amount of neutral arabinan might be a degradation product and is probably not native to the cell wall It is probable that most of the arabinans isolated from other plant sources are also artefacts of extraction conditions

Hot ammonium oxalate soluble polymers (OSP)

Additional pectic substances amounting to 22% of the CWM were extracted with hot ammonium oxalate, from the residue after hot water extraction The sugar composition and DE are given in Table 5 and the amino acid

Table 3 Sugar compositions of fractions obtained from the hot water soluble polymers of cabbage by ion exchange chromatography and by precipitation with CuOAc

Fraction	Sugar composition (μg/mg)						
	Deoxy hexose	Ara	Xyl	Man	Gal	Glc	Uronic acid
Hot H_2O soluble polymers	39	220	11	2	100	16	468
DEAE Sephadex fraction A	33	266	13	2	116	21	215
" " B	10	72	5	4	32	5	564
" " C	11	839	6	7	17	50	50
" " D	25	162	11	1	74	26	194
CuOAc ppt 1	36	186	9	—	94	12	535
" " 2	30	439	9	13	71	53	278
" " 3	36	224	11	—	39	16	388

Table 4 Proportions of arabinose and branched xylose residues present in arabinose-rich pectic polymers from alcohol-insoluble residue of cabbage and in hot water- and oxalate-soluble fractions of cell wall material of fresh cabbage

Partially methylated alditol acetate	From alcohol insoluble residue	Hot water soluble		Hot oxalate soluble	
		Fraction C (Table 3)	CuOAc ppt (2) (Table 3)	As extracted (Table 7)	Fraction OA2 (Table 7)
2,3,5-Me_3Ara*	1 00	1 00	1 00	1 00	1 00
2,3-Me_2Ara	0 68	1 05	1 07	0 98	1 15
2-MeAra	0 31	0 19	0 19	0 15	0 27
Arabinitol	0 37	0 36	0 35	0 32	0 44
3-MeXyl	0 06	0 13	0 19	0 14	0 16

*2,3,5-Me_3Ara = 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methyl arabinitol etc

Table 5 Sugar composition of fractions obtained from the hot oxalate-soluble polymers of cabbage

Fraction	Sugar composition (μg/mg)							Degree of esterification* %
	Deoxy hexose	Ara	Xyl	Man	Gal	Glc	Uronic acid	
Hot oxalate soluble polymers	43	179	8	3	55	12	672	14 5
DEAE Sephacel fractions								
OA1	45	225	8	3	51	7	452	27
OA2	22	111	7	10	26	9	622	25
Insoluble residue	26	146	8	2	40	47	329	

*Mol MeOH/100 mol galacturonate, galacturonate estimated colorimetrically

composition in Table 2 Chromatography on DEAE Sephacel yielded a fraction, OA1, not retained on the column and a more acidic fraction, OA2, which was eluted with a gradient up to 0 5 M sodium chloride Prior to chromatography an insoluble residue was removed which on analysis (Table 5) was found to be similar in sugar composition to OA1 and OA2 (Table 5) though with more glucose, but contained 10% protein compared with 3% and 1% for OA1 and OA2 respectively (Table 2)

Structure of the pectic polysaccharides

(a) *Aldobiouronic acid*—The presence of the very stable aldobiouronic acid Gal*p*A(1 → 2)-Rha*p* has been shown for pectins from relatively few tissues such as soya beans [12, 13], rapeseed hulls [14], lemon peel [9] and sycamore (suspension-cultured cells) [15] The presence of this structure in pectic substances from cabbage cell walls was shown by the following experiments

The WSP acidic fraction B (Table 3) was subjected to partial acid hydrolysis and the acidic oligosaccharides produced were isolated by passage through anion exchange resin (see Experimental) The eluate containing the acidic oligosaccharides was divided into three portions On analysis the first portion was found to contain rhamnose and uronic acid in the molar ratio of 1 4, and very small amounts of arabinose, xylose and galactose The second portion was treated with methanolic hydrochloric acid to form the methyl ester methyl glycosides which were then treated with lithium aluminium [^{2}H]hydride to reduce the carboxyl groups After complete hydrolysis, reduction and acetylation, analysis showed the presence of rhamnose and galactose in the ratio of 1 4 4, with very small amounts of arabinose and xylose GC/MS revealed the incorporation of two ^{2}H atoms at C-6 in the 2,3-di-*O*-methyl galactitol derivative which showed that this was derived from galacturonic acid

The third portion was reduced with sodium boro[^{2}H]hydride and then methylated with [^{2}H$_3$]methyl iodide The latter would reveal the existence of methoxyl groups in C-2 or C-3 of the uronic acid moiety [16] GC of the methylated product on OV-1 gave two main peaks The first (RR_t 0 39, relative to methylated cellobiitol) could not be positively identified by MS but was probably derived from galacturonic acid The second peak (RR_t 0 98, relative to methylated cellobiitol) eluted in the methylated disaccharide alditol region and was identified, (a) by EIMS, based on the presence of diagnostic fragment ions and (b) from the MW deduced from CIMS using the ions $[M+NH_4]^+$ and $[M+1]^+$ The fragmentation pattern was deduced from established principles [17–19] as applied to methylated oligosaccharide alditols derived from plant cell wall polysaccharides [16] The nomenclature for the degradation of the methylated oligosaccharide alditol and the symbols employed correspond to those of Kochetkov and Chizhov [20]

The origins of some pertinent ions obtained by EIMS are shown in Fig 1 CIMS gave ions at *m/z* 497 and 480 corresponding to $[M+NH_4]^+$ and $[M+1]^+$ suggesting a parent disaccharide containing one hexuronic acid and one deoxyhexose unit EIMS gave intense ions at *m/z* 245 and 218 consistent with a hexuronosyl–deoxyhexosyl derivative The nature of the uronic acid can be deduced from the relative intensities of the aA series of ions obtained by EIMS The abundance ratio aA_2/aA_1 = 1 49, suggests that the uronic acid is galacturonic acid, for glucuronic acid the ratio is > 4 [16, 19] The absence of ions at *m/z* 242, 239, 207 and 204 clearly showed that the galacturonic acid does not carry methoxyl groups at C-2 and/or C-3 This inference was confirmed by the relatively intense ion at *m/z* 107 The ions at *m/z* 182 and 147 could only arise from a uronic acid containing derivative The nature of the linkage between galacturonic acid and rhamnose was deduced from the relatively intense ions at *m/z* 156 and 121, which are diagnostic of a methylated rhamnitol substituted at position 2 and hence a (1 → 2)-linkage Methylation analysis of the parent pectin showed that the rhamnose residues were linked through C-2 Thus the parent compound of peak 2 is most probably Gal*p*A-(1 → 2)-Rha*p*

More information on the structure of the pectic polysaccharides and further evidence for the existence of the Gal*p*A-(1 → 2)-Rha*p* linkage was obtained from the oxalate-soluble fraction This was methylated and then divided into three portions One was hydrolysed, converted into partially methylated alditol acetates (PMAA) and examined by GC/MS, another portion was reduced with lithium aluminium [^{2}H]hydride and the third was esterified with diazomethane and then reduced with lithium borohydride After reduction both portions were

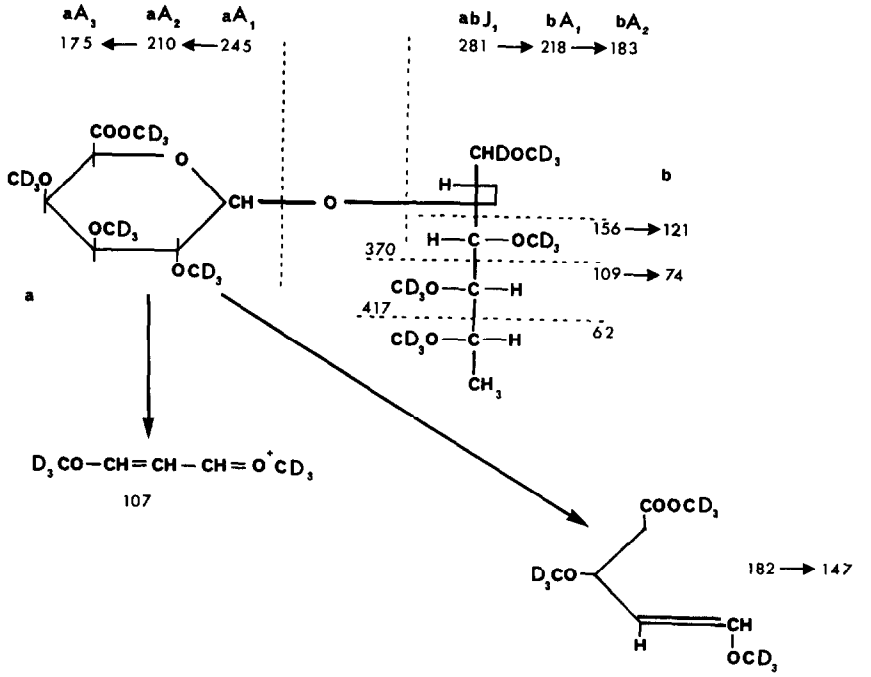

Fig 1 MS fragmentation pattern of methylated aldobiouronic acid, from cabbage pectin, after reduction with $NaBD_4$

hydrolysed, converted to PMAA and examined by GC/MS (Table 6) From the results it can be inferred that, although both methods of reduction of the galacturonic acid residues were effective, there were large apparent losses of galacturonic acid as indicated by the low yield of the 2,3-di-*O*-methyl galactitol derivative Degradation, by β-elimination, of the esterified galacturonic acid residues during initial methylation could have resulted in the production of methylated oligosaccharide fragments containing mainly Gal*p*A which would have been lost during dialysis after this stage The galactitol derivative formed by lithium aluminium [^{2}H]hydride reduction was deuterated at C-6 Because of these losses, to facilitate comparison, the values in parentheses in Table 6 are scaled to correspond with 64 mol % of uronic acid (assumed to be all galacturonic acid) determined colorimetrically on the unmethylated OSP On this basis there is better agreement, overall, with the sugar composition of the unmethylated OSP although some loss of arabinose has also occurred in the reduced samples The proportions of the arabinose residues were the same as for WSP fraction C (Table 3)

In both unreduced and reduced samples (Table 6) there is reasonably good agreement between end groups (T-Ara*f*, T-Gal*p* and T-Xyl*p*) and branch points [(1 → 2,3,5)-linked Ara*f*, (1 → 3,5)-linked Ara*f*, (1 → 2,4)-linked Rha*p* and (1 → 2,4)-linked Xyl*p*] The nature of the small amounts of mannose and glucose residues, indicated by direct analyses (Table 5), could not be positively identified

Fraction OA2 was methylated, esterified with diazomethane, reduced with lithium borohydride partially hydrolysed, reduced with sodium boro[^{2}H]hydride and remethylated with [$^2H_3$]methyl iodide to label the points of attachment of acid labile groups The remethylated material was completely hydrolysed and converted to PMAA The results are given in Table 6, together with those for PMAA from a portion of OA2 after initial methylation

Incorporation of [$^2H_3$]methyl groups into partially hydrolysed and remethylated material

The residues containing [$^2H_3$]methyl groups after methylation are indicated in Table 6 and the deduced points of attachment of the [$^2H_3$]methyl groups are given in Table 7 Removal of acid labile substituents on C-4 of the rhamnose by partial hydrolysis, and replacement with [$^2H_3$]methyl groups after remethylation would have been

Table 6 Partially methylated alditol acetates from polysaccharides present in the oxalate-soluble fraction of cabbage cell wall material

		Relative mol %							
		Oxalate-soluble						DEAE Sephacel acidic fraction OA2	
Alditol acetate	RR_t*	Untreated		Reduced with $LiAlD_4$		Esterified reduced with $LiBH_4$		Untreated	Partially hydrolysed etc †
3,4-Me_2Rha‡	0 87	11 4	(4 1)§	10 7	(5 3)	9 8	(4 4)	7 9	6 0^D
3-MeRha	1 67	3 2	(1 1)	2 4	(1 2)	4 0	(1 8)	2 6	3 3
1,2,3,4-Me_4Ara‖	0 22	—	—	—	—	—	—	—	3 7^D
2,3,5-Me_3Ara	0 41	24 6	(8 8)	12 4	(6 0)	13 6	(6 1)	21 9	27 0^D
2,3-Me_2Ara	1 07	24 2	(8 7)	11 9	(5 9)	14 6	(6 5)	25 3	7 5^D
3,5-Me_2Ara	0 80	1 2	(0 4)	—	—	—	—	0 7	—
2-MeAra	1 93	3 8	(1 4)	3 0	(1 5)	3 4	(1 5)	5 9	—
Arabinitol	2 66	7 9	(2 8)	5 2	(2 6)	5 3	(2 4)	9 6	2 4
2,3,4-Me_3Xyl	0 54	2 4	(0 9)	3 0	(1 5)	2 3	(1 0)	1 9	4 8^D
3-MeXyl	2 15	3 5	(1 3)	2 0	(1 0)	2 8	(1 3)	3 6	—
1,2,3,5,6-Me_5Gal‖	0 44	—	—	—	—	—	—	—	4 5^D
2,3,4,6-Me_4Gal	1 19	3 6	(1 3)	5 4	(2 7)	5 0	(2 2)	2 8	12 6^D
2,3,4-Me_3Gal	2 89	—	—	tr	tr	0 5	(0 2)	—	3 3‖
2,3,6-Me_3Gal¶	2 22	13 1	(4 7)	8 7	(4 3)	11 2	(5 0)	11 6	21 9^D
2,3-Me_2Gal	4 7	—	(64 1)	27 4	(64 1)	19 7	(64 1)	1 5	—
3,6-Me_2Gal	3 2	—	—	—	—	0 7	(0 3)	—	—
2-MeHex		—	—	1 8	(0 9)	3 5	(1 6)	1 4	—
3-MeHex				1 6	(0 8)	1 4	(0 6)		
Hexitol	1 1	(0 4)				2 1	(1 0)	2 4	3 0

*Retention time relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl glucitol on OV-225 at 170°

†Esterified, reduced with $LiBH_4$, partially hydrolysed, reduced with $NaBD_4$, remethylated with CD_3I, hydrolysed and converted to PMAA

‡3,4-Me_2Rha = 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methyl rhamnitol etc

‖Not positively identified

§Values in parentheses are scaled to correspond with a galacturonic acid content of 64 1 mol %

¶May include some 2,3,6-Me_3Glc

^{D}, Deuterium ions present tr, Trace

Table 7 Position of tri-deutero methyl groups in partially hydrolysed* and remethylated fraction OA2

Alditol acetate	Point of attachment of CD_3 groups	Diagnostic fragment ions *m/z* (relative intensity)
3,4-Me_2Rha†	C-4	131(100), 134(68), 92(67), 190(33)
2,3,5-Me_3Ara	C-5	118(100), 129(75), 164(9)
	C-3 + C-5	105(29), 132(11), 165(7), 167(3)
	C-2 + C-3 + C-5	108(5), 121(18), 132(11), 167(3)
2,3-Me_2Ara	C-2	73(20), 105(24), 121 (16), 165(6)
	C-3	75(28), 105(24), 132(24), 165(6)
	C-2 + C-3	76(20), 108(68), 121(16), 168(5), 211(5)
2,3,4-Me_3Xyl	C-2 + C-4	104(14), 105(8), 121(8), 164(3)
1,2,3,5,6-Me_5Gal	C-1 + C-5	92, 93, 137, 148, 173, 208, 256
	C-1 + C-5 + C-6	93, 95, 137, 151, 176, 211, 256
2,3,4,6-Me_4Gal	—	101, 102, 118, 129, 145, 161, 162, 205
	C-4	104(21), 132(31), 148(24), 164(10), 208(13)
	C-6	104(21), 129(46), 148(24), 164(10), 208(13)
	C-4 + C-6	107(8), 132(31), 151(16), 167(8), 211(9)
2,3,6-Me_3Gal	C-6	176(5), 236(15)

*Treatment as in Table 6

†3,4-Me_2Rha = 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methyl rhamnitol etc

expected to increase the yield of the 3,4-di-*O*-methyl rhamnose derivative with a corresponding decrease in the 3-*O*-methyl derivative Although C-4 was labelled with [$^2H_3$]methyl (Table 6) the effect may be masked by the alteration in the proportions of the other PMAA Some of the 3-*O*-methyl derivative remains after remethylation showing that the substituents on C-4 of the rhamnose are not equally susceptible to mild hydrolysis

On GC (OV-225 column) the 2,3,5-tri-*O*-methyl arabinose derivative was only partially separated from a component which, from its fragmentation pattern (Table 7) and R_t (0 44) [21], was deduced to be the 1,2,3,5,6-penta-*O*-methyl galactose derivative This was probably derived from oligosaccharide reducing end group alditols formed on the second reduction Only about 20% of the 2,3,5-tri-*O*-methyl arabinose derivative was deuterated To deduce the positions of the [$^2H_3$]methyl groups, the fragmentation patterns reviewed by Lonngren and Svensson [22] were used

The structures of the labelled derivatives and the main distinguishing ions are shown in Fig 2, and the relative abundance of the diagnostic ions are given in Table 7 The results suggest that the bulk of the [$^2H_3$]methyl groups are carried by the derivative **a** (Fig 2) From these results it could be inferred that partial acid hydrolysis of the methylated product resulted in the cleavage of (1→5)-, (1 → 3,5)- and (1 → 2,3,5)-linked Ara*f* residues These deductions are corroborated by the results of methylation analysis From the relatively high value for T-Ara*f* residues, and the lack of branch points, it could be inferred that a proportion of the methylated Ara*f* residues are hydrolysed to methylated disaccharide fragments The reducing ends of these fragments, on further hydrolysis and derivatization would be converted to the highly volatile 1,2,3,4-tetra-*O*-methyl-5-*O*-acetyl arabinitol Small but significant amounts of this compound were detected (R_t 0 22), but the bulk of it would have been lost during the evaporation step

The yields of fragment ions from the 2,3-di-*O*-methyl arabinose derivative (Table 7) indicated deuteration at C-2, C-3 and C-2 + C-3 These derivatives would have come from (1 → 2,5)-linked, (1 → 3,5)-linked and (1 → 2,3,5)-linked arabinose, respectively The disappearance of the 2-*O*-methyl derivative after partial hydrolysis and the decrease in the yield of arabinitol penta-acetate are in agreement with the above results

The 2,3,4-tri-*O*-methyl xylose derivative was deuterated at C-2 and C-4 From the relative abundance of the diagnostic fragment ions (Table 7) the ratio of labelled to unlabelled derivative could be inferred to be *ca* 2 1 The complete disappearance of (1 → 2,4)-linked xylose residues in the remethylated product is in accordance with the above result which suggests that the residues linked to positions 2 and 4 of the xylose are readily hydrolysable

The yield of 2,3,4,6-tetra-*O*-methyl galactose derivative (**d**, Fig 2) increased after partial hydrolysis and remethylation This increase could be attributed to hydrolysis of (1 → 4)-linked Gal*p* and (1 → 4)-linked Gal*p*A residues From the fragment ions, the [$^2H_3$]methyl groups can be assigned to C-4 (**e**, Fig 2), C-6 (**e'**, Fig 2) and $C_4 + C_6$ (**f**, Fig 2) of the tetra-*O*-Me-galactose derivative(s) The derivative deuterated at C-4 could arise from (1 → 4)-linked Gal*p* residues which are fragmented at C-4 The derivative labelled at C-6 could arise from T-Gal*p*A, and the derivative labelled at C-4 and C-6 could arise from (methylated) Gl*p*A residues which are cleaved at C-4 during partial hydrolysis From the relative abundance of the ions at *m/z* 205, 208 and 211, as well the ions at *m/z* 145, 148 and 151, the approximate ratio of **d** **e** + **e'** **f** could be inferred to be 1 0 8 0 5 The 2,3,6-tri-*O*-methyl galactose derivative also increased in amount after partial hydrolysis and re-methylation and was found to be deuterated at C-6 which indicated that it originated from (1 → 4)-linked galacturonic acid since (1 → 4,6)-linked galactose residues were not detected in the parent compound

Pectic material associated with the α-cellulose fraction

The residue after extraction of the CWM with hot water and oxalate was further sequentially extracted with 1 M

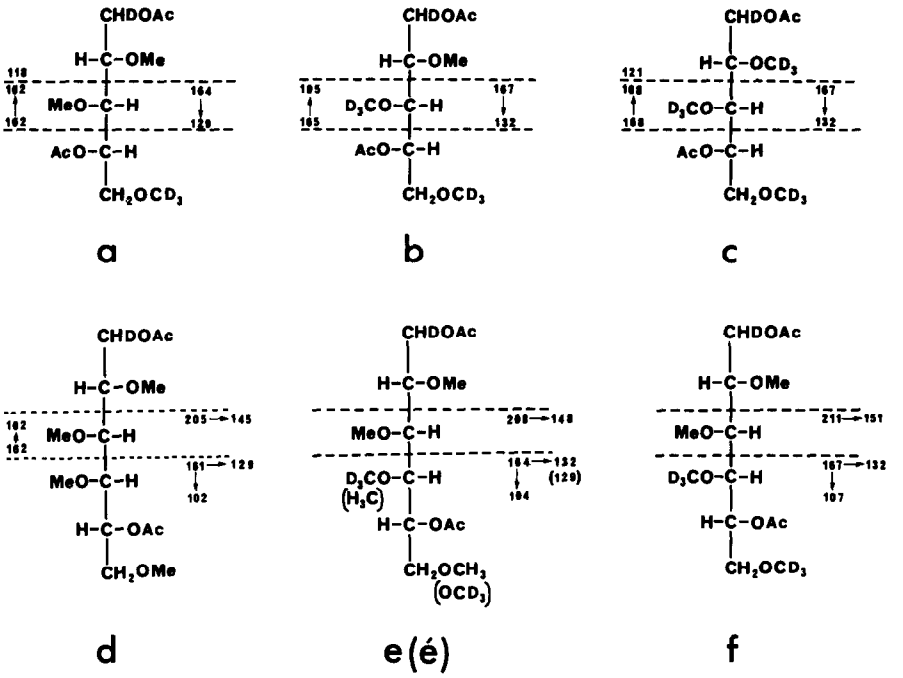

Fig 2 Structures and main distinguishing ions of labelled arabinose (**a**, **b**, **c**) and galactose (**d**, **e**, **f**) derivatives, **e'** corresponds to the derivative carrying $-CD_3$ at C-6, and the corresponding groups and ions are given in parentheses

and 4 M potassium hydroxide to leave a residue (mainly α-cellulose) with the following composition (μg/mg) deoxyhexose 11, Ara 46, Xyl 13, Man 13, Gal 32, Glc 665, uronic acid 113 The yields of these fractions have been reported previously [5] and the composition of the alkali soluble fractions is given in a subsequent paper [23] The α-cellulose residue still contained uronic acid, a feature also observed with CWM from cabbage AIR [2] and other tissues [24–27] After prolonged treatment with cellulase 5% of the original residue remained and 20% was recovered as a non-dialysable soluble fraction The sugar composition of the soluble fraction [(μg/mg) deoxyhexose 31, Ara 104, Xyl 14, Man 17, Gal 113, Glc 166, uronic acid 207] indicated that this contained a pectic substance The protein content of this fraction was 5 5% and it was rich in aspartate and glutamate but poor in hydroxyproline (Table 2) This is in contrast to the hydroxyproline rich protein associated with the α-cellulose fraction of mature runner bean parenchyma [26, 28] The relatively low recoveries of carbohydrate and protein, and the presence of an appreciable amount of glucose, in the soluble fraction, suggest that the pectic polymer is probably linked to the cellulose by phenolic cross linkages

The results of this study show that the cabbage pectin is mainly based on a rhamnogalacturonan backbone to which the bulk of the 'arabinan-rich complexes' are linked via (1 → 4)-linked Gal*p* residues to C-4 of the (1 → 2,4)-linked Rha*p* residues as shown in Fig 3 From the proportion of (1 → 4)-linked Gal*p* to T-Gal*p* residues it can be inferred that the maximum number of galactose residues in a side chain would be *ca* 5 These observations are in agreement with those of previous workers [1] The pectin complex shown in Fig 3 which is partially esterified, can undergo β-elimination during extraction with hot water [29] to give a fragment which is very rich in arabinose Polysaccharides rich in arabinose have been produced from apple pectic substances by a transelimi-

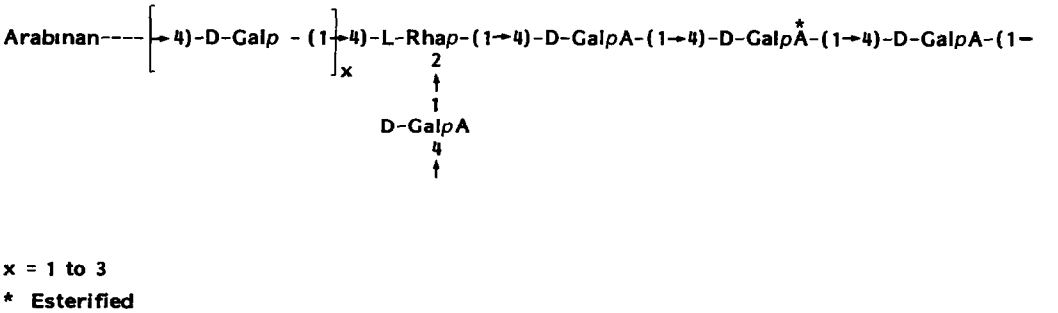

Fig 3 Structural features of the pectic complex from cabbage cell walls

nation reaction [30]

Most of the pectic substances have only small amounts of glycoproteins associated with them The insoluble pectic material from the oxalate-soluble fraction, which has an appreciable amount of protein associated with it, is comparable with the alkali-soluble polysaccharide–protein–polyphenol complexes These are discussed in a subsequent paper [23] Thus this study has not only thrown additional light on the nature of cabbage pectic substances, and the artefacts that could be produced during extraction, but has also given useful leads for studies on cell wall proteoglycan complexes

EXPERIMENTAL

Chemicals $LiAlD_4$, $LiBH_4$, $NaBD_4$ and CD_3I were purchased from Fluka, Switzerland, DMSO, tetrahydrofuran, NaH, $NaBH_4$ were obtained from B D H (Poole, Dorset, U K) DMSO was vacuum distilled over CaH_2 and stored over molecular sieve 3A Tetrahydrofuran was distilled over $LiAlH_4$ and stored under Ar All other chemicals were of the highest purity available DEAE-Sephadex and DEAE-Sephacel were purchased from Pharmacia (Uppsala, Sweden) Cellulase (EC 3 2 1 4) from *Trichoderma viride* CS12 was prepared by the method of Stevens and Payne [31] and partially purified by $(NH_4)_2SO_4$-pptn (30–80% w/v satn)

Plant material Cabbages (var Decema) were grown in experimental plots near the laboratory and harvested when 'mature', about 33 weeks after transplanting Only the immature inner leaves, about 60% of the weight of the head, were used

General methods of analysis Neutral sugars were released by 1 M H_2SO_4 or Saeman hydrolysis for 2 5 hr and estimated as their alditol acetates by GC [11] Uronic acids were estimated colorimetrically by (a) a modified carbazole method [11] and (b) by the method of Blumenkrantz and Asboe-Hansen [32] DE was calculated from the MeOH content, determined by the method of Wood and Siddiqui [33], as a molar proportion of the total uronic acid content estimated colorimetrically Acetyl was estimated by the method of McComb and McCready [32] Amino acids were estimated as their *n*-propyl heptafluorobutyryl derivatives [35] OR was measured using a Bendix Automatic Polarimeter type 143C CH_2N_2 in Et_2O was prepared by the method of Bjerke and Herman [36]

Preparation of CWM CWM was prepared by sequential extraction of the ballmilled fresh tissue with 1% aq Na deoxycholate and PhOH–HOAc–H_2O (2 1 1, w/v/v) as described previously [4]

Sequential extraction of CWM CWM was fractionated by sequential extraction with H_2O at 80°, $(NH_4)_2C_2O_4$, pH 5 0, at 80° then 1 M and 4 M KOH containing 10 mM $NaBH_4$, as described previously [27], to leave a residue of α-cellulose

Purification of WSP (1) Ion exchange—WSP (50 mg) was deesterified at pH 12 for 2 hr at 0° [32], adjusted to 10 mM Pi, pH 6 4, gently stirred for 2 hr with 14 ml of moist DEAE Sephadex A50 (Cl^- form) then packed into a 1 cm diam column, above a 2 cm bed of the same ion-exchanger Elution was with 10 mM K-Pi, pH 6 4, alone initially (20 ml) then with the addition of 0 9 M NaCl (120 ml) Fractions (2 ml) were collected and monitored by reaction with PhOH–H_2SO_4 [38] Appropriate fractions were pooled, dialysed and freeze dried A portion (19 mg) of the material (A) not bound to the column was applied to the same ion exchanger (1 × 6 5 cm column), and eluted with 10 mM Pi pH 6 4 (20 ml) then a linear gradient from 0–0 9 M NaCl (150 ml) containing 10 mM Pi, pH 6 4, followed by further elution with 0 9 M NaCl (80 ml) (2) Precipitation with Cu^{2+} ions—aq $Cu(OAc)_2$ (7% w/v, 5 8 ml) was added to a soln of WSP (171 mg in 34 ml H_2O) The ppt (**1**) which formed was removed by centrifugation, washed with $Cu(OAc)_2$, suspended in 0 5 N HCl (7 ml) and EtOH added to 80% The ppt was removed by centrifugation, dialysed against H_2O and freeze-dried to yield 151 mg EtOH was added to the $Cu(OAc)_2$ supernatant to 80% without pptn Further addition of Me_2CO (1 vol) gave a ppt (**2**) which was removed by centrifugation and washed with aq 80% EtOH containing HCl (5% v/v of conc HCl), then dialysed and freeze dried to yield 15 mg A further portion of WSP (50 mg), deesterified as above, then adjusted to pH 5 0 with HCl, was treated with $Cu(OAc)_2$ to yield a ppt, **3**, (27 mg) which was removed, washed etc as above No further ppt was obtained from the $Cu(OAc)_2$ supernatant

Purification of OSP OSP (214 mg) was stirred with H_2O (15 ml) in the cold, overnight An insoluble residue was removed by centrifugation and freeze-dried to yield 13 mg The supernatant was adjusted to 10 mM Pi, pH 6 4, and applied to a column (1 5 × 25 cm) of DEAE Sephacel (Cl^- form) Elution was with 10 mM KPi, pH 6 4, 120 ml initially, then with this buffer in a linear gradient of NaCl (0–1 M, 200 ml) Fractions (3 ml) were collected, monitored by reaction with PhOH–H_2SO_4 and appropriate fractions combined, dialysed and freeze-dried to yield OA1 (55 mg) and OA2 (77 mg)

Partial acid hydrolysis WSP acidic fraction B was partially hydrolysed by heating with 0 2 N TFA for 2 hr at 120° in a sealed tube TFA was removed by co-distillation with H_2O, under vacuum

Isolation of acidic oligosaccharides and preparation of methyl ester methyl glycoside The isolation of the acidic oligosaccharides by elution from anion exchange resin and the preparation of methyl ester, methyl glycosides were carried out as described in ref [37]

GC/MS GC on OV-225 and ECNSS-M and GC/EIMS analyses of PMAA were carried out as described in ref [39] Methylated oligosaccharides were separated by GC on a 2 8 m × 2 2 mm column of J J's diatomite CQ coated with 4% OV-I, which after 5 min at 190° was temp programmed at 1°/min GC/CIMS, using NH_3 as the reagent gas [40], was performed on an AEI MS30 mass spectrometer

Methylation analysis OSP (20 mg) was methylated as described previously [27], dissolved in $CHCl_3$–MeOH (1 1) and a portion equivalent to 5 mg OSP was converted to PMAA [39] and subjected to GC/MS A similar portion was reduced with $LiAlD_4$ [41] and converted to PMAA after further reduction with $NaBH_4$ [41] The remainder of the methylated OSP was cooled in ice, 2 ml of CH_2N_2 in Et_2O was added and allowed to stand for 0 5 hr Excess CH_2N_2 was removed by evaporation at room temp under a stream of N_2 and the esterified methylated material was reduced with $LiBH_4$ as described in ref [27], and converted to PMAA

Fraction OA2 (20 mg) was methylated, a sample removed for conversion to PMAA, and the remainder was esterified with CH_2N_2 and reduced with $LiBH_4$, as for the OSP The carboxyl reduced OA2 was partially hydrolysed with 90% HCO_2H at 70° for 40 min [22] after which the HCO_2H was removed by co-distillation with H_2O under red pres and the material was reduced with $NaBD_4$ and remethylated using CD_3I The methylated partially degraded material was separated by extraction with CH_2Cl_2 [42] and a sample analysed by GC/EIMS (OV-I column) The remainder of the re-methylated material was completely hydrolysed and converted to PMAA for analysis by GC/MS

Cellulase treatment A portion (54 mg) of the cellulose residue, after sequential extraction of the CWM, was suspended in 10 ml acetate buffer (0 2 M, pH 5 0) and incubated at 37° with 1 ml (110000 units) of cellulase from *T viride* (1 unit produces 1 μg/hr

soluble carbohydrate, estimated as glucose, from ball milled filter paper at pH 5 and 37°) NaN_3 (0·005 M) was added to inhibit bacterial growth After 3 days the supernatant was removed by centrifugation and fresh cellulase and buffer added and incubation continued for a further 3 days after which the suspension was centrifuged, the supernatant filtered through a weighed glass fibre filter (Whatman GF/C) then dialysed and freeze-dried A control was set up with no cellulase

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