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STRUCTURAL INVESTIGATION OF AN ARABINAN FROM CABBAGE (*BRASSICA OLERACEA* VAR. CAPITATA)

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Abstract—An arabinan has been isolated from the hot water-soluble pectic substances of cabbage cell wall material. Methylation analysis involving GC–MS of methylated alditol acetates formed from the methylated arabinan has shown that the parent polysaccharide is highly branched and of the same structural type as other arabinans associated with seed pectins.

INTRODUCTION

As a part of our work on the analysis of dietary fibre, cell wall material (CWM) has been isolated from the alcohol-insoluble residue of cabbage used in clinical feeding trials [1] and its composition studied [2]. Extraction of the CWM with hot water yielded pectic material with a high content of arabinose but only small amounts of other neutral sugars, which indicated the presence of an arabinan. The investigations of the structural features of this arabinan are reported here.

RESULTS AND DISCUSSION

The hot water-soluble pectic substances (H) from cabbage contained an arabinan and pectins which have different degrees of esterification. Although the bulk of the pectins would be removed on a DEAE-Sephadex column, a small proportion of the highly esterified pectins may not be adsorbed and these would contaminate the neutral arabinan fraction. To obviate this problem, H was subjected to the mild de-esterification procedure of Aspinall *et al.* [3]. Although it is possible that the arabinan was bound to the pectic acid before the de-esterification stage, the conditions used were chosen to avoid, or at least minimise, degradation of the pectic substances. De-esterification yielded 3 fractions P_1, P_2 and S, P_1 and P_2 being the precipitates which separated from a solution of de-esterified H, and S the products in the supernatant. S was resolved into a neutral component (N), which was not retained on the DEAE-Sephadex column, and an acidic one (A) which was eluted with the NaCl gradient. The sugar compositions of H, P_1, P_2, N and A are given in Table 1. N contained a high proportion of arabinose, presumably from the arabinan, together with very small amounts of galactose, xylose, glucose, rhamnose and uronic acid from the contaminating traces of pectic acid and starch. In this study, no further purification of the arabinan (N) was attempted.

N was methylated by a modification of the method

Table 1. Sugar compositions of the hot water-soluble pectic substances of cabbage CWM, and those of its component fractions after separation on DEAE-Sephadex A50

	Composition (mol %)				
	Hot water-soluble	Ppts. from H*		Sephadex fractions	
Sugar	H	P_1	P_2	N	A
Rha	5.3	2.9	5.8	0.7	6.3
Ara	39.6	20.3	22.7	88.3	27.0
Xyl	2.6	6.4	2.6	1.7	3.2
Man	0.4	4.1	0.0	0.6	0.1
Gal	4.8	5.1	4.7	3.1	9.0
Glc	0.9	41.0	1.7	1.4	1.7
Uronic acid	46.3	20.1	62.5	4.2	52.9

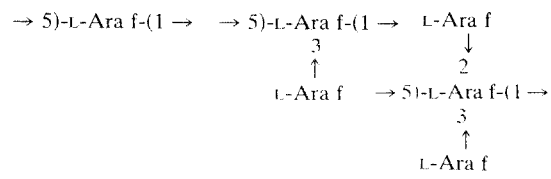
* Ppts. obtained on de-esterification of H.

Table 2. Partially methylated alditol acetates front methylated cabbage arabman (N)

PMAA	RR_t	Mode of linkage	Proportions (mol %) of PMAA
2,3,5-Me_3Ara†	0.43	L-Ara f-(1 →	40.1
2,3-Me_2Ara	1.07	→ 5)-L-Ara f-(1 →	27.4
2-Me Ara	1.85	→ 3,5)-L-Ara f-(1 →	12.6
Arabinitol	2.66	→ 2,3,5)-L-Ara f-(1 →	14.7
2,5-Me_2Ara	0.88		1.0
2-Me Xyl	2.03		2.6
3-Me Xyl	2.03		
2,3,6-Me_3Gal	2.24		1.8

* R_t relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl glucitol on OV 225 at 170°.
† 2,3,5-Me_3Ara = 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methyl-L-arabinitol, etc.

of Hakomori (see Experimental). The methylation appeared to be complete since the methylated polymer showed negligible OH absorption. Methylated N was hydrolysed, reduced, acetylated and the partially methylated alditol acetates were separated by GLC on OV-225 and identified by GC–MS. A summary of the identifiable neutral sugar derivatives obtained from N, their relative retention times, mol per cent composition and the mode of linkage of the sugar residues are shown in Table 2. It is assumed that the small amounts of xylose and galactose derivatives and a comparable proportion (~1%) of the 2,5-di-*O*-methyl arabinose derivative would have resulted from the contaminating pectic acid. The close correspondence between the amount of 2,3,5-tri-*O*-methyl arabinose representing non-reducing arabinofuranose end groups and mono-*O*-methyl arabinose and arabinitol (×2) representing points of branching also confirm the fact that the methylation of N is complete. From the amounts of arabinose derivatives it appears that they represent *ca* 95% of the structural units of N. Although there is no direct evidence from our results about the ring size of the arabinose residues in the polysaccharide other than at the non-reducing end groups, it seems likely that they are all of the same type, i.e. furanose. The configuration of the arabinose, by analogy with related studies, is assumed to be the L-form. The identification of an appreciable amount of unmethylated arabinose from methylated N indicates that there is a significant proportion of (1 → 5)-linked arabinose residues having branch points through positions 2 and 3. It is unlikely that this arabinose arises merely from incomplete methylation of N, since it was observed in about the same concentration in duplicate experiments and is necessary to account for the arabinose end groups. On the basis of the evidence presented, the occurrence of the following structural features in the arabinan could be inferred.



The edible part of cabbage consists of a terminal bud composed of young succulent leaves which would be expected to be rich in pectic polysaccharides. No data appear to be available for arabinans from similar tissues, so strict comparisons are not possible. However, the compositions of some arabinans from seeds [4, 5] are compared with that from cabbage in Table 3. The proportions of linkages present and the absence of → 2,5)-L-Ara f-(1 → residues show that the cabbage arabinan is more similar to arabinans of seeds than to those from bark of trees [6, 7] and suspension-cultured sycamore cells [8].

EXPERIMENTAL

Isolation of CWM from alcohol-insoluble residue (AIR). The AIR of cabbage was first milled so that particles of uniform size (which passed through a 1 mm mesh) were obtained. The milled AIR (20 g) was hydrated in 750 ml H_2O for 5 hr in the cold, blended and then ball-milled in batches of 250 ml in 1 l. pots on a Pascall ballmill at 25 rev/min for 16 hr at 2°. The suspension was centrifuged at 22 000 g for 40 min and residue washed with H_2O (×2). Because it is difficult to extract the coprecipitated cytoplasmic proteins with the usual protein solubilising reagents [9], treatment with pronase was used [2]. The residue was suspended in a

Table 3. Proportions of methylated arabinitol acetates from methylated arabinans (% by GLC)

Arabinitol acetate	Soybean arabinan [4]	Mustard seed arabinan [4]	Rapeseed arabinan [5]	Cabbage arabinan (Present study)
2,3,5-Me_3Ara*	39.2	39.6	34.0	39.2
2,3-Me_2Ara	30.0	25.4	25.7	27.8
2-Me Ara	14.2	28.6	31.5	15.2
3-Me Ara	6.0	T	T	—
Arabinitol	10.5	6.3	8.7	17.8

* Abbreviations as in Table 2.
T—trace.

mixture of H_2O (750 ml) and 0.4 M citrate buffer (750 ml), pH 6.5, containing 3 mM $CaCl_2$ and pronase (600 mg) was added to the mixture. After incubation at 37° for 2 days, the suspension was centrifuged and the residue washed with H_2O. To remove residual proteins the residue was blended with 5 vol. of PhOH—HOAc—H_2O (2:1:1, w/v/v) [9]. After centrifugation and washing with H_2O (×6), the residue was finally treated with 90% v/v aq. DMSO to remove starch [10], washed with H_2O and freeze-dried to yield 8.4 g of CWM.

Hot water extraction. CWM (3.5 g) was suspended in H_2O (250 ml), adjusted to pH 5 with NaOH and then heated at 80° for 1 hr with continuous stirring and occasional sonication. The suspension was centrifuged, the supernatant passed through a sintered filter (No. 3 porosity) and the filtrate concentrated at 30° under vacuum and freeze-dried to yield 130 mg of hot H_2O-soluble polysaccharides (H).

Isolation of arabinan. H was subjected to the mild de-esterification procedure of ref. [3]. H (100 mg) was dissolved in H_2O (20 ml), cooled in ice and the pH adjusted to 12 with NaOH. The mixture was kept at 0° for 2 hr to complete the de-esterification of the pectin. Adjustment of the pH to 3 with H_2SO_4 produced a slight ppt. P_1 (~4 mg) which was removed by centrifugation. After dialysis against H_2O for 24 hr, the supernatant was concentrated under vacuum and K-Pi buffer, pH 6.4, was added to a final concn of 10 mM. The ppt. P_2 (~24 mg) formed was removed by centrifugation and supernatant (30 ml) was slowly applied to a column (17 cm × 1.5 cm) of DEAE-Sephadex A50 equilibrated in the same buffer. The column was eluted with 80 ml of the buffer alone at first, followed by a linear gradient of NaCl up to 1 M. 4 ml fractions were collected from the start of sample application and the fractions monitored by reaction with PhOH—H_2SO_4 [11]. Fractions 1–22 were pooled, dialysed against H_2O and freeze-dried to yield N (21 mg) and fractions 35–65 were likewise treated to yield A (23 mg).

Estimation of sugars. Sugars were released from the polysaccharides by 2N H_2SO_4-hydrolysis, with a heating time of 2.5 hr, and estimated by GLC after conversion to their alditol acetates [12], a correction being applied for incomplete release of rhamnose. Uronic acid content was estimated by the modified carbazole method [12].

Methylation analysis. The arabinan (4 mg) was methylated by a modification of the method of ref. [10] except that 12 hr was allowed for alkoxide formation [13]. The methylated polysaccharides soluble in $CHCl_3$—MeOH, were hydrolysed sequentially with 90% HCO_2H (2 hr) and 0.25 M H_2SO_4 (12 hr) and the partially methylated sugars reduced with $NaBD_4$ and converted to partially methylated alditol acetates (PMAA) [10]. These were separated by isothermal GLC on 3% OV 225 at 170° and temp. programmed—150 to 200° at 0.5°/min; and on 3% ECNSS-M isothermally at 170° and temp. programmed—140 to 200° at 1°/min; other details as in ref. [10]. GC–MS was carried out as described in refs. [10] and [13]. PMAA were identified by, (a) RR_t with reference to 1,5-di-*O*-acetyl-2,3,4,6- tetra-*O*-Me glucitol, (b) the presence of diagnostic fragment ions in the MS and (c) the sugar composition of the H_2SO_4-hydrolysate.

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