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Structural features of hairy regions of pectins isolated from apple juice produced by the liquefaction process*

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ABSTRACT

A high-molecular-weight pectic fraction, released from the cell walls of apple tissue by pectolytic, hemicellulolytic, and cellulolytic enzymes, was isolated from apple juice prepared by the liquefaction process. The fraction, termed modified hairy regions (MHR), was characterized as a highly branched rhamnogalacturonan with arabinose-rich side chains and represented 0.26% of the fresh apple. Arabinose was the most abundant sugar (55 mol%) in MHR, which had a high rhamnose–galacturonic acid ratio (0.29) and degrees of methylation and acetylation of 42% and 60%, respectively. The sugar and linkage compositions, distribution of molecular weights, susceptibility to β-elimination mediated by alkali and 4-methylmorpholine *N*-oxide, and the effect of various technical and pure enzymes on MHR and chemically and enzymically modified MHR have been studied. From the results and ^{13}C-n.m.r. data, a tentative structure for MHR is proposed.

INTRODUCTION

The polysaccharide composition and the structure of apple cell walls have been the subject of many investigations. Pectin, hemicellulose, and cellulose fractions have been obtained and characterized[1–4]. The changes in the cell-wall composition at different stages of ripening of the apple have been studied[5–7]. Fractionation procedures including mild extraction with water, buffer, chelating agents, alkali, chlorite, and various enzymes have been used[3,8–10]. Much effort has been expended on linkage analysis of the cell-wall polysaccharides[3,11,12]. De Vries *et al.*[8,9] investigated the water-, acid-, and oxalate-soluble pectin fractions from apple cell walls and their degradation with pectate and pectin lyases. A new model was proposed in which the pectins were considered to contain homogalacturonan regions comprising 90% of the galacturonic acid residues and so-called "hairy" or ramified regions which contained most of the neutral sugars. These hairy regions are also present in pectins isolated from carrots[13] and the pulp of grape berries[14,15].

We now report on the characterization of a cell-wall polysaccharide fraction isolated from the apple juice of Golden Delicious apples obtained by the so-called

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liquefaction process in which the juice is released from the apple pulp by the combined action of pectolytic and cellulolytic enzymes. This polysaccharide fraction, previously designated ultrafiltration retentate[16], resembled the hairy regions of apple pectin[9]. Since the enzyme preparation used in the liquefaction process contains various pectolytic, hemicellulolytic, and cellulolytic activities, it is probable that the polysaccharide fraction was altered during extraction and therefore it has been renamed modified hairy regions (MHR).

EXPERIMENTAL

Isolation of MHR. — Golden Delicious apples (100 kg, harvest 1981 and 1985) were crushed in an Amos rasp mill (particle size, 3.5 mm) and treated with an experimental enzyme preparation (Rapidase C600, 0.02%) from Gist Brocades (Delft, The Netherlands) for 4 h at 45°. After centrifugation (Pennwalt Sharpless P600 Decanter) and aroma-stripping, the liquid was processed in an ultrafiltration tubular system equipped with a BX3 polysulfone membrane having a mol. wt. cut-off of 60 000 (Paterson Candy Ltd.). The retentate was diluted with 60 L of water and ultrafiltered, and the residue was dialysed, centrifuged (15000*g*, 20 min), and lyophilized.

The MHR-fraction represents 0.26% of the fresh apple and 1.68% of the apple solids.

Chemical treatment of MHR. — The methoxycarbonyl and acetyl groups in MHR were saponified with 0.05M NaOH (1.7% MHR, 24 h, 0°), followed by dialysis and lyophilization, to yield MHR-S (91% of MHR).

The arabinan structures were removed by treatment with acid. The pH of a solution of MHR (1 g) in water (350 mL) was adjusted to 0.4 with aqueous 37% hydrochloric acid. The solution was heated for 2 h at 80°, then dialysed and freeze-dried to give MHR-HCl (~45% of MHR).

MHR was treated[17] with 4-methylmorpholine *N*-oxide for 0.5 and 4 h. Each mixture was dialysed against water and lyophilized. The yields of products were 78% and 66%, respectively.

Analytical methods. — Uronic acid was determined by the colorimetric *m*-hydroxybiphenyl assay[18]. Neutral sugars were determined by g.l.c. after pretreatment (1 h, 30°) with aqueous 72% H_2SO_4 followed by hydrolysis with M H_2SO_4 (3 h, 100°) and conversion of the products into alditol acetates[19]. The alditol acetates were analysed on a glass column (3 m × 2 mm i.d.) packed with Chrom WAW (80–100 mesh) coated with 3% of OV275, in a Carlo–Erba Fractovap 2300 GC.

Methanolysis (24 h, 85°) with methanolic M HCl followed by trimethylsilylation of the resulting methyl glycosides[20] was performed in order to identify uronic acid residues and to confirm the absence of fucose. Methanolysis was also used to check the complete liberation of all sugar residues by hydrolysis with M H_2SO_4 (3 h, 100°) or with 2M trifluoroacetic acid (1 h, 121°). No differences between these methods were observed.

Linkage analysis was established by methylation (Hakomori[21]), followed by hydrolysis with 2M trifluoroacetic acid, and conversion[22] of the products into partially

methylated alditol acetates. Carboxyl reduction was effected by the method of Taylor and Conrad[23]. After three treatments, >90% of the uronic acids had been reduced (*m*-hydroxybiphenyl assay). The products of methylation analysis of the polysaccharides were identified and quantified by capillary g.l.c. and g.l.c.–m.s. For capillary g.l.c., a wall-coated OV-225 column (10 m × 0.32 mm; 0.23-μm layer) and a Carlo–Erba Fractovap 4160 gas chromatograph were used with the temperature programme 150° for 1 min, 150° → 200° at 2°/min, 200° for 2 min. G.l.c.–m.s. was performed with a VG MM 7070F mass spectrometer coupled to a Pye 204 gas chromatograph equipped with a packed column (1.5 m) of 3% of OV-225 on Chromosorb WHP. In order to differentiate 2- and 4-substituted xylose residues, reduction to alditols was also carried out with sodium borodeuteride.

The degrees of methylation and acetylation were determined[24] by h.p.l.c.

Gel-permeation chromatography. — A solution of MHR (50–100 mg) in water (1–3 mL) was applied to a column (100 × 2.2 cm) of Sephacryl S500 (Pharmacia; separation range for dextrans: 4×10^4–2×10^7 Da) or a column (100 × 2.6 cm) of Sephacryl S200 (separation range for dextrans 10^3–8×10^4 Da) and eluted with 0.05M sodium phosphate buffer (pH 7.0). Fractions were assayed by automated methods[25,26] for uronic acid and total neutral sugars. Neutral sugar values (as "anhydroarabinose") were corrected for the contribution of the uronic acid in the orcinol assay.

High performance g.p.c. was performed on a SP8800 HPLC (Spectra Physics) equipped with three columns (each 300 × 7.5 mm) of Bio-Gel TSK in series (40XL, 30XL, and 20XL; Bio-Rad Labs.), in combination with a TSK XL guard column (40 × 6 mm), and elution at 30° with 0.4M acetic acid/sodium acetate (pH 3.0) at 0.8 mL/min. The eluate was monitored using a Shodex SE-61 Refractive Index detector. The system was calibrated with pectins having mol. wts. in the range 10 000–100 000 (as determined by viscosimetry) and oligomers obtained after degradation of polygalacturonic acid by yeast endo-polygalacturonase[27]. The software was obtained from Spectra Physics.

N.m.r. spectroscopy. — The ^{13}C-n.m.r. spectra were obtained with Jeol GX-400 (100.4 MHz for MHR-HCl) and Bruker CXP 300 spectrometers (75 MHz for MHR) at 90° for 3% solutions in D_2O (internal acetone; 31.55 p.p.m. relative to Me_4Si). Data were acquired with broad-band decoupling and acquisition times of 0.65 s and 69 283 transients (MHR-HCl) and 0.65 s and 5724 transients (MHR).

Enzymic hydrolysis. — 0.2% Solutions of MHR and MHR-S in 0.05M sodium acetate buffer (pH 5.0) were incubated with 3.33 nkat of enzyme/mL for 4 h at 30°. Treatment with pectate lyase was carried out in 0.1M glycine buffer (pH 9.4) containing 0.5mM $CaCl_2$. The activities of the hydrolases were measured by the increase in the reducing end-groups[28]. Lyase action was determined[29] by the increase in absorbance at 235 nm. The following enzymes were used: two endo-(1→4)-β-glucanases (EC 3.2.1.4; a specific glucanase active only on cellulose substrates and a non-specific glucanase that was also active on xylans[30]), endo-(1→4)-β-galactanase (EC 3.2.1.89), endo-polygalacturonase (EC 3.2.1.15) of fungal and yeast origin, endo-pectate lyase (EC 3.2.2.2), endo-pectin lyase (EC 4.2.2.10), and fungal pectin methylesterase (EC 3.1.1.11).

Enzymic degradation of MHR by arabinanases was performed as follows. A

solution of MHR (1 g) in 0.1M sodium acetate (pH 5.0; 70 mL) was incubated with 1620 nkat of α-L-arabinofuranosidase type B (EC 3.2.1.55, activity measured against *p*-nitrophenyl α-L-arabinofuranoside) and 12 nkat of endo-(1→5)-α-L-arabinanase [EC 3.2.1.99, activity measured against linear α-(1→5)-L-arabinan[31]] for 48 h at 35°. After dialysis and freeze-drying, 45% of MHR was recovered as MHR-ARA.

The effect on MHR of 45 commercial enzyme preparations involved incubation of 0.5% solutions in 0.05M sodium acetate buffer (pH 5.0) with 0.01% of the enzyme preparation. Digests were analyzed by high performance g.p.c.

RESULTS AND DISCUSSION

Characterization of MHR. — A fraction (MHR, modified hairy regions) that represented 0.26% of the fresh apple appeared to be a high-molecular-weight polysaccharide that was resistant to further degradation by the liquefying enzyme preparation. The sugar compositions of MHR and modified MHR are shown in Table I. MHR contained mainly arabinose but was also rich in rhamnose, xylose, galactose, and galacturonic acid. The absence of glucuronic acid residues was proved by methanolysis and analysis of the resulting methyl glycosides after trimethylsilylation. This analysis also confirmed that rhamnose was the only 6-deoxyhexose present. The rhamnose:galacturonic acid ratio of 0.29 was high in comparison with those of other apple pectin fractions (0.029 and 0.038, respectively, for water- and oxalate-soluble pectin fractions[3] and 0.004–0.026 for cold-buffer-soluble pectin fragments[8]). High rhamnose:galacturonic acid ratios have been reported for enzyme-treated pectin fractions of apples[9] (0.22), carrots[13] (1.0), and grape berries[15] (0.8).

The sugar composition revealed that MHR polysaccharide resembled the so-called "hairy regions" as described by De Vries *et al.*[9] (rhamnose:galacturonic acid ratio

TABLE I

Sugar composition (mol%) of modified MHR

Sugar	*MHR*	*MHR-S*	*MHR-HCl*	*MHR-ARA*
Rha	6 (5.0)[a]	6 (4.3)	13 (4.0)	16 (3.7)
Ara	55 (42.6)	55 (35.3)	5 (1.3)	7 (1.5)
Xyl	8 (5.7)	7 (4.6)	18 (4.8)	8 (1.7)
Man	0 (0)	0 (0)	0 (0)	2 (0)
Gal	9 (9.7)	10 (8.5)	19 (6.4)	15 (4.0)
Glc	1 (0.6)	1 (0.7)	1 (0.3)	0 (0)
GalA	21 (19.6)	21 (18.6)	44 (16.2)	52 (15.7)
OMe	42 (1.5)	0 (0)	33 (1.0)	14 (0.4)
OAc	60 (4.0)	0 (0)	10 (0.6)	65 (3.5)
Total sugars	83%	79%	73%	60%
Rha:GalA	0.29	0.29	0.30	0.31

[a]Gram quantities per residue in the MHR substrates originating from 100 g of MHR.

0.22, arabinose 50%, xylose 5%, galactose 13%, rhamnose 5%, and galacturonic acid 23%). However, MHR contained less methoxycarbonyl groups, and the presence of acetyl groups was not reported by De Vries *et al.*[9]. MHR was isolated after treatment of the entire cell wall with various polysaccharide-degrading enzymes present in the experimental enzyme preparation used. De Vries *et al.*[9] obtained their hairy regions by extraction of a pectin fraction under mild conditions followed by degradation with a pure pectate lyase.

The relatively large proportion of MHR isolated indicated that it may originate from the soluble pectins from the apple cell wall and the insoluble proto-pectin fraction. Voragen *et al.*[32] reported that the concentration of uronic acids in liquefaction juice increased 7 times as compared to juice obtained by conventional pressing, whereas the arabinan content was 20 times higher[16]. These data illustrate the degradation and solubilization of insoluble cell-wall polysaccharides by the action of enzymes in the liquefaction process. MHR could be isolated in 100-g quantities, whereas De Vries *et al.*[9] obtained only mg quantities of the hairy regions. Arabinan-rich pectic hairy regions, isolated after treatment of cell walls with pectolytic enzymes, have been described for carrot cell walls[13] (40 mol% arabinose) and grape berries[14,15] (30–50 mol% arabinose).

McNeil *et al.*[33,34] and Lau *et al.*[35] have described rhamnogalacturonan I, isolated from suspension-cultured sycamore cells, that had a rhamnose:galacturonic acid ratio of 0.5 and a backbone that consisted partly of alternating rhamnose and galacturonic acid residues.

No protein or polyphenols were detected in MHR and, based on the uronic acid content, a degree of methylation of 35% was calculated. The degree of acetylation was 60% and such a value has not been reported hitherto for apple pectin fractions; commercial apple pectins have values of 3–4% (ref. 24).

The methoxycarbonyl and acetyl groups could be saponified to give MHR-S without changing the sugar composition (Table I) and with only a slight loss of sugars (max. 10%).

Exhaustive treatment of MHR with a combination of endo-arabinanase and arabinofuranosidase B removed most of the arabinose without loss of acetyl groups (Table I, MHR-ARA). Also, 70% of the xylose residues and 60% of the galactose residues could be removed enzymically. These results indicated that the acetyl groups were linked to the galacturonosyl residues. MHR-ARA also had a decreased methoxyl content. The low sugar content can be explained by the addition of large amounts of enzyme protein (40 mg), which may account for 10–15% of this fraction. Arabinose could also be removed from MHR by treatment with hydrochloric acid. The product (MHR-HCl) had a low content of arabinose, but 75% of all the other sugars were retarded. The degree of methylation was reduced by 33% and only 15% of the acetyl groups survived.

The molecular weight and the homogeneity of MHR were determined by using high performance g.p.c. (Fig. 1). MHR consisted of three distinct populations (A–C), with different molecular weights, which were isolated by fractionation (Sephacryl S500 and S200). Data on these fractions are shown in Table II. The rhamnose:galacturonic

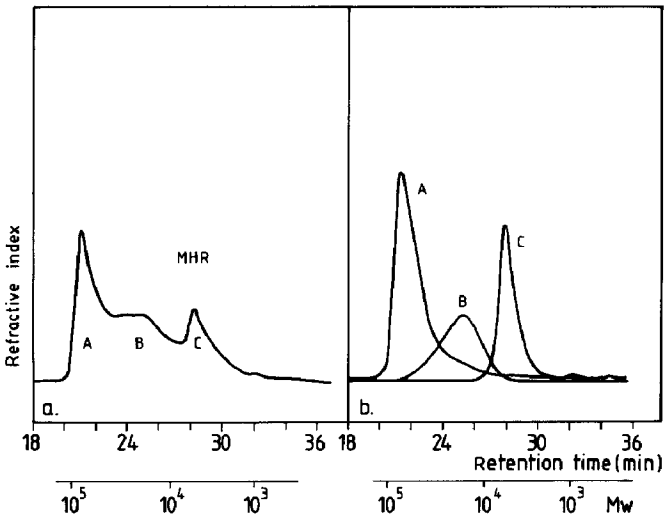

Fig. 1. High performance g.p.c. of (*a*) MHR, and (*b*) the fractions of MHR isolated by chromatography over Sephacryl S200 and S500.

TABLE II

Sugar composition (mol%) of the fractions of MHR isolated by chromatography over Sephacryl S200 and S500

Sugar	*MHR*	*A*	*B*	*C*
Rha	6	5	6	10
Ara	55	50	59	47
Xyl	8	11	5	3
Man	0	0	0	0
Gal	9	10	13	7
Glc	1	0	0	0
GalA	21	24	17	33
OMe	42	28	84	100
OAc	60	55	57	21
Rha:GalA	0.29	0.21	0.35	0.30

acid ratios for A–C were 0.21, 0.35, and 0.30, respectively, and there were differences in the xylose and methoxyl contents. Although variations were observed in the sugar composition (minor) and hydrodynamic volumes (major), MHR was considered to be a mixture of similar polysaccharides.

However, by elution from Sephacryl S500 with water, Voragen *et al.*[32] isolated a neutral fraction that comprised 6–7% of the MHR and which was identified as an arabinan (degree of branching, 18%). Since our investigations were concerned with the structure of the backbone of MHR and since this arabinan did not interfere in the chemical and enzymic analyses, it was not removed. This arabinan is not present in MHR-ARA and MHR-HCl.

TABLE III

Glycosidic linkage composition of MHR and MHR-HCl

Sugar residue		*Glycosidic linkage composition*[a]	
		MHR	*MHR-HCl*
Rhamnose	1,2-Rha*p*[b]	-	14.3
	1,2,4-Rhap	4.4	15.0
		(4.4%)	(29.3%)
Arabinose	T-Ara*f*	10.7	3.7
	1,2-Ara*f*	0.4	-
	1,3-Ara*f*	6.3	-
	1,5-Ara*f*	30.8	1.9
	1,3,5-Ara*f*	8.8	-
	1,2,5-Ara*f*	1.9	-
	1,2,3,5-Ara*f*	3.0	-
		(61.9%)	(5.6%)
Xylose	T-Xyl*p*	6.5	6.1
	1,4-Xyl*p*	2.9	-
	1,2-Xyl*p*	0.3	-
		(9.7%)	(6.1%)
Galactose	T-Gal*p*	3.6	16.2
	1,3-Gal*p*	1.5	1.4
	1,4-Gal*p*	1.1	3.8
	1,6-Gal*p*	0.3	4.8
	1,2,4-Gal*p*	0.3	-
	1,3,6-Gal*p*	1.1	2.3
	1,2,3,4-Gal*p*		1.1
		(7.9%)	(29.6%)
Galacturonic acid[c]			
	1,4-Gal*p*A	12.8	24.2
	1,3,4-Gal*p*A	3.2	4.9
		(16%)	(29.1%)
Ratio terminal/branching		0.98	1.07

[a] Linkage types in mol%; the figures after methylation analysis are given in brackets. [b] 1,2-linked Rha, *etc.* T connotes a terminal group. [c] After carboxyl reduction.

Table III shows the results of the methylation analyses of MHR and MHR-HCl after reduction of the galacturonic acid residues. The MHR-associated arabinans were less branched (27%) than arabinans (40–45%) isolated from apple cell walls that had not been treated with enzyme[16].

Methylation analyses of MHR showed the rhamnose residues to be branched at C-4. However, only 73% of the rhamnose residues were recovered as methylated alditol acetates. Of the xylose residues, 65% were terminal and the remainder were (1→4)-linked. Almost 50% of the galactose residues were (1→4), (1→3), (1→6), or (1→3,6)-

linked, and 45% were terminal. The high proportion (45%) of terminal galactose residues, compared to that (4–5%) in the hairy regions reported by De Vries *et al.*[12], indicates that, due to endo-galactanase activity in the liquefaction process, longer galactan sequences are removed. The residual short side chains were not degraded by the galactanases.

All of the galacturonosyl residues were α-(1→4)-linked with 20% branched at C-3. The high proportion (6.5%) of terminal xylose residues cannot be explained by the presence of a highly branched xylan. No xyloglucans were present (no glucose derivatives were detected in the methylation analysis). Part of the terminal xylose residues might be accounted for by the presence of a rhamnogalacturonan that carries side chains of single xylosyl units as described by De Vries *et al.*[12,43]. Methylation analysis of MHR-HCl revealed only 50% of the rhamnose residues to be branched at C-4 and all

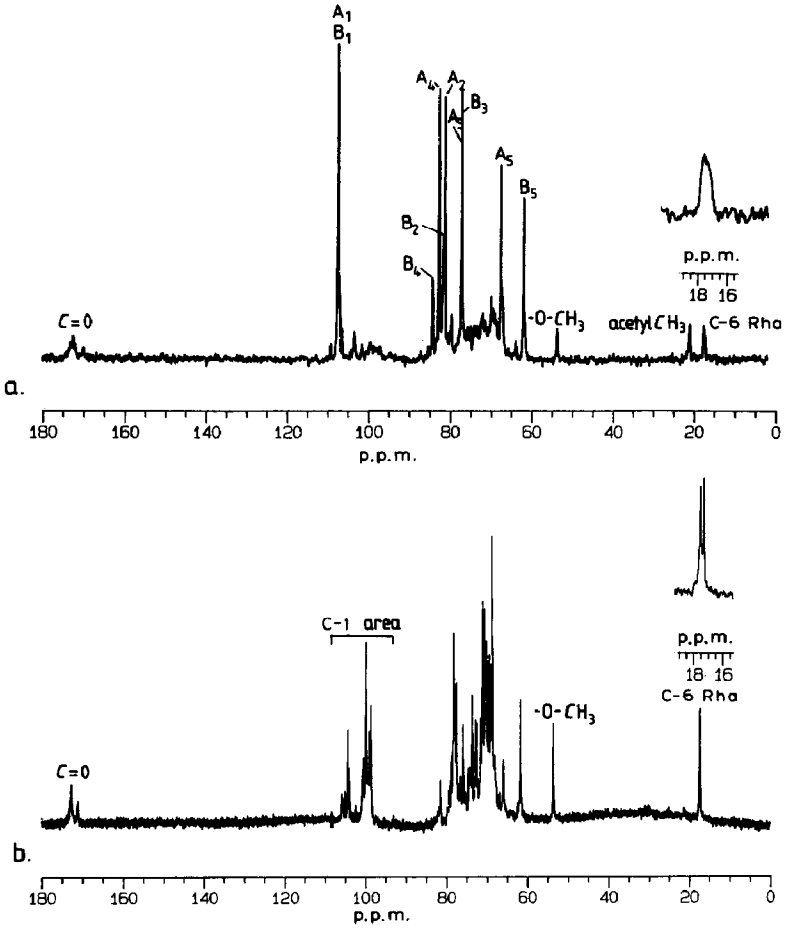

Fig. 2. ^{13}C-N.m.r. spectra of (*a*) MHR and (*b*) MHR-HCl. A, α-(1→5)-Ara*f*: 108.82 (C-1), 82.14 (C-2), 78.05 (C-3), 83.63 (C-4), 68.20 (C-5); B, Ara*f*: 108.78 (C-1), 82.57 (C-2), 77.88 (C-3), 85.33 (C-4), 62.50 p.p.m. (C-5).

xylose residues to be terminal. However, the recovery of the sugar residues in the methylation analysis (especially of xylose and galacturonic acid) was not in full agreement with the sugar composition as analysed as alditol acetates. Methylation analysis of MHR-ARA dit not give trustworthy results.

The ^{13}C-n.m.r. spectrum of MHR (Fig. 2a) resembled those of branched arabinans. The arabinose side chains (55% of the total sugar residues) were highly flexible and gave strong signals, the chemical shifts of which were in agreement with data reported[36]. The backbone structure, consisting of galacturonic acid and rhamnose, was rigid, which resulted in the broadening and weakening of signals. This effect has been reported for pectic substances[37,38]. The signals of the backbone are more pronounced in the ^{13}C-n.m.r. spectrum of MHR-HCl (Fig. 2b) since there were no signals for arabinose. The peak at 53.73 p.p.m. is characteristic for the COOMe group of galacturonate residues[38] and there were no peaks for acetyl groups. The signals for C-6 in the region 16–18 p.p.m. revealed two differently linked rhamnose residues. In general, ^{13}C-n.m.r. spectra of polysaccharides are difficult to assign completely.

MHR was fairly resistant to base-catalysed β-elimination under the conditions described by Thibault[39], but was degraded on treatment[17] with 4-methylmorpholine *N*-oxide. Table IV shows the recovery of the sugars, methyl esters, and acetyl groups. After treatment for 0.5 h, ~80% of the sugar residues were recovered but, after 4 h, xylose, galacturonic acid, and methoxyl groups in particular were lost, probably during dialysis. These observations confirm the presence of xylogalacturonan regions and that demethylation proceeds faster than deacetylation.

Enzymic degradation of MHR. — MHR was resistant (Table V) to most pectic enzymes and specific and non-specific endo-(1→4)-β-D-glucanases. Fungal endo-(1→4)-poly-α-D-galacturonase cleaved ~6% of the galacturonosyl linkages. Pectin esterase was not able to make MHR a more suitable substrate for either fungal or yeast-poly-galacturonases. Endo-(1→4)-β-D-galactanase hydrolysed 10% of the galactosyl linkages. Mild saponification of MHR gave MHR-S which was degraded slightly by endo-polygalacturonase. Of 45 different commercial enzyme preparations, only five

TABLE IV

Recovery of the sugar residues, methoxyl, and acetyl groups of MHR after treatment with 4-methylmorpholine *N*-oxide

Sugar residue	*Recovery (%) after treatment.*	
	0.5 h	*4 h*
Rha	83	77
Ara	80	77
Xyl	67	18
Gal	77	77
GalA	73	38
OMe	55	0
OAc	80	57

could degrade MHR to some extent, *e.g.*, Hemicellulase Reg. II, Gist Brocades (Fig. 3, enzyme A). Arabinanases were responsible for this degradation, since the presence of arabinose and its oligomers was established by h.p.l.c. according to Voragen *et al.*[40]. These five enzyme preparations were active towards linear and branched arabinans but not towards MHR-HCl and MHR-ARA. Arabinofuranosidase activity was observed by the release of small amounts of arabinose when MHR was incubated with Rapidase

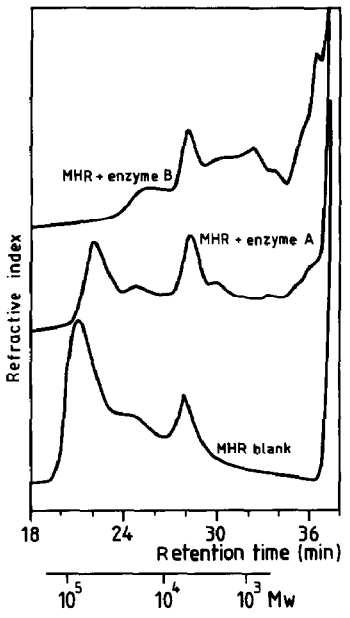

Fig. 3. High performance g.p.c. of MHR, before and after degradation with commercial enzyme preparations: *A*, Hemicellulase Reg II; B, Ultra Sp.

TABLE V

Degradation of MHR by various purified enzymes during 4 h at 30°

Enzyme	*Degradation (%)*	
	MHR	*MHR-S*
Pectin esterase (PE)	0	0
Yeast Polygalacturonase (PG)	0	3.4[a]
Fungal Polygalacturonase	6.0	14.5
Yeast PG + PE	0	3.1
Fungal PG + PE	6.6	13.8
Pectate lyase	2.3	4.6
Pectin lyase	0	0
Endo-galactanase	10.3[b]	17.4
Cellulase:xylanase	0	0

[a]GalA linkages split. [b]Gal linkages split.

C600. The lack of arabinose oligomers after incubation with MHR or pure linear arabinan indicated that Rapidase C600 had poor endo-arabinanase activity. Incubation with polysaccharides isolated from plant cell walls revealed that this enzyme mixture contained a wide spectrum of pectolytic and cellulolytic enzymes in addition to endo-xylanases and endo-galactanases.

High performance g.p.c. of MHR after treatment with enzyme A (Fig. 3) showed an almost identical behaviour of the MHR backbone that remained after removal of the arabinan residues which make up > 50% of MHR. This phenomenon can be explained by assuming a minor change in hydrodynamic volume of MHR after removal of 50% of its constituent sugars.

Only one of the enzyme preparations (Ultra SP, Novo Ferment AG) degraded the rhamnogalacturonan backbone of MHR (Fig. 3, enzyme B). Most of the molecules of high molecular weight were degraded to oligomeric fragments but some were resistant to enzyme B. A novel enzyme was isolated from the crude enzyme preparation B which catalysed the degradation of the backbone of MHR (see following paper[41]).

Although a heterogeneous molecular-weight distribution was established for MHR, a possible structure is proposed (Fig. 4). The presence of a region with alternating rhamnose and galacturonic acid residues was demonstrated by ^{13}C-n.m.r. spectroscopy of tetra-, penta- and hexa-saccharide fragments produced with the above novel enzyme[42].

The position of the acetyl groups at C-2 and/or C-3 of the galacturonic acid was confirmed by the enzymic degradation studies with rhamnogalacturonase as described in the following paper[41]. Most of the xylose residues were linked to the galacturonic acid

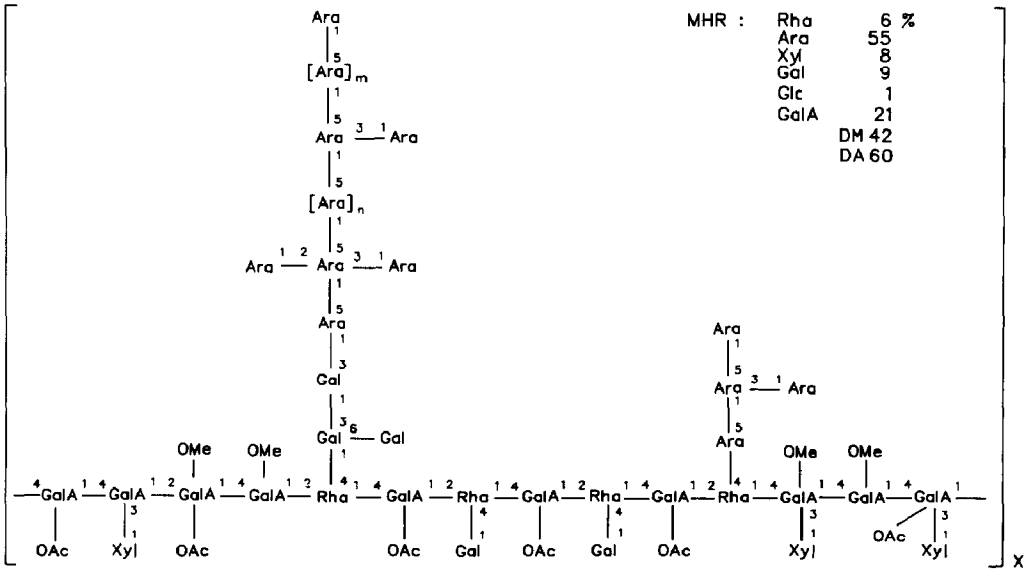

Fig. 4. Possible structure of MHR.

backbone and almost all of the rhamnose residues were branched with either galactose or arabinose side chains. These arabinan side chains were α-(1→5)-linked with a degree of branching that was ~50% lower than that of native apple arabinans[16]. It is recognized that structures other than that in Fig. 4 are possible. It is concluded that the complex hairy regions are composed of different repeating units as earlier stated by De Vries[43].

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