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METHYLATION ANALYSIS AND MILD ACID HYDROLYSIS OF THE "HAIRY" FRAGMENTS OF SUGAR-BEET PECTINS*

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ABSTRACT

The fragments of the rhamnogalacturonan backbone carrying neutral side-chains ("hairy" fragments) of sugar-beet pectins have been subjected to methylation analysis and mild acid treatment. The fragments have similar structures which consist of a rhamnogalacturonan backbone carrying highly branched (1→5)-linked arabinans and linear (1→4)-linked galactans of low d.p. together with (1→3,6)-linked galactans in low proportions. Of the feruloyl groups, 50–60% are ester-linked to the neutral side-chains and are removed together with arabinose or galactose residues.

INTRODUCTION

Pectic molecules are heteropolysaccharides characterised by the presence of galacturonic acid, arabinose, rhamnose, galactose, xylose, and glucose[1–4], and, occasionally[5], by minor amounts of 2-*O*-methylfucose, 2-*O*-methylxylose, and apiose[6]. The molecules comprise a partly methyl- and acetyl-esterified[7–12] (1→4)-linked α-D-galacturonan backbone interspersed with (1→2)-linked α-L-rhamnose. Neutral sugar side-chains are thought[1–4] to be bound mainly to O-4 of the L-rhamnosyl residues.

The extraction, purification, and characterisation of sugar-beet pectins from an alcohol-insoluble material have been described[9,10]. The acid-soluble (HP) and alkali-soluble pectins (OHP) had molecular weights of 45,800 and 39,200, respectively, and were highly acetylated (23.5 and 10.7%); they contained neutral sugars (20.2 and 26.1%), mainly arabinose with intermediate proportions of galactose and rhamnose, and also small proportions of feruloyl ester groups (<1%) and hydroxyproline-rich protein (~4%). The action of an endopolygalacturonase showed[10,11] the demethylated and deacetylated pectins to consist of large homogalacturonan

*Structural Investigation of the Neutral Sugar Side-Chains of Sugar-Beet Pectins, Part I.

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blocks (91–86.5% of the galacturonic acid), which carried most of the acetyl groups (~75%)[13], and small rhamnogalacturonan blocks (9–13.5% of the galacturonic acid) carrying neutral sugar side-chains with almost all of the feruloyl groups and ~30% of the hydroxyproline-rich proteins.

We now report on the structure of these neutral sugar side-chains, using methylation analysis and mild acid hydrolysis.

EXPERIMENTAL

Pectins. — Acid-soluble (HP) and alkali-soluble pectin (OHP) were obtained by sequential extraction of sugar-beet pulp with 0.05M HCl (80°) and 0.05M NaOH (4°), respectively, and purified[10] by precipitation with cupric ions. The purified pectins were demethylated and deacetylated in cold alkali, and their "hairy" fragments (29–35% of the purified pectins) were isolated from the endopolygalacturonase digests by gel-filtration chromatography[10].

Mild acid hydrolysis. — "Hairy" fragments (10 mg) were treated with 0.05M or 0.1M trifluoroacetic acid (0.5 mL) in a sealed tube for 1 h at 100°. The acid was evaporated *in vacuo* and the residue was dissolved in 1 mL of water.

Gel-filtration chromatography. — Each acid hydrolysate (0.5 mL) was injected onto a column (203 × 1.6 cm) of Bio-Gel P-2 and eluted (descending) at 60° with degassed water at 50 mL/h. The eluate was monitored for galacturonic acid and neutral sugar. The appropriate fractions (5 mL) were combined and analysed for galacturonic acid, neutral sugars, and feruloyl groups. The results are expressed as a function of the elution volume.

The polysaccharides which were eluted in the void volume were loaded onto a column (50 × 1.6 cm) of Sepharose CL-6B and eluted (ascending) with 0.1M sodium acetate buffer (pH 4.0) at 20 mL/h. Fractions (3–4 mL) were assayed for galacturonic acid, neutral sugars, and feruloyl groups. The void (V_o) and total (V_t) volumes of the column (43 and 105 mL, respectively) were determined with *O*-(carboxymethyl)cellulose and D-galacturonic acid, respectively. The results are expressed as a function of $K_{av} = (V_e - V_o)/(V_t - V_o)$, where V_e is the elution volume of the fraction.

H.p.l.c. — The above monomeric and oligomeric fractions isolated from Bio-Gel P-2 were analysed[13] by h.p.l.c., using an Aminex HPX 87P column and water as eluent.

Analytical methods. — Galacturonic acid and total neutral sugars (expressed as arabinose) were determined by automated 3-hydroxybiphenyl[14] and orcinol[15] methods, respectively, the latter being corrected for interfering galacturonic acid. The neutral sugars were determined after hydrolysis with M H_2SO_4 (2 h, 100°) as their alditol acetates by g.l.c.[16]. Feruloyl groups were determined spectrophotometrically at 375 nm on freshly prepared solutions of pectins, the pH being adjusted to 10 by M NaOH; a molar extinction coefficient at 31,600 was used[17]. In some minor fractions, the feruloyl groups were determined spectrophotometrically di-

rectly on aqueous solutions at 325 nm, using 22,900 as the molar extinction coefficient[10]. All the values were calculated on a moisture-free basis.

Polysaccharides were methylated by the Hakomori[18] method as modified by Lomax and Conchie[19] and Harris *et al.*[20]. The partially methylated alditol acetates were analysed by g.l.c. on two capillary columns packed with OV-1 (Delsi Instruments, 50 m × 0.32 mm i.d., 0.20-μm film thickness; on-column injection at 40°, then 15°/min to 190°; detector temperature, 250°; helium carrier gas) and OV-225 (DB-225, J. W. Scientific, 30 m × 0.32 mm i.d. 0.25-μm film thickness; 150° for 1 min, then 2°/min to 200°, 200° for 2 min; cooled injection on the column; helium gas at 0.48 atms.). Peaks were identified on the basis of retention time, using authentic partially methylated alditol acetates.

TABLE I

PARTIALLY METHYLATED ALDITOL ACETATES DERIVED FROM HP, HP "HAIRY" FRAGMENTS, AND HP "HAIRY" FRAGMENTS DEGRADED WITH TRIFLUOROACETIC ACID

	Relative mol %				
Methylated alditol[a]	*HP*	*HP ("hairy")*	*HP ("hairy") 0.05M acid*	*HP ("hairy") 0.1M acid*	*Linkage*
2,3,5-Me_3-Ara[b]	22.5	21.5	7.6	5.4	Ara*f*-(1→
2,3,4-Me_3-Ara	tr	1.0			Ara*p*-(1→
3,5-Me_2-Ara	0.7	0.6			→2)-Ara*f*-(1→
2,5-Me_2-Ara	1.5	1.7	3.5	2.5	→3)-Ara*f*-(1→
2,3-Me_2-Ara	20.6	20.7	20.4	10.2	→5)-Ara*f*-(1→
2-Me-Ara	18.7	13.7	4.6	2.5	→3,5)-Ara*f*-(1→
3-Me-Ara	2.3	2.1			→2,5)-Ara*f*-(1→
Ara	1.6	2.0	1.0		→2,3,5)-Ara*f*-(1→
Total	67.9 (67.5)[c]	63.3 (67.6)	37.1 (39.3)	20.6 (23.9)	
2,3,4,6-Me_4-Gal	5.2	7.6	17.4	13.9	Gal*p*-(1→
2,4,6-Me_3-Gal	3.8	4.1			→3)-Gal*p*-(1→
2,3,6-Me_3-Gal	8.0	10.6	20.1	25.6	→4)-Gal*p*-(1→
2,3,4-Me_3-Gal	1.1				→6)-Gal*p*-(1→
2,6-Me_2-Gal	1.9	1.1	5.2	5.9	→3,4)-Gal*p*-(1→
2,4-Me_2-Gal	2.8	2.2			→3,6)-Gal*p*-(1→
Total	22.8 (22.0)	25.6 (25.4)	42.7 (38.0)	45.4 (39.2)	
3,4-Me_2-Rha	5.6	7.2	11.6	15.5	→2)-Rha*p*-(1→
3-Me-Rha	3.7	3.9	8.6	18.5	→2,4)-Rha*p*-(1→
Total	9.3 (10.5)	11.1 (14.2)	20.2 (19.4)	34.0 (35.0)	

[a]Some minor components, the identities of which were not confirmed, are omitted. [b]2,3,5-Me_3-Ara denotes 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylarabinitol, *etc.* [c]Values in parentheses are based on the analysis of alditol acetates.

TABLE II

PARTIALLY METHYLATED ALDITOL ACETATES DERIVED FROM OHP, OHP "HAIRY" FRAGMENTS, AND OHP "HAIRY" FRAGMENTS DEGRADED WITH TRIFLUOROACETIC ACID

	Relative mol %				
Methylated alditol[a]	*OHP*	*OHP ("hairy")*	*OHP ("hairy") 0.05M acid*	*OHP ("hairy") 0.1M acid*	*Linkage*
2,3,5-Me_3-Ara[b]	21.5	19.2	8.4	3.3	Ara*f*-(1→
2,3,4-Me_3-Ara	1.0	1.3			Ara*p*-(1→
3,5-Me_2-Ara	0.6	0.6			→2)-Ara*f*-(1→
2,5-Me_2-Ara	1.7	3.0			→3)-Ara*f*-(1→
2,3-Me_2-Ara	20.7	22.3	12.3	6.7	→5)-Ara*f*-(1→
2-Me-Ara	13.7	10.6	2.1		→3,5)-Ara*f*-(1→
3-Me-Ara	2.1	1.1			→2,5)-Ara*f*-(1→
Ara	2.0	1.7	2.2		→2,3,5)-Ara*f*-(1→
Total	63.3 (67.6)[c]	59.8 (55.4)	25.0 (20.3)	10.0 (10.1)	
2,3,4,6-Me_4-Gal	7.6	9.8	17.9	19.1	Gal*p*-(1→
2,4,6-Me_3-Gal	4.1	4.5			→3)-Gal*p*-(1→
2,3,6-Me_3-Gal	10.6	13.4	30.0	19.5	→4)-Gal*p*-(1→
2,3,4-Me_3-Gal					→6)-Gal*p*-(1→
2,6-Me_2-Gal	1.1	1.9	5.1	6.7	→3,4)-Gal*p*-(1→
2,4-Me_2-Gal	2.2	3.2			→3,6)-Gal*p*-(1→
Total	25.6 (25.4)	32.8 (34.6)	53.0 (58.7)	45.3 (42.0)	
3,4-Me_2-Rha	7.2	4.1	7.8	18.5	→2)-Rha*p*-(1→
3-Me-Rha	3.9	3.3	14.2	26.2	→2,4)-Rha*p*-(1→
Total	11.1 (14.2)	7.4 (9.5)	22 (20.9)	44.7 (45.7)	

[a]Some minor components, the identities of which were not confirmed, are omitted. [b]2,3,5-Me_3-Ara denotes 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylarabinitol, *etc.* [c]Values in parentheses are based on the analysis of alditol acetates.

RESULTS

Methylation analysis of pectins and their "hairy" fragments. — OHP was partly (66%) soluble in methyl sulfoxide, but the compositions in neutral sugars of the soluble and insoluble fractions were similar to that of OHP. The soluble fraction was therefore considered to be representative of OHP. Pectic materials (initial and "hairy" fragments) gave mainly the same partially methylated derivatives (Tables I and II). Only the derivatives from arabinose, galactose, and rhamnose are reported since they account for >90% of the neutral sugars. The xylose and glucose derivatives were formed in amounts too low to be characterised unambiguously. Methylation was assumed to be complete because the ratios of terminal to branched derivatives were close to unity. There was good agreement between the relative proportions of the parent sugars determined as the alditol acetates and as the partially methylated alditol acetates.

The distribution of linkages in pectins was similar to that in their "hairy" fragments, which indicated that the side-chains were not modified during the isolation procedure. Arabinofuranose, the major neutral sugar, was mainly (1→5)- and (1→3,5)-linked and terminal; small amounts of (1→3)-linked and terminal arabino-

pyranose residues were also detected. This pattern is typical of a highly branched arabinan. As judged from the values of the molar ratio of (1→5)- to (1→3,5)-linked Araf (~1 and ~2 in the HP and OHP fractions, respectively) and from the relative proportion of terminal arabinose residues, the side-chains in OHP may have a d.p. lower than those in HP. Possibly, some arabinose residues may occur also as single units attached to the other side-chains.

Galactose residues were mostly (1→4)-linked and terminal, but some (1→3), (1→3,4), and (1→3,6) linkages were detected, indicating the presence of both type I [(1→4)-linked with few branches at C-3] and II (arabino) galactans. The high proportion of terminal residues could only be accommodated by the occurrence of short galactose side-chains.

Rhamnose occurred as (1→2)- and (1→2,4)-linked residues as expected for pectic material containing neutral sugar side-chains. The increase of the ratio of (1→2,4)- to (1→2)-linked rhamnose in OHP hairy fragments when compared to OHP suggested that some unsubstituted rhamnosyl residues were interspersed in part of the smooth regions and were removed during the isolation of the "hairy" fragments.

Mild acid hydrolysis. — The "hairy" fragments, after hydrolysis with 0.05 and 0.1M trifluoroacetic acid, were chromatographed on Bio-Gel P-2. The chromatograms (*cf.* Fig. 1) showed material excluded from the gel, a main peak eluted at 350 mL, and small peaks at 315 and 285 mL. The material in each peak was analysed (Tables III and IV).

Analysis showed that peak 1 of the 0.05M acid hydrolysate contained mainly arabinose and galactose. Chromatography on Aminex HPX 87P revealed that ~66 and ~76% of the initial arabinose, and ~13 and ~8% of the initial galactose of the

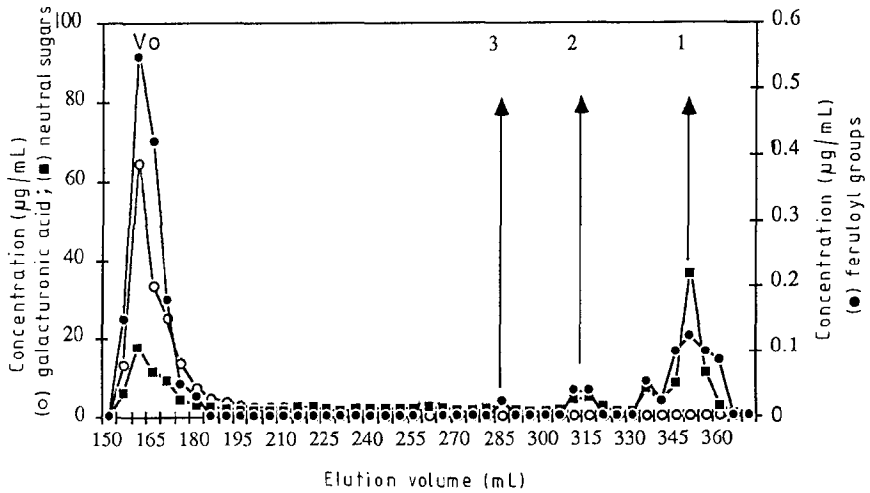

Fig. 1. Chromatography on Bio-Gel P-2 of the material obtained by hydrolysis of HP "hairy" fragments with 0.05M trifluoroacetic acid for 1 h at 100°.

TABLE III

SUGAR COMPOSITION AND FERULIC ACID CONTENT OF THE FRACTIONS DERIVED FROM THE HP "HAIRY" FRAGMENTS BY PARTIAL HYDROLYSIS WITH TRIFLUOROACETIC ACID

	0.05M Acid				*0.1M Acid*			
	Fractions				*Fractions*			
	1	*2*	*3*	V_o	*1*	*2*	*3*	V_o
Recovery	32.7[a]	3.1	2.1	54.7	49.6	2.4	1.7	36.0
Gal A				32.2				48.9
Rha				13.2	1.3			18.0
Ara	91.9[b]	28.7	26.8	27.0	51.4	30.7	61.2	12.2
Xyl				1.1				0.8
Gal	8.1	71.3	73.2	26.0	47.3	69.3	38.8	20.0
Ferulic acid	1.1	0.3	4.7	2.4	1.7	2.1		2.5

[a]Expressed relatively to the material injected onto the Bio-Gel P-2 column. [b]Expressed in relative weight percentages.

TABLE IV

SUGAR COMPOSITION AND FERULIC ACID CONTENT OF THE FRACTIONS DERIVED FROM THE OHP "HAIRY" FRAGMENTS BY PARTIAL HYDROLYSIS WITH TRIFLUOROACETIC ACID

	0.05M Acid				*0.1M Acid*			
	Fractions				*Fractions*			
	1	*2*	*3*	V_o	*1*	*2*	*3*	V_o
Recovery	30.9[a]	5.0	5.8	52.1	48.2	14.5	1.3	33.1
Gal A				37.8				55.1
Rha				12.8	1.3			20.5
Ara	92.6[b]	22.5	24.4	12.4	70.4	45.0	46.9	4.5
Xyl				1.1				0.9
Gal	7.4	77.5	75.6	35.9	28.3	55.0	53.1	18.9
Ferulic acid	0.7	1.3	1.5	1.7	1.1	1.3	0.9	1.7

[a]Expressed relatively to the material injected onto the Bio-Gel P-2 column. [b]Expressed in relative weight percentages.

HP and OHP hairy fragments, respectively, were released as monomers. The u.v. spectra of the material in this peak showed a change from a double absorption at 300 and 325 nm at pH 4.8 towards a single peak at 375 nm when the pH was adjusted to 10. This is indicative[21] of feruloyl ester groups. H.p.l.c. did not reveal carbohydrate–ferulic acid compounds, because they accounted for only a small part

of the material and were probably retained on the guard column. The delayed elution of the material containing feruloyl groups on Bio-Gel P-2 might be due to interactions of the phenolic compounds and the polyacrylamide matrix. Peaks 2 and 3 contained small amounts of material with galactose as the preponderant neutral sugar. H.p.l.c. demonstrated that these peaks contained a mixture of arabinose and galactose oligomers, mainly di- and tri-mers (Fig. 2). Ferulic esters were detected in a negligible quantity relative to that of the initial "hairy" fragments. The material at the void volume was enriched in galacturonic acid, galactose, and rhamnose at the expense of arabinose. Peaks 2 and 3 contained 73 and 68%, respectively, of the initial feruloyl groups.

The 0.1M acid hydrolysates also gave three peaks and a fraction eluted in the void volume (Tables III and IV). Peak 1 contained a higher proportion of material than that obtained from the 0.05M acid hydrolysates and was composed of ~88 and ~92% of the arabinose, ~48 and ~49% of the galactose, and ~47 and ~41%, respectively, of the feruloyl groups of HP and OHP "hairy" fragments. Material found in peaks 2 and 3 was in similar amount to that obtained after hydrolysis with 0.05M acid. There was a decrease in the material eluted in the void volume, which had a lower content of neutral sugars and a higher content of galacturonic acid than after the hydrolysis with 0.05M acid. This material was enriched in rhamnose at the expense of arabinose and galactose.

Materials in the void volume of the Bio-Gel P-2 column were eluted from a Sepharose CL-6B column, and two of the chromatograms obtained are shown in Fig. 3. For all the materials, neutral sugars, galacturonic acid, and feruloyl groups co-eluted in a single peak, the elution volume of which moved towards the total volume as compared to that of the initial "hairy" fragments and this shift was more marked after the hydrolysis with 0.1M acid. The K_{av} increased from 0.49 for the HP

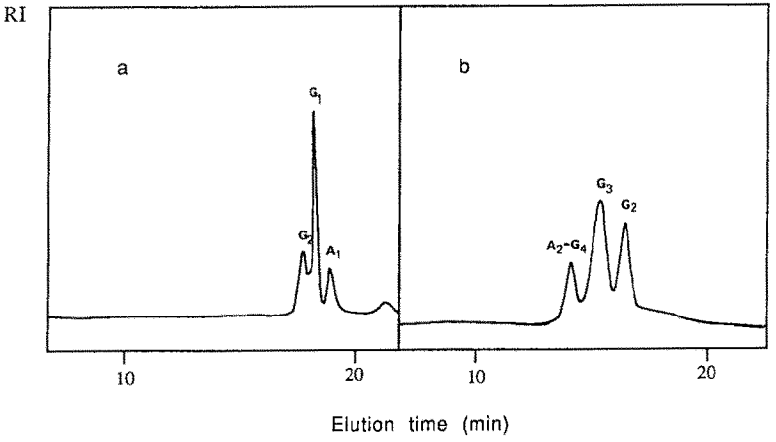

Fig. 2. H.p.l.c. of the neutral sugars in (a) peak 2 in Fig. 1 (from HP "hairy" fragments), and (b) the corresponding peak 3 from OHP "hairy" fragments: A_1, arabinose; A_2, arabinobiose, G_1, galactose; G_2, galactobiose; G_3, galactotriose.

"hairy" fragments to 0.7 and 0.9 for the HP "hairy" fragments degraded with 0.05 and 0.1M acid, respectively, and from 0.49 to 0.66 and 0.93 for the OHP "hairy" fragments. The small initial fraction of "hairy" fragments that eluted in the void volume disappeared after acid treatment.

Methylation analysis of the "hairy" fragments degraded with 0.05 and 0.1M trifluoroacetic acid. — The results are given in Tables I and II. The amounts of sugars calculated from the analyses of the alditol acetates and partially methylated alditol acetates were in good agreement. The proportions of branched and terminal residues were equivalent, showing that methylation was complete. The nature of small amounts of xylose and of glucose residues could not be determined. As anticipated, a comparison of the composition of the products obtained on acid hydrolysis of HP and OHP with that of initial "hairy" fragments showed that the arabinose side-chains were more affected than the galactose side-chains and the rhamnogalacturonic backbone. Of the total arabinose, ~32 and ~17% were recovered from the HP and OHP "hairy" fragments, respectively, after hydrolysis with 0.05M acid, which indicated a more marked degradation of the OHP arabinose side-chains. This difference was confirmed by the distribution of the linkages; the relative proportion of (1→5)-linked Ara*f* increased by 79%, whereas that of terminal and (1→3,5)-linked Ara*f* decreased by 41 and 51.3%, respectively, in the HP

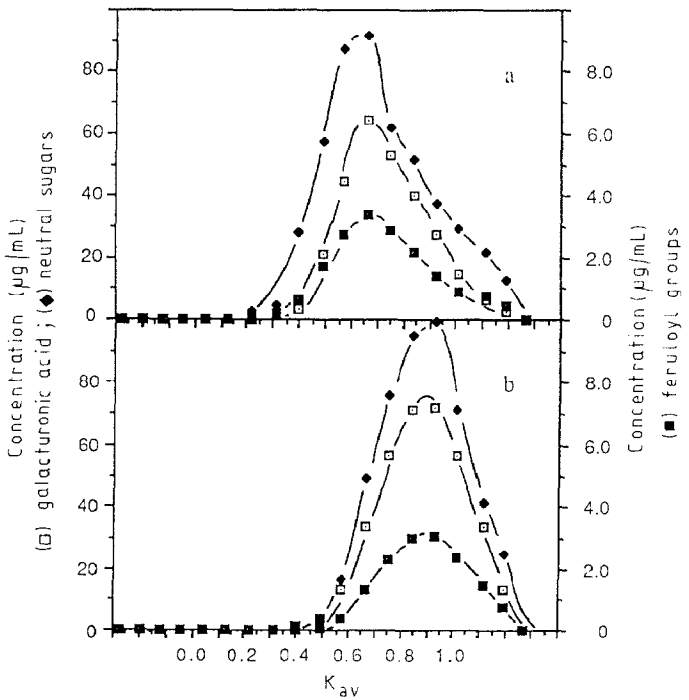

Fig. 3. Chromatography on Sepharose CL-6B of the high-molecular-weight material from HP "hairy" fragments after hydrolysis with (a) 0.05M and (b) 0.1M trifluoroacetic acid.

"hairy" fragments. (1→5)-Linked and terminal Ara*f* increased by 32 and 4.7%, respectively, whereas (1→3,5)-linked Ara*f* decreased by 52.5% in OHP "hairy" fragments. In both "hairy" fragments and especially in HP, the bonds of non-reducing terminal units were cleaved faster than the inner bonds. The degradation was more extensive with 0.1M acid, as only 9.6 and 4%, respectively, of the total arabinose were recovered in HP and OHP "hairy" fragments. (1→5)-Linked arabinose residues were also the main residues, but inner bonds were also cleaved. The contents of galactose of the degraded "hairy" fragments increased and this was more marked with 0.1M acid. As observed for arabinose, galactose residues were more extensively hydrolysed in the OHP "hairy" fragments; (1→3)- and (1→6)-linked galactose were not found in degraded "hairy" fragments. Proportions of terminal and (1→4)- and (1→3,4)-linked galactose in "hairy" fragments after hydrolysis with 0.05M acid were close to those in initial "hairy" fragments. An enrichment in (1→4) linkages at the expense of those involving terminal units were noticed with 0.1M acid, but still some branch points were found.

"Hairy" fragments degraded with acid were all enriched in rhamnose. No terminal rhamnosyl units were identified in the methylated derivatives, and the ratio of (1→2)- to (1→2,4)-linked Rha*p* was close to those previously obtained for the initial "hairy" fragments.

DISCUSSION

Methylation analysis shows the presence of terminal, (1→5)- and (1→3,5)-linked Ara, terminal, (1→4)-, (1→3)-, (1→3,6)- and (1→3,4)-linked-Gal, and (1→2)- and (1→2,4)-linked Rha as the major structural units of the neutral sugar residues of HP and OHP. Among the methylated derivatives, those from arabinofuranose preponderated and accounted for at least 60% of the neutral residues. Partial hydrolysis gave an acidic fraction that was excluded from the Bio-Gel P-2 column and three neutral peaks. Mainly arabinose residues were removed by hydrolysis with 0.05M acid, indicating that they are mainly furanose. Lower amounts of galactose and feruloyl groups were released. Arabinose and galactose were mostly released as monomers together with feruloylated sugars, the nature of which was not established. Galacto-oligosaccharides were not obtained. (1→5)-Linked and terminal arabinose, terminal and (1→4)-linked galactose, and (1→2)- and (1→2,4)-linked rhamnose were the main derivatives in "hairy" fragments after hydrolysis; (1→3)-, (1→6)-, and (1→3,6)-linked galactosyl residues were not recovered. Arabinose and galactose side-chains were more extensively degraded with 0.1M trifluoroacetic acid as shown by the enrichment in rhamnose of the residues. The shift of the elution volumes of the degraded "hairy" fragments towards the total volume on chromatography on Sepharose CL-6B suggested that some degradation occurred in the rhamnogalacturonic backbone. However, analysis showed no variations in the proportions of the rhamnosyl derivatives and in the ratio of galacturonic acid to rhamnose.

Mild acid hydrolysis did not remove all the feruloyl groups, since ~70% and ~40% remained after the treatments with 0.05 and 0.1M acid, respectively. The 30% released by 0.05M acid were assumed to be attached to the arabinosyl terminal units, which were eliminated faster than the other glycosyl units. The subsequent fraction (30%) removed with 0.1M acid would arise from the breakdown of the galactose side-chains. The remainder of the feruloyl groups may be associated with residual galactosyl residues that are still linked to the rhamnogalacturonic backbone. These results accord with previous results on spinach pectins[30].

The foregoing results suggest that HP and OHP had similar structural features. The backbone of the "hairy" fragments consists of[1-6] (1→4)-linked α-D-galactopyranosyluronic acid residues interspersed with (1→2)-linked L-rhamnosyl residues. Side-chains were attached to the backbone *via* position 4 of the rhamnosyl units. The arabinose side-chains were highly branched with a main core of (1→5)-linked-Ara*f* carrying 2-O- or 3-O-substituents. The distribution of the linkages in initial and degraded "hairy" fragments and the difference in the extent of degradation of the arabinose side-chain on acid hydrolysis suggested that they were longer in HP than in OHP. Galactose was involved in two types of structure, namely, in (1→4)-linked galactans with few branching points at positions 3, which constitute the major galactose side-chains, and highly branched (1→3,6)-linked galactans representing the minor galactose side-chains. The high proportions of terminal galactose residues could be accommodated only by the occurrence of short side-chains of galactose. The anomeric configuration of the linkages was not established. The arabinose side-chains in sugar-beet pectins have a structure close to those described in other pectins or in a neutral arabinan[22-26]. The terminal arabinopyranosyl units in HP and OHP and in their "hairy" fragments are released by treatment with trifluoroacetic acid. This sugar has been observed in commercial arabinan from sugar beet[27], pectins from spinach[17,28], and neutral arabinans from white willow[29], Marshmallow[30], and *Rosa glauca*[31]. These pyranose residues were proved[28] to carry the feruloyl groups in the arabinan side-chains of spinach. Such evidence has not been obtained in our experiments. (1→4)-Linked galactose side-chains are more similar to those described in apple pectins[26] than those encountered in soya bean[32], lupine[33,34], potato tuber[35], or tobacco pectins[36]. The higher acid degradability of the OHP galactose side-chains, when compared to those of the HP "hairy" fragments, may be explained by the differences in the amounts of feruloyl groups attached to galactose residues and by the presence of galactosyl units attached to some galacturonic acid residues. (1→3,6)-Linked galactose residues have been found in significant amounts in pectins from different sources[38-43] and originate from the arabinogalactan side-chains. According to Clarke *et al.*[44], the arabinogalactans associated with pectins may correspond to the carbohydrate moiety of the arabinogalactan-proteins. In purified beet pectins, hydroxyproline-rich proteins have been found, but only ~30% were recovered in the "hairy" fragments[10] and there was no marked decrease in the (1→3)- and (1→3,6)-linked galactose residues. This finding suggests that all the proteineous fractions were not covalently linked to pectins through

arabinogalactan side-chains. After hydrolysis with trifluoroacetic acid, (1→3)- and (1→6)-linked galactose residues were not recovered in the "hairy" fragments. The origin and the nature of the linkages between pectins and these arabinogalactans are not clear, and there is no evidence that they function as covalent bridges between pectins and proteins within the walls. Further studies are necessary in order to characterise these proteins and their association with pectins.

In the following paper[45], the use of enzymes and the analysis of the products confirm these results and give more information on the location of the feruloyl groups.

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