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Registry No. Pectin, 9000-69-5; arabinose, 147-81-9; rhamnose, 3615-41-6; galactose, 59-23-4; mannose, 3458-28-4; glucose, 50-99-7; galacturonic acid, 685-73-4; sucrose, 57-50-1; fructose, 57-48-7; sorbitol, 50-70-4; malic, 6915-15-7; citric, 77-92-9; quinic, 77-95-2.

Studies on Vegetables. Investigation of an Arabinan from Parsnip (*Pastinica sativa*)†

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An arabinan isolated from parsnip was shown by sedimentation studies to be homogeneous, and methylation revealed a highly branched structure. Hydrolysis of the fully methylated polysaccharide yielded 2,3,5-tri-*O*-methyl-L-arabinose (11 mol), 2,3-di-*O*-methyl-L-arabinose (20 mol), 3-*O*-methyl-L-arabinose (trace), 2-*O*-methyl-L-arabinose (7 mol), and L-arabinose (2 mol). The general structural features of the arabinan are discussed.

Arabinans are widely distributed in the plant kingdom, being found in, among other things, seeds, vegetables, and fruits. These polymers tend to fall into two groups: those that are associated with pectins and may be released by alkaline degradation during the isolation and fractionation procedures and those believed to be natural homoglycans such as those found in mustard, soybean, rapeseeds, and parsnip.

In order to eliminate the possibility of degradation due to β-elimination, very mild conditions were used for the removal of starch and subsequent fractionation and purification of an arabinan from parsnip. The present communication deals with the general structural features of this polysaccharide.

EXPERIMENTAL SECTION

The general experimental methods have been reported previously (Siddiqui, 1990).

Removal of Starch. The 80% ethanol insoluble residue (60 g) (Siddiqui, 1990) from parsnip in 0.2 M acetate buffer (pH 4.50) (2.5 L) was treated with (800 mg) amyloglucosidase (*Aspergillus orysae*; Sigma). The surface was layered with toluene, and the suspension was stirred for 48 h, at room temperature. The digested material was dialyzed (Spectrapore membrane tubing, diameter 32 mm, molecular weight cutoff 12 000–14 000) for 48 h against running tap water and 4 h against distilled water. The sample following concentration to a thick slurry (1.5 L) was added to ethanol (4 volumes) and allowed to settle. The clear supernatant was removed by siphoning and filtration, and the insoluble residue was washed with ethanol and acetone and air-dried for 24 h to yield a dry fluffy residue (25 g).

The clear supernatant was concentrated to a small volume and freeze-dried to yield the 80% ethanol soluble fraction (1 g). Acid hydrolysis of a portion (5 mg) with 72% sulfuric acid (general methods) showed mainly glucose and arabinose with traces of galactose, mannose, xylose, and uronic acid.

Table I. GLC and CI-MS Data for Sugars from Methylated Arabinan

compound	ret time (acetate), min	molar ratio	CI-MS [MH$^+$]	mode of linkage
2,3,5-trimethylarabinitol	0.77	11	279	L-Ara*f*-(1→
2,3-dimethylarabinitol	0.86	20	307	→5)-L-Ara*f*-(1→
2-methylarabinitol	0.93	7	335	→3,5)-L-Ara*f*-(1→
3-methylarabinitol	0.95	trace	335	→2,5)-L-Ara*f*-(1→
arabinitol	1.00	2	303	→2,3,5)-L-Ara*f*-(1→

Fractionation of Polysaccharides. The polysaccharide (~1 g, ethanol soluble fraction) was fractionated on a column (3 × 35 cm) of DEAE-cellulose (borate form) (Neiekom and Kuendig, 1965). Elution with water (600 mL), with collection of effluent in 10-mL fractions, yielded two water-eluted fractions, W_1 (tubes 15–27, negative rotation) and W_2 (tubes 29–50, positive rotation). Gradient elution with 0–0.5 M sodium metaborate (1 L) gave an acidic fraction. The neutral fractions were dialyzed (20 h against running tap water, 4 h against distilled water) and freeze-dried to yield materials W_1 (124 mg) and W_2 (154 mg). The borate fraction was acidified with acetic acid and similarly recovered following dialysis and freeze-drying to yield acidic material (180 mg).

GLC of alditol acetates prepared from the hydrolysates of W_1 and W_2 following reduction and acetylation (general methods) showed arabinose (83.2%), galactose (7.4%), glucose (8.2%), and mannose (1.2%) in the former and arabinose (16.9%), galactose (2.3%), and glucose (80%) in the latter.

Further Purification of the Arabinan. Fraction W_1 (119 mg) was fractionated on a column (2.5 × 30 cm) of Sephadex G-75 (Granath, 1965). Elution with water, with collection of effluent in 10-mL fractions, yielded fraction 1 (tubes 1–25, positive rotation) and fraction 2 (tubes 27–45, negative rotation). Fraction 2 (68 mg) was recovered following concentration and freeze-drying.

Paper chromatography of a hydrolysate revealed arabinose with a trace of glucose.

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Table II. Comparative Data of Some Arabinans of Plant Origin

acetate derivative	soybean[a]	lemon peel[a]	mustard seed[a]	rapeseed[b]	rapeseed[c]	apple juice[d]	apples[e]	cabbage[f]	parsnip[g]
2,3,5-tri-*O*-methylarabinitol	39.2	30.0	39.6	40.7	32	2	36	39.2	27.5
2,3,4-tri-*O*-methylarabinitol						3			
2,3-di-*O*-methylarabinitol	30.0	38.4	25.4	25.9	32	93	32	27.8	50.0
2,5-di-*O*-methylarabinitol							4		
2-*O*-methylarabinitol	14.2	15.0	28.6	25.9	28	3	20	15.2	17.5
3-*O*-methylarabinitol	6.0	4.6	trace	trace					trace
arabinitol	10.5	12.0	6.3	7.4	8		8	17.8	5.0
$[\alpha]_D$,[h] deg	−108	−140	−173	−181	−111	−127.3	−180		−122.3
	(−130)	(−153)		(−165.7)					(−99.7)

[a] Aspinall and Cottrell, 1970, 1971. [b] Siddiqui and Wood, 1974. [c] Lar et al., 1975. [d] Churms et al., 1983. [e] Aspinall and Fannous, 1984. [f] Stevens and Selvendran, 1980. [g] Present study. [h] $[\alpha]_D$ for methylated polysaccharides in parentheses.

Analysis of Parsnip Arabinan. The arabinan had $[\alpha]^{25}_D$ −122° (*c* 0.37, water). Sedimentation analysis (Svedberg and Pedersen, 1940), using a synthetic boundary cell and a 1.2% solution in 0.1 M sodium chloride at 28 000 rpm, showed a single symmetrical peak. The arabinan (3.0 mg) was hydrolyzed with 0.25 M sulfuric acid (0.5 mL) for 3 h at 100 °C. The hydrolysate was reduced, acetylated, and examined by GLC in the usual way (general methods). The results showed the presence of only arabinitol pentaacetate.

Acid Hydrolysis of the Arabinan. The arabinan (22 mg) was hydrolyzed with 0.25 M sulfuric acid (1 mL) at 100 °C for 3 h. Neutralization ($BaCO_3$), filtration, and evaporation yielded a syrup (12 mg) that crystallized from 95% aqueous ethanol, giving β-L-arabopyranose: mp and mixed mp 149–151 °C; $[\alpha]^{24}_D$ +116° (5 min) → 105° (equilibrium) (*c* 0.33, water).

Methylation of Arabinan. The dried arabinan (26 mg) was dissolved in dry dimethyl sulfoxide (1.2 mL) by stirring at 50 °C. The solution was flushed with nitrogen, and a 2 M solution (0.3 mL) of methylsulfinyl carbanion (Hakomori, 1965) was added dropwise. The viscous solution was stirred magnetically and periodically on a Vortex mixer for 4.5 h. Methyl iodide (0.2 mL) was added dropwise, and the solution was stirred for 1 h. After a second and a third addition of methylsulfinyl carbanion and methyl iodide (second addition, 0.3 mL of carbanion (16 h) and 0.2 mL of iodide (1 h); third addition, 0.3 mL of carbanion (6 h) and 1.5 mL of iodide (1 h)), the solution was poured in water (5 mL) and mixture dialyzed (Spectrapore tubing, molecular weight cutoff 6000–8000) for 18 h and concentrated to a syrup: 30 mg; $[\alpha]^{25}_D$ −99.7° (*c* 1.0, chloroform); no IR absorption for hydroxyl.

Analysis of Methyl Sugars. The methylated arabinan (25 mg) in 3% methanolic hydrogen chloride (10 mL) was boiled under reflux for 24 h. After neutralization (silver carbonate), filtration, and evaporation, the syrupy product was hydrolyzed with 0.5 M sulfuric acid (2.5 mL) at 100 °C for 30 h. The hydrolysate was neutralized (barium carbonate), filtered, and concentrated to a syrup (22 mg). PC (solvent C) of a solution of methyl sugars showed four components: R_f 0.5, 0.19, 0.42, and 0.85, corresponding to arabinose, 2-methyl, 2,3-dimethyl, and 2,3,5-tri-*O*-methyl ethers of arabinose.

A portion (3 mg) of the methylated sugars was reduced with sodium borohydride, and products were acetylated and examined by GLC (general methods). The identity of the components (Table I) was confirmed by GLC–EI-MS (Bjorndal et al., 1967) and the respective $[MH]^+$ ions by GLC–CI-MS (Horton et al., 1974).

RESULTS AND DISCUSSION

The 80% aqueous ethanol insoluble residue from parsnip (*Pastinica sativa*) following removal of starch on fractionation with ethanol yielded a starch-free residue (41%) insoluble in 80% ethanol and a soluble material (1.6%). Acid hydrolysis of the latter fraction produced inter alia major amounts of arabinose and glucose.

Fractionation of the polysaccharide material from the ethanol soluble fraction on DEAE-cellulose (borate form) yielded two neutral fractions, W_1 and W_2, and an acidic fraction. The water-eluted fractions, on acid hydrolysis, gave mainly arabinose (83%) in the former fraction and mainly glucose (80%) in the latter fraction.

A final purification of the arabinan (fraction W_1) was effected on Sephadex G-75, giving the pure arabinan, in an overall yield of 4% of the ethanol soluble material, which on hydrolysis produced arabinose with negligible traces of glucose.

The parsnip arabinan had $[\alpha]^{25}_D$ −122°. Sedimentation analysis (Svedberg and Pedersen, 1940) showed a single symmetrical peak. After mild hydrolysis with acid, L-arabinose was recovered in a crystalline form.

The polysacchride was methylated, then methanolyzed, and hydrolyzed. The identities and quantitative composition of the components were determined by GLC and were further confirmed by GLC–EI-MS (Bjorndal et al., 1967) and GLC–CI-MS (Horton et al., 1974).

Methylation analysis showed that the molecule has a highly branched structure, containing eight terminal, nonreducing L-arabinose residues. The disparity in the number of reducing ends to branch points (0.7:1 instead of 1:1) is ascribed to a preferential loss of the more volatile arabinose methyl ether during evaporation. After correction for losses (Table I), an average statistical unit consisted of 40 sugar residues, containing 11 terminal, nonreducing L-arabinose residues. There are 9 L-arabinose involved in branching, including 7 through positions 3 and 5 and 2 through positions 2, 3, and 5. The nonterminal residues consist of 20 (1→5)-linked L-arabinose residues. The molecular weight of mustard seed arabinan, determined by sedimentation equilibrium and vapor pressure osmometry corresponds to a molecule containing about 45 sugar units (Rees and Steele, 1966), a figure very close to that found in the present study. The highly negative specific rotations of the unmethylated ($[\alpha]_D$ −122°) and methylated ($[\alpha]_D$ −99.7°) polysaccharides suggest that the majority, if not all, of the sugar residues are those of the α-L type.

The differences between the various arabinans (Table II) except the one from apple juice (Churms et al., 1983) are on the whole minor. The configuration of the linkages is the same in each polysaccharide, but there are some quantitative differences in the proportion of linkages. The absence, or detection only in traces, of 3-*O*-methylarabinose in a majority of cases, however, may provide a distinction between the soybean and lemon peel arabinans and those from other sources.

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Chemical Structures of Green Coffee Bean Polysaccharides

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The chemical structures of green coffee bean polysaccharides have been determined by methylation analysis of whole beans and of extracted polymer-rich fractions. Ground beans were completely solubilized in 4-methylmorpholine *N*-oxide, and the Hakomori methylation procedure was applied directly. The polysaccharide fraction in Robusta beans constitutes 48% of the dry weight and is principally composed of three polymers: arabinogalactan, mannan, cellulose. The arabinogalactan has a β1→3-linked galactan main chain with frequent arabinose and galactose residue containing side chains, whereas the mannan resembles cellulose in that it has a linear β1→4-linked structure. Only occasional single-residue galactose side chains are present in the mannan fraction. Polysaccharide compositions of Robusta and Arabica beans are similar; the only significant difference is the higher content (approximately 3%) of arabinogalactan in the former type.

On a dry-weight basis, almost half of the green coffee bean is made of polysaccharides. In spite of the obvious importance of this fraction with respect to coffee processing, a complete structural characterization of the polysaccharides in green coffee beans has not yet been published. The most detailed structural studies are those of Wolfrom et al. (1960, 1961) and Wolfrom and Patin (1965) who identified an arabinogalactan, a mannan, and cellulose in green coffee. The arabinogalactan was characterized as having a β1→3-linked galactan main chain with two unit (Araβ1→3-Gal1–) side chains. The mannan was shown to be a linear, β1→4-linked polymer with a comparatively low molecular weight (7000) and a low degree of side chain substitution. Hashimoto (1971) used galactanase enzymes to probe the structure of coffee arabinogalactan. He verified the β1→3-galactan backbone and concluded that about two-thirds of the galactose residues were substituted at C6 either with (Araβ1→3-Gal1–) side chains or single arabinose (furanoside or pyranoside forms) or galactose residues.

These studies utilized pure polymers isolated from coffee beans in small yields, and the structures determined were not necessarily representative of the whole polysaccharide fraction that produced arabinose, galactose, and mannose on hydrolysis. A number of publications on coffee polysaccharides have been produced by Thaler's group [see, for example, Thaler (1979) and references therein] and characterization is as the hydrolysis products: arabinose, galactose, mannose, and glucose. Both mannan and galactomannan have been referred to as components of coffee beans (Clifford, 1985); however, structural evidence for the latter polymer type has not been produced. One of the objectives of this study was to characterize the polymeric fraction that yields mannose on acid hydrolysis.

By applying high-resolution GC–MS, the classical methylation procedure can be used for the structural analysis of complex polysaccharide mixtures. Also, it has been shown that, by nondegradative presolubilization in the *N*-oxide solvent, 4-methylmorpholine *N*-oxide, it is possible to utilize the Hakomori methylation procedure to obtain a linkage analysis of cell wall material (Joseleau et al., 1981). In this publication, the application of these methods to define the structure of coffee bean polysaccharides is described.

EXPERIMENTAL SECTION

Polysaccharide Content. Ground, green Ivory Coast Robusta coffee beans were Soxhlet-extracted overnight with chloroform/

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