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Note

Primary structure of two arabinogalactans from the water-soluble polysaccharides of field-bean *(Dolichos lablab)* hulls

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As part of our studies on the chemical, functional, and nutritional role of food carbohydrates, various starchy and nonstarchy polysaccharides of the field-bean (*Dolichos lablab*, variety lignosus) endosperm and hulls have been isolated and characterized[1]. Some structural features of the cold-water-soluble polysaccharides are described in this communication.

The cold water-soluble fraction obtained in 0.83% yield from the 70% alcohol-insoluble residue contained ~25% protein contamination and was purified by proteolysis with pronase. The resulting carbohydrate-rich fraction on hydrolysis contained arabinose and galactose in 2:1 ratio, and had a uronic acid content of 12.5%. The fraction was thus an arabinogalactan type of polysaccharide.

Chromatographic resolution on DEAE-cellulose (PO_4^{3-}) resolved the polysaccharide into four fractions (Table I). Fractions eluted with water and 0.1M phosphate in yields of 37 and 41% were designated arabinogalactans 1 and 2. The basis for the charge differences of these two groups of arabinogalactans is not known at present. Although the carbazole method showed a uronic acid content of 2.5% in fraction 2, chromatographic examination of the acid hydrolyzate revealed no acidic sugars. Charge heterogeneity has been observed in arabinogalactans from other plant sources[2,3]. Both arabinogalactans were electrophoretically homogeneous[4] and gave a single, symmetrical peak on sedimentation analysis. Molecular-sieve analysis on a precalibrated column of Bio-gel P-200 also indicated homogeneity, with $\overline{M}_n$ values of 93,000 and 1,200,000, respectively. Acid hydrolysis of the fractions and g.l.c. of the derived alditol acetates showed arabinose and galactose in molar proportions of 1:2 and 1:1.2, respectively. Partial hydrolysis of the arabinogalactans released most of the arabinose residues, indicating their labile furanosidic nature and also their presence in the side-chain.

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TABLE I

CHEMICAL COMPOSITION (%) OF ARABINOGALACTAN FRACTIONS AFTER SEPARATION ON DEAE-CELLULOSE

Polysaccharide fraction	*Yield (%)*	[α]D *(degrees)*	*Total sugar*	*Constituent sugars*			*Galactose: arabinose ratio*	*Periodate reduced (mol/sugar residue)*	*Formic acid released (mol/sugar residue)*
				Gal	*Ara*	*GalA*			
1	37.0	+180 (*c*, 1 in H_2O)	89.4	62.6	26.8		2.34	0.60	0.13
2	41.0	+120 (*c*, 0.5 in 0.1M NaOH)	89.5	45.0	44.5	2.5	1.00	1.03	0.25
3	9.5	[a]	90.4	56.0	28.0	6.5	2.00	[a]	[a]
4	12.8	[a]	92.2	65.1	16.3	10.8	3.90	[a]	[a]

[a]Not determined.

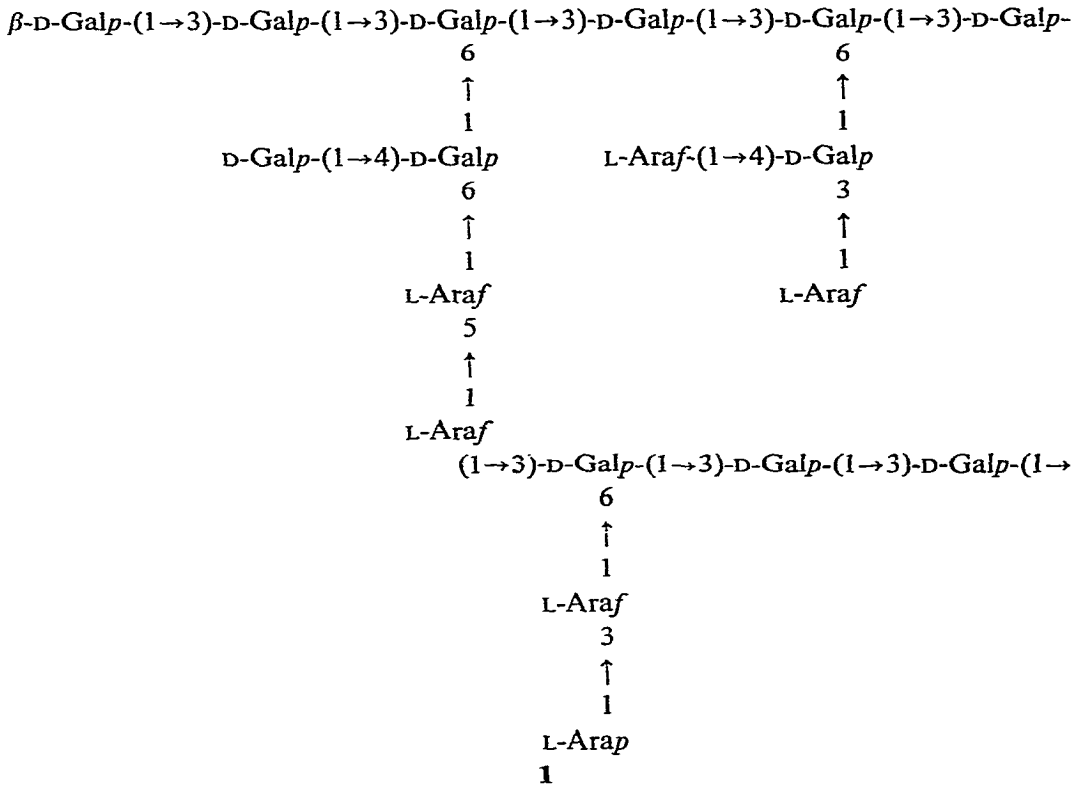
TABLE II

METHYLATION ANALYSES OF ARABINOGALACTANS 1 AND 2 FROM FIELD-BEAN HULLS

Parent sugar	*Peak no.*	*Methyl ether*	*Mode of linkage*	R_T	*Relative molar yield*
Arabinogalactan 1					
Arabinose	1	2,3,5-tri	L-Ara*f*-(1→	0.48	3.7
	2	2,3,4-tri	L-Ara*p*-(1→	0.73	1.1
	3	2,5-di	→3)-L-Ara*f*-(1→	1.10	0.7
	4	2,3-di	→5)-L-Ara*f*-(1→	1.14	5.1
Galactose	5	2,3,4,6-tetra	D-Gal*p*-(1→	1.25	3.4
	6	2,4,6-tri	→3)-D-Gal*p*-(1→	2.28	8.0
	7	2,6-di	→3,4)-D-Gal*p*-(1→	3.60	3.2
	8	2,3-di	→4,6)-D-Gal*p*-(1→	5.68	1.0
	9	2,4-di	→3,6)-D-Gal*p*-(1→	6.35	3.8
Arabinogalactan 2					
Arabinose	1	2,3,5-tri	L-Ara*f*-(1→	0.48	6.0
	2	2,3-di	→5)-L-Ara*f*-(1→	1.14	9.7
Galactose	3	2,3,4,6-tetra	D-Gal*p*-(1→	1.25	0.4
	4	2,3,4-tri	→6)-D-Gal*p*-(1→	3.40	8.8
	5	2,4-di	→3,6)-D-Gal*p*-(1→	6.35	5.6

After methylation of the arabinogalactans by the method of Hakomori[5], the products showed no i.r. absorption for unsubstituted hydroxyl groups. The permethylated polysaccharides were sequentially hydrolyzed, reduced, and *O*-acetylated[6], and the identities and molar proportions of the sugars determined by g.l.c. and combined g.l.c.–m.s.[7] (Table II).

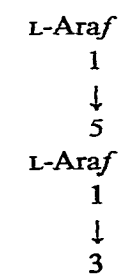
Arabinogalactan 1 was thus found to have an average repeating-unit of 30 sugar residues, of which there are eight terminal, non-reducing end groups (4 L-arabinofuranosyl, 1 L-arabinopyranosyl, and 3 D-galactopyranosyl), besides five (1→5)-linked and one (1→3)-linked L-arabinofuranose residues. There are eight residues of (1→3)-linked D-galactose and eight residues of D-galactose involved in branching, four through O-6, three through O-3, and one through O-4. The molar ratios of arabinose and galactose in the native (1:2) and methylated (1:1.8) polysaccharides, as well as the proportions of terminal (non-reducing) and branching sugar-residues (1:0.98), were in good agreement. Methylation data thus demonstrated the presence in arabinogalactan 1 of D-galactosyl residues mutually joined by (1→3) and (1→6) linkages, the former preponderantly in interior chains and the latter mainly in exterior chains. Residues of L-arabinofuranose, and to a smaller extent L-arabinopyranose, terminated some of the outer chains, as depicted in the partial structure (**1**) (the sugar sequence is purely arbitrary).

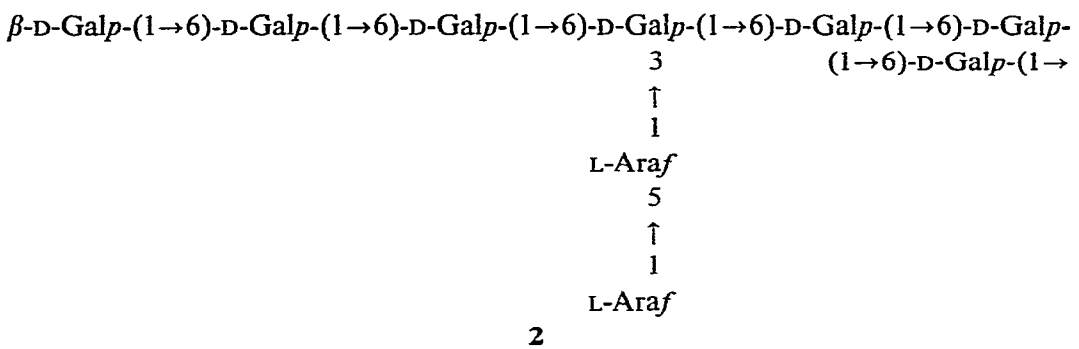


Both furanosidic and pyranosidic L-arabinose residues have been noted in arabinogalactans from other sources[8].

The results of periodate oxidation were in good agreement[8] with the proposed structure: periodate consumption, ~0.60 mol, expt 0.63 mol; and formic acid liberated, ~0.13 mol, expt 0.10 mol, per sugar residue. Hydrolysis of the reduced oxopolysaccharide yielded glycerol and threitol, together with intact galactose, indicating again a branched structure for the arabinogalactan 1. The high positive $[\alpha]_D$ value (+180°) for the unmethylated polysaccharide, as well as the release of galactose on treatment with β-D-galactosidase, point to glycosidic links of the β-D type.

In contrast, methylation-analysis data on arabinogalactan 2 demonstrated a (1→6)-linked backbone of D-galactose residues with branch points at O-3. Doubly substituted residues of L-arabinofuranose terminated the branching centres, as shown in the partial structure (2) (the sugar sequence is purely arbitrary).





This structural assignment was substantiated by the results of periodate oxidation and borohydride reduction followed by acid hydrolysis. Glycerol was the major product, together with galactose. The periodate consumption (1.03 mol) and formic acid liberation (0.25 mol) per sugar residue were in reasonable agreement with the calculated molar values of 0.98 and 0.20.

The release of galactose after incubation of the fraction with β-D-galactosidase suggested β-D-galactose residues at the non-reducing terminal.

Arabinogalactans of plant origin, found either as a general hemicellulosic constituent of coniferous woods, as in larch[9] or as a cell-wall constituent of pectic substances as in the soybean[10–12], have been studied in considerable depth. Fieldbean hull arabinogalactans resemble larch arabinogalactan in that both are highly branched polymers containing β-(1→3) and β-(1→6) glycosidic links in the backbone, and have a variety of sidechain linkages. Two highly branched arabinogalactans have been characterized in rapeseed flour[2,13]. One resembles larch arabinogalactan but contains, in addition, terminal residues of D-glucuronic acid and thus appears to be closely related to the arabinogalactan from coffee beans[14]. The other arabinogalactan is a neutral polysaccharide having a much lower content of galactose[13]. In contrast, the arabinogalactans from lucerne (alfalfa), gum tragacanth, lemon peel, and centrosema seed are distinguished by all three inter-galactosidic linkages[15]. The arabinogalactans in plant tissues have been implicated in such diverse functions as cell adhesion, nutrition of growing pollen-tubes, response to microbial infections, and markers of identity expressed in the terminal sequences of the saccharide chains[16].

EXPERIMENTAL

General methods. — Descending paper-chromatography was carried out on Whatman Nos. 1 and 3 MM papers with solvent systems (*a*) 10:1:2 butyl alcohol–ethanol–water; (*b*) 8:2:1 ethyl acetate–pyridine–water; and (*c*) 7:1:2 propyl alcohol–ethanol–water. T.l.c. was performed on Polygram-Sil G, precoated sheets (Macherey Nagel Co., Germany) with (*d*) 5:2 ethanol–benzene or (*c*). Sugars were detected by spraying with *p*-anisidine · HCl[17], or ammoniacal silver nitrate[18] or aniline phthalate[19] reagents. G.l.c. was carried out on a Varian Aerograph series 1400 gas chromatograph with columns of (*e*) 3% ECNSS-M on Gas Chrom Q (100–120 mesh) at

170°, or (*f*) 3% OV-225 on Chromosorb WHP (80–100 mesh) at 180°. Retention times (R_T) are given relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol as standard. For combined g.l.c.–m.s., a U-shaped column (*f*) was used in a Finnigan quadrupole system mass spectrometer (model 3200 E) at an operating temperature of 170°, ionization potential 70 eV, mass range 40–300, and integration time 7 ms/scan. Electrophoresis[4] was performed either on cellulose acetate membranes (Beckman Microzone electrophoretic cell, model 101) or Millipore Phoroslide electrophoresis strips in (*g*) ammonium carbonate buffer (0.05M, pH 9.3) at an applied voltage of 180 V. I.r. spectra were recorded with a Hilger–Watts Infragraph spectrophotometer. Specific rotations were measured in aqueous solution by using a Carl Zeiss polarimeter. Unless otherwise stated, all evaporations were conducted *in vacuo* at 40°. Total sugar, pentose, and uronic acid were estimated by phenol–sulfuric acid[20], phloroglucinol[21], and modified carbazole[22] methods, respectively. The polysaccharides were hydrolyzed with 0.5M sulfuric acid for 8 h at ~100°, made neutral (solid barium carbonate), and deionized (Amberlite IR-120, H^+ resin). Field-bean seeds were purchased in a local market during December 1977. The hulls were removed manually from the freeze-dried seeds.

Fractionation of crude, water-soluble polysaccharide. — The crude fraction (1.25 g) was fractionated on a column (2 × 80 cm) of DEAE-cellulose (PO_4^{3-} form). Elution with water followed by successive elutions with 0.1→0.5M phosphate buffer, and collection of the effluent in 15-mL portions afforded four fractions. Acid hydrolysis of fractions 1 and 2 revealed arabinose and galactose, whereas fractions 3 and 4 contained considerable amounts of galacturonic acid, in addition to these two sugars.

Electrophoresis of the dyed[4] (Procion Brilliant Red 2BS) fractions 1 and 2, as well as sedimentation analysis using a synthetic boundary cell and a 1% solution in 0.1M sodium chloride at 57,600 rev./min indicated their homogeneity. Molecular-sieve chromatography on a pre-calibrated column (1 × 120 cm) of Bio-gel P-200 gave $\overline{M}_n$ of 93,000 and 1,200,000, respectively, for fractions 1 and 2. Partial hydrolysis (0.05M sulfuric acid, 1 h at 90°) of the arabinogalactans released most of the arabinose residues.

Methylation analyses. — Polysaccharides (2 mg) were permethylated by the method of Hakomori. The fully methylated products, after purification by passage through Sephadex LH-20, showed a very weak i.r. absorption for hydroxyl groups. The methylated products were depolymerized, and the resulting methyl ethers reduced and *O*-acetylated. The derived alditol acetates were analysed by g.l.c. and g.l.c.–m.s. Methylated alditol acetates were also identified by single-ion mass-fragmentographic monitoring[23].

Periodate oxidation. — Arabinogalactans 1 and 2 (5 mg) were dissolved in water (1 mL) and 0.1M sodium metaperiodate was added and the solutions kept at 4° in the dark. Aliquots (50 μL) were withdrawn at regular intervals and examined for consumption of periodate[24]. Formic acid liberated was estimated by the method of Brown[25].

The oxopolysaccharides from periodate oxidation were treated with ethylene glycol (a few drops), dialyzed, and the solutions concentrated. The concentrates were reduced with sodium borohydride for 18 h at room temperature. The excess of borohydride was decomposed with dilute acetic acid and the solutions deionized and concentrated. Borate ions were removed by evaporation of methanol from the residue, which was then hydrolyzed (125mM sulfuric acid for 15 h at 100°).

Enzymic hydrolysis. — Solutions of polysaccharides (10 mg of each) in phosphate buffer (0.05M, pH 7.2) were incubated at 37° with β-D-galactosidase (11 units/mg protein) for 16 h with a drop of toluene. The reaction was stopped by adding alcohol, and the mixture deionized and concentrated. Both p.c. and t.l.c. revealed considerable amounts of free galactose.

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