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Technical Note

Structural Features of an Arabinan from Cowpea (*Vigna sinensis*)

ABSTRACT

The purified arabinan, isolated by 10% trichloroacetic acid extraction of cowpea endosperm, consisted of 1,5-linked L-*arabinofuranosyl residues in the main chain, some of which were further involved in branching through O-2 and/or O-3.*

INTRODUCTION

Earlier reports on the cowpea (*Vigna sinensis*) carbohydrates are confined only to the analysis of 70% alcohol-soluble sugars (Akpapunam & Markakis, 1979; Longe, 1980; El Faki *et al.*, 1983*a*), physico-chemical characterization of starch (El Faki *et al.*, 1983*b*) and preliminary nutritional studies (*in vitro* and *in vivo*) (El Faki *et al.*, 1983*c*) of the various dietary fibre components. No information is available, as such, on the compositional and structural features of the mucilaginous polysaccharide-complex of cowpea. This note summarizes the main features of the molecular structure of a water-eluted fraction of cowpea polysaccharide-complex.

EXPERIMENTAL

General methods

The arabinan was hydrolyzed with N H_2SO_4 at 100°C for 6 h and other polysaccharides were hydrolyzed by the 72% H_2SO_4 solubilization method

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(Paramahans & Tharanathan, 1982). Quantitation of the released sugars as alditol acetates was done by isothermal (190°C) GC on OV-225, 3% on a Chromosorb W-packed column (6 ft × $\frac{1}{8}$ in). Combined GC–MS was performed with a Finnigan MAT 1020 instrument equipped with a CP-Sil 5 capillary WCOT column (Paramahans *et al.*, 1984). Total sugar (McKelvy & Lee, 1969) and uronic acid (Knutson & Jeanes, 1968) were estimated by standard methods. The sedimentation behaviour of the arabinan in 0·1 M NaCl solution was carried out in a Spinco Model E analytical ultracentrifuge at a rotor speed of 57 600 rpm. Molecular sieving was effected on Sephadex G-200 and Sephacryl S-400 which were calibrated with the dextrans of known $\bar{M}_n$. Microzone electrophoresis (Anderson *et al.*, 1971) was performed on cellulose acetate membranes (Phoroslides) in a Beckman microzone electrophoretic cell and using ammonium carbonate–sodium chloride buffer (pH 4·8, 0·05M) at 180 V. Optical rotation of the arabinan (1% solution in water) was measured in a Perkin-Elmer 243 polarimeter.

Isolation of arabinan

Cowpea endosperm (500 g, 60 mesh powder) was repeatedly extracted with 10% trichloroacetic acid (TCA) (3 litres) for 6 h at 4°C. The extract was precipitated with acetone (three volumes). The precipitate in water was dialyzed and lyophilized. The polysaccharide-complex (1 g) was fractionated on DEAE-cellulose (CO_3^{2-}) by successive elutions with water, ammonium carbonate (0·1 and 0·2M) and sodium hydroxide (0·2 and 0·3M). The flow rate was 60 ml h^{-1} and 10 ml fractions were collected. The elution was monitored by the phenol–sulphuric acid method (McKelvy & Lee, 1969).

Methylation analysis

The arabinan (2 mg) was permethylated by the Hakomori (1964) method, acid hydrolyzed and derivatized (1-$^2H_1$ alditol acetate).

Periodate oxidation and Smith degradation

The arabinan (10 mg/10 ml) was oxidised with sodium metaperiodate (20 mM) at 4°C in the dark. At regular time intervals aliquots (10 μl) were removed and iodate consumed was determined by Avigad's (1969) method. After 96 h the oxopolysaccharide was sodium borohydride (20 mg) reduced (Abdel-Akher *et al.*, 1952), the excess borohydride decomposed and the product hydrolyzed (0·5N H_2SO_4 at room temperature for 48 h).

RESULTS AND DISCUSSION

Extraction, with 10% TCA, of cowpea endosperm furnished a polysaccharide-complex (PC) in 1·1% yield. An undegraded pentosan had earlier been isolated by 15% TCA extraction of wheat flour (Tran & Nordin, 1977). The TCA extraction method is claimed to be superior for the isolation of high molecular weight, protein-free polysaccharide and also, due to its low pH, the recovered material is not subjected to any inadvertent enzymic modification. Sugar analysis revealed arabinose to be the major constituent, together with a large proportion of xylose and galactose and smaller amounts of rhamnose, mannose and galacturonic acid.

DEAE–Cellulose (CO_3^{2-}) fractionation of PC and water elution gave a neutral polysaccharide followed by four acidic polysaccharides eluted with ammonium carbonate (0·1 and 0·2M) and sodium hydroxide (0·2 and 0·3M). The yield of the fractions and their carbohydrate profile (see Table 1) suggested, in particular, considerable heterogeneity of all the acidic fractions. Fraction 1 (Fra. 1) appeared to be an arabinan-type polysaccharide. On a 100 g endosperm basis, the yield of Fra. 1 was 0·25%.

Examination by cellulose acetate membrane electrophoresis, sedimentation analysis and molecular sieve chromatography of Fra. 1 showed it to be homogeneous. Its $\bar{M}_n$, determined on a precalibrated Sephacryl S-400 column, was ~11 600 which was comparable with the values reported for several of the arabinans (Capek *et al.*, 1983). On acid hydrolysis and alditol acetate derivatization, GC analysis showed exclusively arabinose.

An examination by GC–MS of the *O*-methyl ethers of permethylated Fra. 1 showed the presence of 2,3,5-tri, 2,3-di, 2- and 3-mono-*O*-methyl derivatives of arabinose and free arabinitol (Table 2). The latter derivatives are not due to any undermethylation as there was a molar agreement in

TABLE 1
Carbohydrate Composition (%) of Cowpea Polysaccharide Fractions Separated on DEAE–Cellulose

Fraction	*Yield (%)*	*Uronic acid*	*Constituent sugars*[a]				
			Rha	*Ara*	*Xyl*	*Man*	*Gal*
1	22·8	—	—	100	—	—	—
2	32·6	7	8	68	6	—	11
3	12·3	18	9	45	16	—	12
4	15·4	22	17	6	19	24	12
5	11·3	[b]	3	28	62	5	2

[a] Quantitation based on integration of GC peak areas.
[b] Not determined.

TABLE 2
Methylation Analysis of Arabinan

O-Methyl ether of arabinose[a]	R_T[b]	*Per cent of total carbohydrate*[c]	*Molar ratio*[d]	*Mode of linkage*	*Diagnostic mass fragments m/z*
2,3,5-tri	0·54	23·0	8·2	L-Araf-(1→	162, 161, 129 118, 102, 101, 45
2,3-di	1·11	60·0	19·6	→5)-L-Araf-(1→	233, 189, 162 129, 118, 102, 87
2-mono-	1·77	7·8	2·3	→3,5)-L-Araf-(1→	261, 201, 118
3-mono-	1·88	3·3	1·0	→2,5)-L-Araf-(1→	190, 189, 130, 129, 87
Arabinitol	2·44	5·6	1·5	→2,3,5)-L-Araf-(1→	290, 218, 217, 188, 187, 146, 145

[a] 2,3,5-tri = 1,4-Di-*O*-acetyl-2,3,5-tri-*O*-methyl-L-arabinitol, etc.
[b] Relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl glucitol.
[c] Based on integration of GC peak area.
[d] With respect to 3-mono-*O*-methyl-L-arabinose.

the proportion of non-reducing terminal and branching residues and exactly similar results were observed in duplicate experiments. By analogy with several reported arabinans (Joseleau *et al.*, 1983), as well as by its easy hydrolytic cleavage, the ring size of the glycosyl residues was assumed to be L-furanosidic. The high negative optical rotation (−80°) for unmethylated Fra. 1 suggested that the glycosidic linkages are mainly α-L. The high proportion of 2,3-di-*O*-methyl sugar shows that the backbone is essentially 1,5-linked and carries occasional sidechain branches through *O*-2 or *O*-3 terminated by additional non-reducing L-arabinofuranose units. However, the branching is considerably less in comparison with earlier reports on other arabinans (Siddiqui & Wood, 1977). No arabinopyranosyl methyl ethers were detected in cowpea arabinan.

The methylation evidence showed that Fra. 1 has a highly branched structure consisting of a 1,5-linked arabinan chain, 30% of glycosyl units being singly (at *O*-2 or *O*-3) or doubly (*O*-2/*O*-3) substituted with additional arabinose residues. Such a polysaccharide would theoretically consume 0·70 mole IO_4^- per anhydrosugar, in good agreement with the experimental IO_4^- uptake of 0·68 mole. On borohydride reduction and mild hydrolysis, the oxopolysaccharide gave mainly glycerol and arabinose in close correspondence with the structure assigned for the polysaccharide.

L-Arabinans of plant origin that have been studied in some detail can be classified into two groups. The first group (Hough & Powell, 1960; Barrett & Northcote, 1965) includes those that are associated with pectins and supposedly are released by alkaline degradation procedures. The second group, which includes mustard (Rees & Richardson, 1966), soyabean (Aspinall & Cottrell, 1971) and rapeseed (Siddiqui and Wood, 1974) arabinans are believed to be natural undegraded homoglycans. Normally, arabinans are highly branched polysaccharides, occasionally containing a very low proportion of arabinopyranosyl residues. Recently, an L-arabinan was isolated by a simple water extraction of defatted mustard (*Brassica juncea*) seed meal and characterized by methylation analysis (Tharanathan *et al.*, 1985). Cowpea arabinan is another example of naturally occurring pure arabinan. The possibility that the cowpea arabinan may be a degradation product derived during the TCA extraction step was ruled out by the fact that commercial pectin (from citrus) and arabinogalactan (from larch wood) on treating similarly (10% TCA, 4°C for 6 h) did not show any detectable degradation products in the extract (R. N. Tharanathan and U. Ramadas Bhat, unpublished results).

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