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Note

Isolation and characterization of an arabinogalactan from wheat flour

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Wheat flour contains a small percentage of nonstarchy polysaccharides soluble in cold water[1]. These carbohydrates are composed mostly of D-xylose and L-arabinose; they are therefore usually referred to as wheat-flour pentosans or wheat-flour gums and are responsible for the high viscosity of aqueous flour-extracts. The crude pentosan preparations always contain considerable amounts of soluble starch (dextrans), proteins, small amounts of bound ferulic acid, and polysaccharide fractions containing D-galactose as building units. The nature of the latter polysaccharides has been uncertain, owing to difficulties in separating the D-galactose-containing fractions from the L-arabino-D-xylans. It will be shown that D-galactose occurs in wheat flour as an L-arabino-D-galactan and that it can be separated from the L-arabino-D-xylans by fractionation with ammonium sulfate, a method rarely used for polysaccharide fractionation[2,3].

For the isolation and purification of wheat-flour arabinogalactan, white wheat-flour was mixed with twice the amount of water and extracted by vigorous stirring (Homorex) for 10 min. Centrifugation of the aqueous flour suspension yielded a viscous, clear flour-extract. This was saturated with ammonium sulfate and kept overnight at 4°. The precipitate (arabinoxylans and proteins) was removed by centrifugation. The solution containing the arabinogalactan and starch was dialyzed, concentrated, and lyophilized to obtain a crude arabinogalactan; yield 0.2%, based on flour. This preparation contained about 50% of soluble starch, which was degraded by crystalline α-amylase (*Bacillus subtilis*, Calbiochem AG, Lucerne, Switzerland) in a dialysis bag, the starch fragments being continuously removed by dialysis[4]. After precipitation and removal of the enzyme, the final solution was lyophilized to obtain the purified arabinogalactan; yield 35%, based on the crude arabinogalactan (0.07–0.1%, based on flour).

The composition of the crude and purified arabinogalactan is shown in Table I. The sugars were analyzed by g.l.c. of their alditol acetates obtained by hydrolysis of the polysaccharides with 0.5M sulfuric acid for 4 h at 110°, and reduction of the monosaccharides with sodium borohydride[5]. The figures show that treatment with α-

TABLE I

COMPOSITION OF CRUDE AND PURIFIED WHEAT-FLOUR L-ARABINO-D-GALACTAN

Components	*Arabinogalactan*	
	Crude[a]	*Purified*[b]
Protein (%)	5.0	6.1
Polysaccharides (%)[c]	95.0	93.9
Arabinose	16.3	40.8
Xylose	0.7	0.8
Mannose	1.6	trace
Galactose	23.3	58.4
Glucose	58.0	trace
Ratio of galactose to arabinose	1.43	1.43

[a]Before α-amylase treatment. [b]After α-amylase treatment. [c]By difference.

amylase afforded a practically pure arabinogalactan having a galactose to arabinose ratio of 1.4:1; for every 7 galactose residues there are 5 arabinose residues. It is interesting to note that the small amount of D-mannose in the crude product also disappeared upon treatment with enzyme (fractionation of the crude product on DEAE-cellulose indicated that mannose is probably combined with glucose or xylose, or both). It is not known whether the small amount of protein in the preparations is an impurity or is chemically bound to the polysaccharide*.

The arabinogalactan was, at first, methylated, according to the procedure of Hakamori[6], as described by Sandford and Conrad[7], with methylsulfinyl anion and methyl iodide. The purified, methylated arabinogalactan was hydrolyzed with

TABLE II

PRODUCTS OF HYDROLYSIS OF THE METHYLATED ARABINOGALACTAN[a]

Products of hydrolysis	*Molar proportion*	*Mode of linkage*
2,3,5-Tri-*O*-methyl-L-arabinitol	1.00	L-Ara*f*-(1→
2,4-Di-*O*-methyl-D-galactitol	0.99	→3) →6)]-D-Gal*p*-(1→
2,4,6-Tri-*O*-methyl-D-galactitol	0.14 } 1.18	→3)-D-Gal*p*-(1→
2,3,4-Tri-*O*-methyl-D-galactitol	0.05	→6)-D-Gal*p*-(1→

[a]Determined as *O*-methylglycitols.

formic acid–sulfuric acid[8]; the resulting methylated monosaccharides were identified and determined as their alditol acetates by gas–liquid chromatography[9]. The identity of all the *O*-methylglycitols was confirmed by combined g.l.c. and m.s. (see Table II).

*Very recently, G. B. Fincher, W. H. Sawyer, and B. A. Stone [*Biochem. J.* 139 (1974) 535] isolated, from wheat endosperm, an arabinogalactanpeptide that is most likely identical with the arabinogalactan described in this paper.

The terminal position of the L-arabinosyl groups, as shown in Table II, could be confirmed by enzymic degradation of the arabinogalactan with an α-L-arabinofuranosidase described earlier[10]. This enzyme removed about 90% of the L-arabinose residues, leaving the galactan backbone-chain to which the L-arabinose residues were attached. The specific rotation $[\alpha]_D^{20}$ changes from −43.5° of the original arabinogalactan to −13° after removal of the L-arabinose residues. The methylation studies (Table II) and the values of the optical rotation suggest that the galactan is linked either β-D-(1→3), or β-D-(1→6), or both. Periodate oxidation of the galactan gave a consumption of 1.23 mole of periodate per unit, whereas the arabinogalactan consumed 1.08 mole. These results indicate that about 60% of the D-galactose residues are linked β-D-(1→6) and have L-arabinosyl residues linked to C-3; the remainder of the D-galactose residues are most likely partially substituted by branches having β-D-(1→3) and β-D-(1→6) linkages. Postulation of the exact structure must await methylation studies of the D-galactan moiety. The investigations carried out so far show that the arabinogalactan from wheat flour belongs to the Type II L-arabino-D-galactans according to Aspinall[11]. Type I L-arabino-D-galactans[11] contain linear chains of β-D-(1→4)-linked D-galactose residues and seem to be associated with pectic substances; this class of polysaccharides is however absent in cereal grains.

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