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Structural Studies on a Water-soluble Arabinogalactan Isolated from Rapeseed (*Brassica napus*)

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A highly branched arabinogalactan isolated from rapeseed flour has been separated into a low molecular and a high molecular fraction by gel chromatography. The fractions had similar chemical compositions. For the structural investigation, methylation analysis of the original arabinogalactan and of products obtained after partial acid hydrolysis and after Smith degradation have provided the essential information in this study. A tentative structure for the arabinogalactan is presented.

RESULTS AND DISCUSSION

In a previous report,[1] the isolation of an arabinan and an arabinogalactan from rapeseed was described and structural studies on the arabinan were reported. Fractionation of the arabinogalactan on a Sepharose 2B column has now revealed that the arabinogalactan fraction contained at least two polysaccharides of different molecular weights. (Fig. 1). Similar observations on arabinogalactans from other sources have been reported elsewhere in the literature.[2] After repeated chromatographic separations on Sepharose 2B a high molecular weight fraction (A_H), a low molecular weight fraction (A_L) and a mixed fraction A_M were collected. (Fig. 1). All three fractions on hydrolysis yielded L-arabinose and D-galactose in the ratio 9:1 and these sugars accounted for more than 95 % of the materials. IR spectroscopy, paper chromatography and paper electrophoresis of the hydrolysates from A_H, A_M and A_L showed that no other sugar or uronic acid residues except for very small amounts of xylose were present. The optical rotations were similar and there was a close resemblance between the methylation analyses of A_H, A_L and A_M (Table 1). It therefore seems reasonable to assume that A_H, A_M and A_L differ only in their molecular weights and the following discussion is confined to structural studies of A_M. A sample of A_M, in 0.05 M aqueous sulfuric acid was kept at 82 °C and the change in optical rotation was followed. A rapid increase from $[\alpha]_{578}$ −111° to +20° over 5 h, followed by a slower increase to +65° in 29 h, was observed. This showed that the arabinose units in A_M are α-linked,[3] and have the L-configuration. Treatment of the hydrolysate with D-galactose oxidase, which should not oxidize L-galactose,[4] revealed that galactose had the D-configuration.

Methylation analysis [5] of A_M (Table 1, column 1) showed that A_M consisted of terminal L-arabinofuranosyl units and further of either L-arabinofuranosyl residues substituted at O−5 and O−2 or L-arabinopyranosyl units substituted at O−4 and O−2. The methylation analysis also demonstrated that A_M contained 3,6-di-*O*-substituted D-galactopyranosyl units and small amounts of either L-arabinofuranosyl units substituted at O−5 or L-arabinopyranosol units substituted at O−4.

A_M was subjected to partial acid hydrolyses under conditions designed to achieve partial and complete cleavage, respectively, of the furanosidic linkages. Methylation analysis of partially hydrolysed and reduced A_M (Table 1, column 4) revealed that furanosidic and small amounts of pyranosidic terminal L-arabinosyl units had been formed after the mild acid treatment. This indicates that A_M also contains, in addition to furanosidic L-arabinose units, small amounts of L-arabinopyranosyl units. The presence of both furanosidic and py-

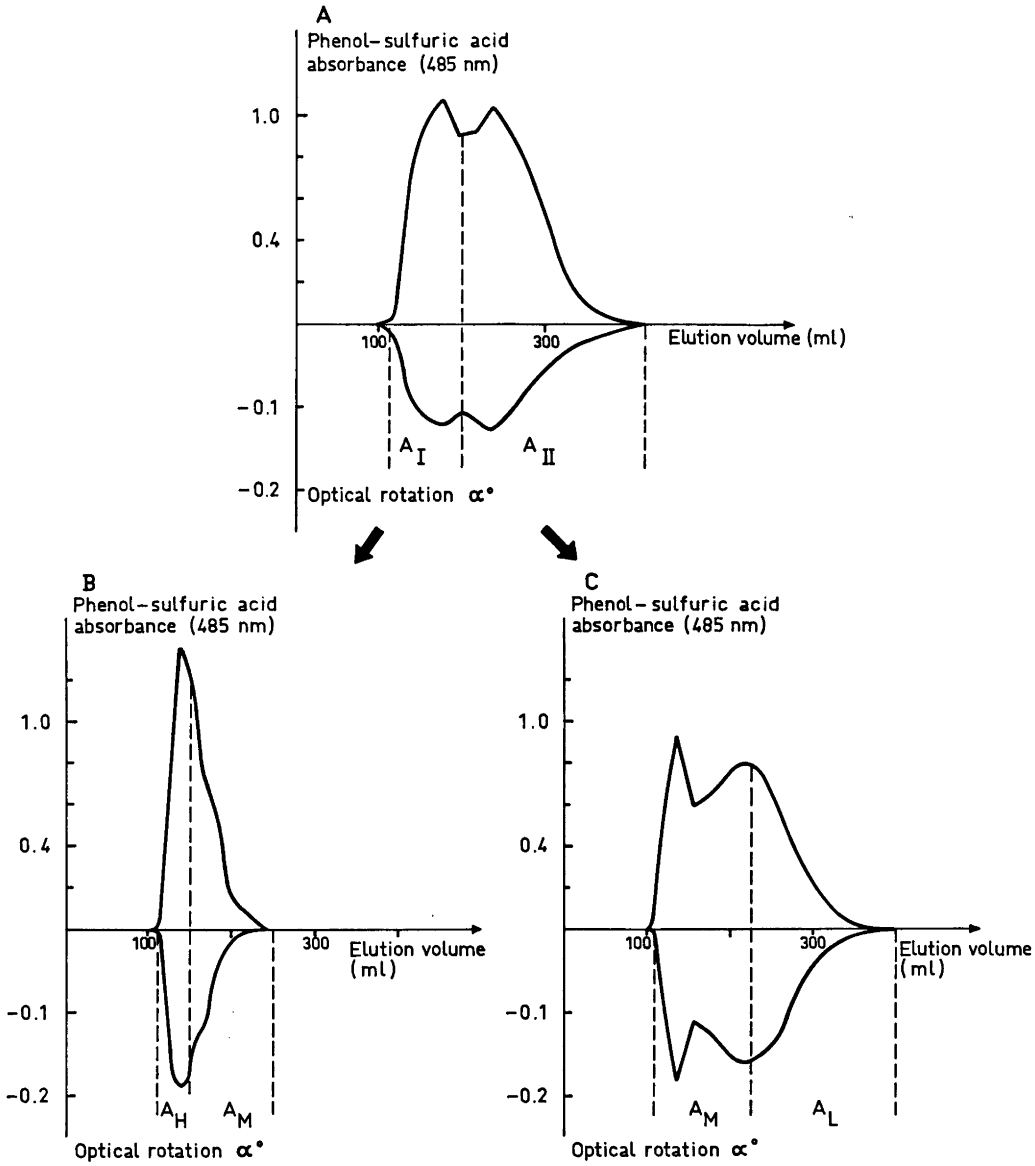

Fig. 1. Separation of a water-soluble arabinogalactan on Sepharose 2B into a high-molecular fraction (A_H) and a low molecular fraction (A_L).

ranosidic L-arabinose units has earlier been observed in arabinogalactans from other sources.[6] Methylation analyses of the more strongly degraded and reduced polymer (Table 1, columns 5 and 6) showed that a mixture of oligosaccharides containing D-galactose remained after the acid hydrolyses of A_M. Further, more 2,3,4-tri-*O*-methyl-D-galactose than 2,4,6-tri-*O*-methyl-D-galactose had been formed during the hydrolyses, indicating that the easily hydrolysed furanosidic L-arabinose units had been linked to O-3 of D-galactose. This indicates that A_M contains a backbone of (1→6) linked D-galactose units to which L-arabinose units are linked.

Table 1. Hydrolysis products (%) of (1) methylated A_H, (2) methylated A_M, (3) methylated A_L, (4) partially hydrolysed (2.5 h), reduced and methylated A_M, (5) partially hydrolysed (10 h), reduced and methylated A_M, (6) partially hydrolysed (21 h), reduced and methylated A_M, (7) periodate oxidized, reduced and methylated A_M, and (8) periodate oxidized, reduced, Smith degraded and methylated A_M.

Sugars	r^a	1	2	3	4	5	6	7	8
2,3,5-Tri-*O*-methyl-L-arabinose	0.40	48	46	46	55	12		9	35
2,3,4-Tri-*O*-methyl-L-arabinose	0.52	1	2	1	9	1			
3,5-Di-*O*-methyl-L-arabinose	0.75				10				16
2,3-Di-*O*-methyl-L-arabinose	1.03	3	3	3	6				16
3-Mono-*O*-methyl-L-arabinose	2.11	40	41	42	10			77	18
2,3,4,6-Tetra-*O*-methyl-D-galactose	1.17				3	32	34		2
2,4,6-Tri-*O*-methyl-D-galactose	2.01					8	17		
2,3,4-Tri-*O*-methyl-D-galactose	2.90				4	29	33		2
2,4-Di-*O*-methyl-D-galactose	5.10	8	8	8	3	18	16	14	11

[a] Retention time of the corresponding alditol acetate on the OV-225 column, relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-*D*-glucitol.

A_M was oxidized with periodate and part of the oxidized polymer was reduced and subjected to a methylation analysis.[7] (Table 1, column 7). The presence of 2,3,5-tri-*O*-methyl-L-arabinose in the methylation analysis of oxidised and reduced A_M showed that the oxidation had not gone to completion or that glycosidic linkages had been broken during the process, thereby generating new terminal units. The results of the methylation analysis of periodate oxidized, Smith degraded polysaccharide are summarized in Table 1, column 8. The presence of both 2,3-di-*O*-methyl-L-arabinose and 3,5-di-*O*-methyl-L-arabinose and small amounts of 2,3,4-tri-*O*-methyl-D-galactose in the analysis shows that oxidizable units have been linked to both O-2 and O-5 of the branching L-arabinose units and to some extent to O-3 of the D-galactose units. The latter result, further confirms the results from the partial acid hydrolysis experiment. The results from the analyses are presented in Fig. 2, which, however, does not necessarily represent the exact structure of the polysaccharide.

Arabinogalactans of plant origin have been studied extensively [7–15] and the function of arabinogalactans in the cell wall has been discussed.[16,17] One group of plant arabinogalactans are those related to the pectins.[14,15] They contain (1→4) linked β-D-galactopyranose residues with side chains of L-arabinofuranosyl residues. There are also arabinogalactans containing a backbone of branched β-D-galactopyranose units substituted at O-3 and O-6. The side chains are terminated with L-arabinofuranosyl residues or sometimes D-glucuronopyranosyl residues.[8–13] The acidic arabinogalactan from turnip rapeseed (*Brassica campestris*) [12] and an

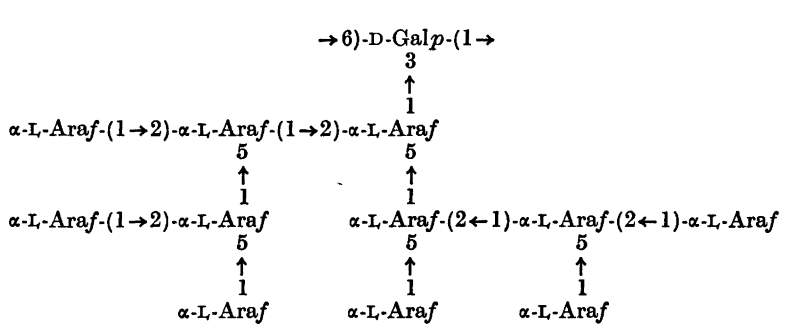

Fig. 2. A tentative structure of the arabinogalactan.

arabinogalactan from wheat flour[13] residues belong to this group. The arabinogalactan investigated in this work has structural similarities with the latter arabinogalactans, but it is more branched and has a much lower percentage of galactose.

EXPERIMENTAL

General methods, preparation of the arabinogalactan, sugar and methylation analysis and acid hydrolysis in the polarimeter are described in our investigation of a water-soluble arabinan isolated from rapeseed (*Brassica napus* var. *Sinus*).[1] Additional procedures were as follows.

Isolation of A_H, A_M and A_L. The arabinogalactan (200 mg) was fractionated on a Sepharose 2B column (1.5 × 75 cm) which was irrigated with water. The eluted carbohydrate material was detected by the phenol-sulfuric acid method [8] and by optical rotation. A high molecular fraction A_I and a low molecular fraction A_{II} were collected (Fig. 1). A_I and A_{II} were separated again and fractions were collected as shown in Fig. 1. The high molecular fraction A_H (10 mg), $[\alpha]_{578}^{20}$ −105° (*c* 0.1, water) contained arabinose: galactose in the ratio 89:11. The middle fraction A_M (85 mg), $[\alpha]_{578}^{20}$ −112° (*c* 0.1, water) contained arabinose: galactose in the ratio 91:9 and the low molecular fraction A_L (18 mg), $[\alpha]_{578}^{20}$ −115° (*c* 0.1, water), contained arabinose: galactose 91:9. In the hydrolysates from A_H, A_M and A_L small amounts of xylose could be detected.

Enzymatic oxidation of D-*galactose.* A_M (10 mg) with *myo*-inositol as internal standard was treated with 0.25 M sulfuric acid (4 ml) for 16 h at 100 °C. The hydrolysate was neutralized ($BaCO_3$), filtered, and treated with D-galactose oxidase (GALAX from KABI, Sweden). After 6 h the material was concentrated to dryness and silylated and the sugars were analysed by GLC. Only arabinose remained after the enzymatic treatment.

Analysis of partially hydrolysed A_M. A_M (64 mg) was hydrolysed in sulfuric acid (0.045 M) at 82 °C. Samples were withdrawn after 2.5, 10 and 21 h. The hydrolysates were neutralised ($BaCO_3$) and reduced ($NaBH_4$) and subjected to methylation analysis (Table 1, columns 4, 5 and 6). The residues from the hydrolyses were analysed for oligosaccharides. Paper chromatography and GC of the trimethylsilylated materials [9] revealed that no di- or trisaccharides were present in the hydrolysate from the material that was hydrolysed for 21 h. However, higher oligomers could be detected.

Analysis of periodate oxidized, reduced and Smith degraded A_M.[7] A_M (35 mg) was dissolved in 0.04 M sodium metaperiodate (12.0 ml), propanol (0.5 ml) was added and the mixture was kept in the dark at room temperature. The reaction was followed by optical rotation. When the oxidation was complete (28 h), ethylene glycol and then an excess of potassium borohydride were added and the solution was kept at room temperature for 24 h. The reaction mixture was neutralised (Dowex 50, H^+ form), filtered, dialysed against distilled water and concentrated. The residue was hydrolysed in sulfuric acid (0.5 M, 20 ml) at room temperature and samples were withdrawn after 0 and 20 h, neutralized ($BaCO_3$) and subjected to methylation analysis (Table 1, columns 7 and 8).

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