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

OLLE LARM, OLOF THEANDER and PER ÅMAN

Department of Chemistry, Agricultural College of Sweden, S-750 07 Uppsala 7, Sweden

The structure of an arabinan, isolated from rapeseed flour, has been investigated. Methylation analysis on the original polysaccharide and on products obtained after mild acid hydrolysis and after Smith degradation of the polysaccharide, have provided the essential information in this study. A tentative structure for the polysaccharide is presented

RESULTS AND DISCUSSION

Oil-free, heat-treated and coat-free rapeseed flour from *Brassica napus* var. *Sinus* was extracted first with ethanol and then with hot water. The aqueous fraction was precipitated with ethanol. The precipitate was removed and from the concentrated ethanol phase an arabinogalactan (A) $[\alpha]_{578}$ $-111°$ and an arabinan (B) $[\alpha]_{578} - 83°$ were isolated by gel chromatography (Fig. 1). The structural investigation of the arabinan will be presented in this paper.

The arabinan showed a symmetrical elution curve on both Sepharose 2B and Sephadex G-200. IR spectroscopy, paper chromatography and paper electrophoresis of a hydrolysate of the polymer revealed that it was a pure arabinan devoid of uronic acid residues. A GLC analysis showed that arabinose accounted for 95 % of the polysaccharide. As the optical rotation of the polymer changed from a large negative value to a large positive value on acid hydrolysis under mild conditions, it is assumed that the polysaccharide is composed of α-L-arabinofuranose units.[1]

The arabinan was fully methylated and hydrolysed, and the methylated sugars were converted into their alditol acetates and analysed by GLC-MS.[2] (Table 1, column A). From the methylation analysis, it was concluded that the arabinan consisted of (1→5)-linked arabinofuranoside residues and that about 33 % of the sugar residues were further substituted at O-3. A small amount of L-arabinose, substituted in the 2,3 and 5 positions was also found. It is reasonable to assume that part of the volatile 2,3,5-tri-*O*-methylarabinose and its derivatives was lost during the concentrations. This explains the poor agreement between terminal and branching L-arabinose units in the methylation analysis. No 3-*O*-methylarabinose was detected.

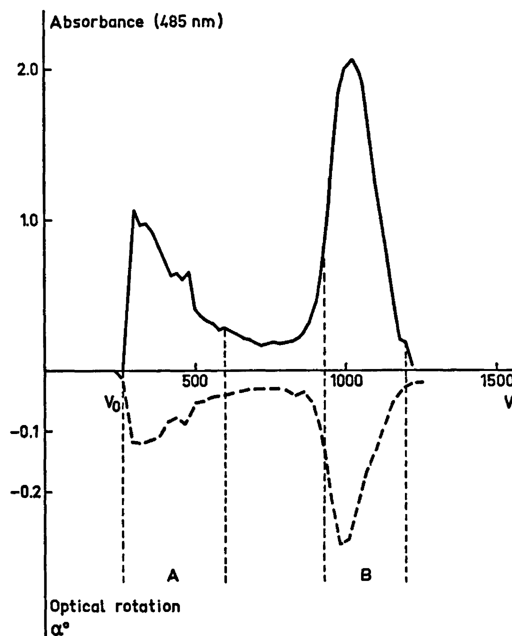

Fig. 1. Purification of a water-soluble arabinan on Sepharose 2 B. Absorbance at 485 nm in phenol-sulfuric acid. Elution volume (*V*) in ml.

Table 1. Hydrolysis products of (A) methylated arabinan, (B) partially hydrolysed, deuterium reduced and methylated arabinan, (C) periodate oxidised, reduced and methylated arabinan, (D) periodate oxidised, Smith degraded (20 h), and methylated arabinan, and (E) periodate oxidised, Smith degraded (69 h) and methylated arabinan.

Sugars	r^a	Mol % A	B	C	D	E
1,2,3,4,5-Penta-*O*-methyl-L-arabinitol [b]	0.05		13			
1,2,3,4-Tetra-*O*-methyl-L-arabinitol [b]	0.17		} 5			
1,2,4,5-Tetra-*O*-methyl-L-arabinitol [b]	0.17					
1,2,4-Tri-*O*-methyl-L-arabinitol	0.29		4			
2,3,5-Tri-*O*-methyl-L-arabinose	0.40	32	33		67	68
3,5-Di-*O*-methyl-L-arabinose	0.75		2			
2,5-Di-*O*-methyl-L-arabinose	0.84		5			
2,3-Di-*O*-methyl-L-arabinose	1.01	32	24		33	32
2-Mono-*O*-methyl-L-arabinose	1.79	28	11	77		
L-arabinose	2.52	8	3	23		

[a] Retention times of the corresponding alditol acetate on the 3 % OV-225 column, relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. [b] The quantitation of these volatile sugar derivatives is uncertain.

The 100 MHz ^{1}H NMR spectrum of the original arabinan, dissolved in pyridine showed *inter alia* three signals of similar intensity at δ 5.45, 5.64 and 5.77. Each of these signals integrated for one proton and they were assigned to the anomeric protons from the terminal, the chain and the branched arabinose residues.

In order to verify that the L-arabinose units are furanosidic, the polysaccharide was subjected to a mild acid treatment, generating new terminal units. The partially hydrolysed arabinan was reduced with sodium borodeuteride. Methylation analysis (Table 1, column B) yielded *inter alia* 1,2,4-tri-*O*-methyl-L-arabinitol and 2,3,5-tri-*O*-methyl-L-arabinose but no 2,3,4-tri-*O*-methyl-L-arabinose. The 1,2,4-tri-*O*-methyl-L-arabinitol was deuterated at C-1, and derived from the reducing end of the degraded polysaccharide. The tetra- and penta-*O*-methyl ethers were also deuterated which facilitated their identification on MS. The absence of 2,3,4-tri-*O*-methyl-L-arabinose ($r = 0.48$) demonstrates that all the terminal units in the degraded polysaccharide are furanosidic, indicating that all the arabinose units in the arabinan are furanosidic. In order to investigate the distribution of the branched arabinose units, the polysaccharide was subjected to a Smith degradation,[3] that is periodate oxidation, borohydride reduction and acid hydrolysis under mild conditions. Only the branched arabinose units should remain intact and their glycosidic linkages should not be degraded by this treatment. The products of the Smith degradation were examined by methylation analysis (Table 1, columns C, D, and E).[4] If every second residue in a chain was branched, only 2,3,5-tri-*O*-methyl arabinose should have been obtained in the methylation analysis of the degraded polymer. If the branching residues were concentrated in block structures, 2,3-di-*O*-methyl arabinose would have predominated. As neither of these alternatives were observed, it is evident that the arabinan has both isolated and adjacent branching residues. The structural features observed are summarized in Fig. 2, which does not, however, represent the exact structure of the polysaccharide. It is unlikely that the polysaccharide has an ordered structure and the length of the side chains has not been deduced.

Genuine arabinans of plant origin, generally associated with pectin in the cell wall, have been studied earlier.[4–8] The structural features of these arabinans were discussed mainly on the basis of methylation analysis data on the original arabinan. Rees and Richardson,[4] however, used Smith degradation together with methylation analysis in their investigation of arabinan from white mustard. The mode of linkage is the same in all arabinans of plant origin, but the detection of only traces of 3-*O*-

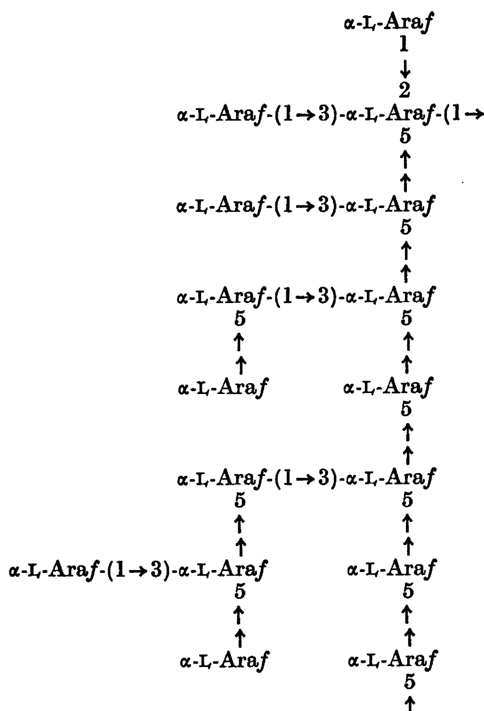

Fig. 2. Structural features of the arabinan.

methyl-arabinose in the methylation analysis of arabinans from the mustard/rape-family seems to be characteristic. The degree of branching, the molecular weight and the optical rotation differs between arabinans from different sources. However, these differences are small between arabinans isolated from the mustard and the rape family.[4,6]

EXPERIMENTAL

Concentrations were performed at reduced pressure at bath temperature not exceeding 40 °C. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. NMR spectra were recorded on a Varian HA-100 D spectrometer and IR spectra with a Perkin-Elmer 337 spectrometer. GLC was conducted with a Varian model 2700 instrument fitted with a column containing 3 % OV-225 on Gas-Chrom Q. For GLC-MS, a Varian CH7 gaschromatograph-mass spectrometer was used. Paper chromatograms were run on Whatman No. 1 papers, using the following systems (v/v): (a) ethyl acetate-acetic acid-water, 3:1:1, (b) pyridine-ethyl acetate-water, 2:8:1, (c) butanol-acetic acid-water, 6:2:1. The components were detected with *p*-anisidine hydrochloride or silver nitrate-sodium hydroxide.

Preparation of the polysaccharide. Oil-free, coat-free, heat-treated and milled rapeseed from *Brassica napus* var. *Sinus* (1.9 kg dry weight) was extracted (6 × 20 min) with 80 % ethanol (6 × 5 l) on a boiling waterbath under reflux. The ethanol insoluble fraction was extracted (5 × 2 h) with hot water (5 × 5 l) and the water extract was concentrated to 3 l, centrifuged and precipitated with 4 volumes of ethanol. The precipitate was dissolved in water (3 l) and reprecipitated with 2 volumes of ethanol. The ethanol phases from the precipitations were concentrated and deproteinised by the hot phenol-water procedure of Westhpal *et al.*[9] The aqueous phase was dialysed against distilled water, concentrated and precipitated with 6 volumes of ethanol. The precipitate (1.8 g), on acid hydrolysis yielded arabinose and galactose in the relative proportions 24:1; traces of mannose were also found in the hydrolysate. The material (0.9 g) was dissolved in water (20 ml) and applied on a Sepharose 2 B column (3.5 × 100 cm) which was irrigated with water. 10 ml fractions were collected and the separation was monitored by the phenol sulfuric acid method [10] and with optical rotation. The polysaccharide fractions, A and B (Fig. 1) were concentrated, dialysed and freeze-dried. A, (243 mg), $[\alpha]_{578} - 111°$, (*c* 0.54, water), on hydrolysis yielded arabinose and galactose in the relative proportions 10:1. B, (423 mg), $[\alpha]_{578} - 83°$ (*c* 0.88, water), yielded on hydrolysis only arabinose. Molecular weight determinations using calibrated Sepharose columns [11] gave $\overline{M}_w$-values of 7×10^6 and of 15×10^3 for A and B, respectively.

Sugar analysis of the arabinan. A mixture of the arabinan (B) (5 mg) and *myo*-inositol (5 mg) was treated with 0.25 M sulfuric acid, for 16 h at 100 °C. The hydrolysate was neutralised ($BaCO_3$), reduced (KBH_4), acetylated, and analysed by GLC-MS. The sugar analysis revealed that arabinose accounted for 95 % of the material.[12] The identity of the sugars was confirmed by paper chromatography, system a, b, and c.

Methylation analysis of the arabinan. The polysaccharide (10 mg) was methylated in dimethyl sulfoxide by treatment with sodium methylsulfinyl carbanion and methyl iodide.[13] The resulting solution was dialysed against distilled water, concentrated, and the product was hydrolysed, reduced, converted into partially methylated alditol acetates, and analysed by GLC-MS.[2] The results are summarised in Table 1, column A.

Analysis of partially hydrolysed arabinan. The arabinan (25 mg) was hydrolysed in 10 ml sulfuric acid (0.045 M) for 2.5 h at 82 °C. The hydrolysate was neutralized ($BaCO_3$) and reduced ($NaBD_4$). Methylation analysis of the material was performed as described above with

the exception that the methylated products were isolated by partition between chloroform and water. The results are summarised in Table 1, column B.

Methylation analysis of periodate oxidized, reduced and Smith degraded arabinan.[4] The arabinan (38 mg) was dissolved in 0.0400 M sodium metaperiodate (13.3 ml), propanol (0.5 ml) was added and the mixture was kept in the dark at room temperature. The reaction was followed by measurement of the optical rotation. When the oxidation was complete (36 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 by the addition of Dowex 50 (H^+ form), filtered, dialysed against distilled water and concentrated. The product was dissolved in 0.5 M sulfuric acid (20 ml) and left at room temperature for 70 h. Samples were withdrawn after 0, 20, and 70 h, neutralised ($BaCO_3$) and subjected to methylation analyses. The methylation analyses were performed as described above. The results are presented in Table 1, columns C, D, and E.

Acid hydrolysis of the arabinan. A solution of the arabinan (26 mg) in water (5 ml) was diluted with the same volume of cold 0.09 M sulfuric acid, transferrred to a 1 dm polarimeter tube, thermostated at 82 °C and the optical rotation was determined at intervals. An increase of $[\alpha]_{578}$ from -111 to $+83°$ in 16.5 h was observed.

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