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ARABINANS FROM THE ROOTS OF HORSEBEAN (*Vicia faba*)

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ABSTRACT

Young roots from the horsebean (*Vicia faba* L.) show a very high content of arabinose among their constituent cell-wall carbohydrates. Two water-soluble arabinans have been isolated from the cell-wall material. Their structures have been established by chemical methods and by ^{13}C-n.m.r. spectroscopy and showed to consist of an α-(1→5)-linked backbone of L-arabinofuranose residues; arabinopyranose residues are absent. The latter feature make these polysaccharides slightly different from arabinans from other origins that are characterized by their high degree of branching.

INTRODUCTION

Arabinans have been isolated from cell walls of different plant sources[1]. A few pure arabinans have been characterized by methylation analysis and shown to be highly branched[2]. They all are characterized by the predominance of L-arabinofuranose residues; a very low proportion of pyranose ring-form is present in some cases[3,4]. When pyranose residues are present, they are believed to occupy terminal positions. Furanose residues are principally α-L-(1→5)-linked in the main chain, with short side-chains having α-L-(1→2)- and/or, α-L-(1→3)-linked branch points. Characteristic negative optical rotations are consistent with α-L-glycosidic linkages. Additional evidence has been obtained by ^{13}C-n.m.r. spectroscopy[5] which allowed unambiguous assignment of the anomeric configuration of the glycosidic linkages and of the furanose form of the internal, α-L-(1→5)-linked residues. Pectic polysaccharides, including arabinans from root cell-walls, have not been extensively studied, although they may play an important role in the metabolic exchanges that take place between the plant and the soil.

This paper describes the isolation of arabinans from the young roots of *Vicia faba* and their chemical structure is demonstrated by methylation analysis and by ^{13}C-n.m.r. spectroscopy.

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RESULTS AND DISCUSSION

Horsebean seeds were germinated in tap water and the 3-cm-long roots that developed were inserted in the holes of a grid resting above a basin containing a solution of inorganic salts. After 5 days in the dark, the roots (5–8 cm long) were harvested. Cell walls were prepared according to Kivilaan *et al.*[6] or according to Esquerre-Tugaye[7]. Total carbohydrate analysis showed the cell walls of *Vicia faba* roots to be particularly rich in pectic material, with ~21% of the wall being uronic acids and 6% being arabinose, two characteristic sugar constituents of pectins. Extraction of the cell-wall material with water gave a polysaccharide mixture that was precipitated with ethanol and gave an ethanol-soluble fraction and an ethanol-insoluble one. Acetone precipitation of the former afforded a fraction containing arabinose as the main constituent (arabinan I). The latter, which contained 56% of acidic material, was subsequently purified on a column of DEAE-Trisacryl-CM successively eluted with M sodium chloride, 0.25M sodium hydroxide, and then 0.5M hydrochloric acid. Elution with the acidic solution afforded an arabinan-enriched fraction that contained 87% arabinose among the neutral sugars and 20% of uronic acid material.

Carboxyl reduction, followed by hydrolysis, showed the uronic acid to be galacturonic acid. When the carboxyl-reduced mixture was redeposited on the DEAE-Trisacryl-CM column, successive elution with water, and then 0.5M hydrochloric acid gave no clear separation of the arabinan and the galactan, as most of the material remained adsorbed on the column. This result suggests that adsorption of the arabinan on the column might have been independent of the presence of uronic acids in the molecule.

Arabinans I and II were not further purified, as their structures could be studied readily without complete purification, a procedure which often results in partial degradation of the polysaccharides.

Two arabinans were permethylated by the Hakomori procedure[8] followed by two methylations according to Purdie[9]. The partially methylated sugars obtained upon hydrolysis of the fully methylated polysaccharides were analyzed by g.l.c. of their alditol acetate derivatives, and their identities confirmed by g.l.c.–m.s. The proportions of the different methylated derivatives and the corresponding linkage-analysis is given in Table I. It appears from these results that arabinans I and II are similar polysaccharides. In contrast to many of the arabinans thus far studied, the present polysaccharides exhibit a lower degree of branching and have only one type of internal linkage, corresponding to (1→5)-linked arabinofuranosyl residues, as indicated by the presence of 2,3-di-*O*-methylarabinose as the only dimethyl ether. Examination of the ^{13}C-n.m.r. spectra of arabinans I and II confirmed these two characteristic features. The spectrum showed five strong signals, at δ 108.1 (C-1), 82.9 (C-4), 81.5 (C-2), 77.5 (C-3), and 67.6 (C-5, linked). These could be readily assigned by reference to model compounds[5] and by application of empirical rules concerning the chemical shifts induced by the presence of an *O*-alkyl substituent

on the different carbon atoms of a glycoside[10]. Those five signals correspond to the carbon atoms of the internal α-L-(1→5)-linked arabinofuranosyl residues which, according to the methylation analysis, are expected to be the most abundant in the arabinans. Other signals are more difficult to assign with certainty, as they correspond to proportionally less abundant residues. However, signals at δ 84.6, 77.2, and 61.9 p.p.m. may be tentatively ascribed to the terminal groups of the side chains, which are the next most prevalent components in the polymers. The signal at δ 84.6 is assigned to C-4, which is expected to appear at lower field in the unsubstituted residues, the signal at δ 77.2 is assigned to C-3, and that at δ 61.9 to C-5 of the free CH_2OH group of the terminal, nonreducing residues. The signals of those residues bearing the side chains at O-2 or/and O-3 were not differentiated from the other signals in the spectrum, or were not visible because of their low proportion in the arabinan.

Arabinopyranose residues have been reported in many arabinans thus far investigated[11–13]. In all instances, the proportion of the arabinopyranose residues was only a few percent of the arabinofuranose form and these residues appeared

TABLE I

METHYLATION ANALYSIS OF ARABINANS

Methylated sugar[a]	*Mole %*			
	Arabinan I		*Arabinan II*	
2,3,5-Me_3-Ara	13.5	1	16.5	1
2,3-Me_2-Ara	55	4.07	61	3.7
2-Me-Ara	5	0.37	8.5	0.51
3-Me-Ara	24.5	1.81	14	0.85
L-Arabinose	2	0.15	trace	trace

[a]2,3,5-Me_3-Ara = 1,4-Di-*O*-acetyl-2,3,5-tri-*O*-methyl-L-arabinitol, etc.

TABLE II

PRINCIPAL SIGNALS IN THE ^{13}C-N.M.R. SPECTRUM OF THE ARABINANS FROM *Vicia faba* L.

δ (*p.p.m.*)[a]	*Carbon atoms*	*Indications for the arabinose residues*
108.1	1	α-L-furanose
84.6	4	terminal residue
82.9	4	5-linked residue
81.5	2	5-linked residue
77.5	3	5-linked residue
77.2	3	terminal residue
70.2		unassigned
67.6	5	5-linked
61.9	5	free OH of terminal residue

[a]Chemical-shifts are expressed in p.p.m. relative to Me_2SO (39.6 p.p.m.)

TABLE III

GLYCOSYL LINKAGE COMPOSITIONS (MOL %) OF ARABINANS OF DIFFERENT ORIGINS

Methylated derivative[a]	*Maritime pine*[11]	*Aspen*[3]	*White willow*[13]	*Rape seed*[4]	*Rosa glauca*[5]	*Horse bean*	
						Arabinan I	*Arabinan II*
2,3,5-Me_3-Ara	1	1	1	1	1	1	1
2,3,4-Me_3-Ara	0.04	0.09	0.06		0.04		
2,3-Me_2-Ara	2.44	1.26	0.59	1	0.9	1.1	3.7
2,5-Me_2-Ara	0.28		0.11		0.2		
3-Me-Ara	0.13	0.21	0.14		0.17	1.8	0.85
2-Me-Ara	0.45	0.37	0.22	0.87	0.4	0.37	0.51
t-Ara	0.11	0.31	0.34	0.25	0.25	0.15	trace

[a] 2,3,5-Me_3-Ara = 1,4-Di-*O*-acetyl-2,3,5-tri-*O*-methyl-L-arabinitol

only in terminal, non-reducing positions. It is therefore questionable as to whether the pyranose residues are really part of the polysaccharides or if they are artefacts arising during the methylation analysis. It has recently been demonstrated[14] that some methyl α-L-arabinopyranoside is formed on treatment of methyl α-L-arabinofuranoside with alkali. In an arabinan, the terminal arabinofuranosyl residues may be regarded as simple glycofuranosides, and consequently if any tautomeric rearrangement were to take place, it would be on the terminal residues, and not on the internal residues where the furanose ring-form is fixed by the (1→5) linkages.

The authors[14] estimated the actual amount of pyranoside formed during the treatment of methyl arabinofuranoside to be >2%. Although their experimental conditions were more drastic, with treatment of the furanoside for 10 h at 170° in 2.65M aqueous sodium hydroxide, we checked the possibility of pyranoside formation under the conditions of the Hakomori methylation, which takes place in the presence of strong base. Methyl α-L-arabinofuranoside, the purity of which had been previously established by g.l.c. of its triacetate, was subjected to prolonged contact with dimsyl ion and was then methylated under the conventional conditions. Analysis of the product by g.l.c. following reduction and subsequent acetylation did not reveal any 2,3,4-tri-*O*-methyl arabinopyranoside accompanying the permethylated furanoside. As the 6-membered ring could be present only in very small amount in the background of the g.l.c. trace, a g.l.c.-m.s. recording with multiple-ion detection was performed, monitoring the ions at m/z 45, 101, and 117. At the retention time of the 2,3,5-tri-methyl ether there was a strong peak at each of the three selected mass-values, whereas at the retention time of the 2,3,4-tri-methyl ether no ion at m/z 45 could be observed accompanying those at the m/z 101 and 117. The proportion of pyranoside that could have been formed was <1%, and therefore it could not have accounted for the detection as an artefact of arabinopyranose residues in many arabinans or arabinogalactans, when the pyranose form is present, the 2,3,4-tri-methyl ether obtained upon methylation analysis usually constitutes 3–5% of the total.

Additional evidence for the absence of arabinopyranose residues in the arabinans from broad bean is from the ^{13}C-n.m.r. spectrum, which did not show any signal at ~105 p.p.m. where C-1 resonance of an α-L-pyranosyl residue would be expected[5], although this technique is not accurate when a species exists in only low percentage.

The structural similarity of the two arabinans isolated from broad-bean roots shows that a family of these polysaccharides having similar structures is associated to the pectic polymers present in the cell walls of the roots. It is to be noted that ^{13}C-n.m.r. spectroscopy is particularly suitable for the structural investigation of arabinans which, because of their α-L-furanoid constituents, exhibit very characteristic spectra. This was clearly illustrated when a mixture of arabinan and arabinogalactan was examined. The C-1 signal for the α-L-arabinofuranose residue of the arabinan resonated at 108.1 p.p.m., whereas that for the β-L-arabinopyranose residues of the arabinogalactan resonated at 104.4 p.p.m. It is therefore easy to differentiate the two different kinds of arabinose-containing polysaccharides in such pectic extracts by ^{13}C-n.m.r. spectrosopy; in arabinogalactans the terminal arabinose residues are pyranoid and β-linked[2]. The same would apply to the oligo-β-arabinofuranoside structures present in the plant glycoproteins.

The two arabinan fractions described here certainly originate in the same pectic material, but display slight structural differences, particularly in the proportion of branching at O-2 and O-3 of the internal residues. This variation might be sufficient to explain the differences in their solubility and therefore their extraction behavior. The main structural features of the two polysaccharides are well shown in their ^{13}C-n.m.r. spectra, which permit clear distinction between the α-arabinose residues of an arabinan and the β-arabinose of an arabinogalactan. The behavior of arabinan II during purification on the column of DEAE-Trisacryl, and its association with acidic material at the end of the stepwise elution suggests that linkages might exist between this arabinan and the acidic pectic material.

EXPERIMENTAL

General methods. — The arabinans were hydrolyzed with 2M trifluoroacetic acid (4 h, 100°). The hydrolyzate was reduced with sodium borohydride and acetylated in pyridine–acetic anhydride (1:1 v/v, 2 h, 100°). The resulting alditol acetates were analyzed by g.l.c. with a Packard–Becker 417 instrument on glass columns (200 × 0.15 cm) containing 3% SP 2340 on Chromosorb W, AW-DMCS (100–120° mesh) at 205°. The methylated polysaccharides were hydrolyzed with 90% formic acid (1 h, 100°) followed by 2M trifluoroacetic acid (4 h, 100°). The alditol acetates of the partially methylated sugars were analyzed by g.l.c. on the same column (2 min at 180°, and then 2°/min to 180°, and then isothermal). G.l.c.–m.s. analysis was performed with a Girdel 3000–MS 30 AEI instrument fitted with a capillary glass-column packed with SP 2340 (25 m × 0.25 mm).

The ^{13}C-n.m.r. spectra were obtained with a WP-100 Bruker F.-t. instrument at 25.18 MHz; all spectra were recorded at 80° with Me_2SO-d_6 as the internal reference. Samples were dissolved in D_2O (28 mg/mL).

Isolation of the arabinans. — Seeds of horsebean were grown in a nutritive, aqueous medium containing calcium carbonate, plus magnesium sulfate and potassium hydrogenphosphate at a 0.01mM concentration. Cultures were kept for 5 days in the dark at 27–30°, and the roots were then excised. The corresponding cell-walls were prepared according to Kivilaan[6] or Esquerre-Tugaye[7]. The cell-wall material was extracted with water (1 L per 5 g for 4 h at 90°). The extraction was repeated four times. The lyophilized extract corresponded to 19.5% of the total cell-wall.

Arabinan I. — An aqueous solution of the extract was precipitated with ethanol and the supernatant solution by precipitation with acetone (3 vols) to give arabinan I, which contained >85% of arabinose in its constituent sugars.

Arabinan II. — The ethanol precipitate was washed with 0.15M sodium hydroxide in ethanol, solubilized in water, and then placed on top of a column (35 × 2 cm) of DEAE-Trisacryl-CM eluted successively with M sodium chloride (250 mL), 0.25M sodium hydroxide (250 mL), and then 0.5M hydrochloric acid (400 mL). The elution was monitored by the Molisch test and by optical rotation. Arabinan II was collected in the latter eluant and contained 87% arabinose among the neutral sugars and ~20% of uronic acid.

Methylation analysis. — Arabinan I was methylated by two successive Hakomori[8] methylations, whereas arabinan II was methylated by a single Hakomori methylation followed by two treatments according to Purdie[9].

Carboxyl reduction. — Arabinan II (4 mg) in water (5 mL) was carboxyl-reduced according to Taylor and Conrad[15]. The mixture was dialyzed, freeze-dried, and a portion hydrolyzed and the sugar analyzed conventionally. The galactose released upon hydrolysis corresponded to the reduction of the galacturonic acid residues.

The reduced mixture was placed on top of a column of DEAE-Trisacryl-CM, but no significant carbohydrate material could be eluted with water or with 0.5M hydrochloric acid.

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