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EXTRACELLULAR ARABINOGALACTAN FROM SUSPENSION-CULTURED RED KIDNEY BEAN ROOT CELLS

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Abstract—An arabinogalactan ($[\alpha]_D^{22}$ −50°) containing L-arabinose and D-galactose in a molar ratio of 1:2·7 was isolated from the culture medium of suspension-cultured red kidney bean root cells (*Phaseolus vulgaris*). The polysaccharide was homogeneous by moving boundary electrophoresis and by sedimentation in the ultracentrifuge. The molecular weight was estimated to be *ca.* 25 000. Methylation and hydrolysis of the polysaccharide yielded the following *O*-methyl ethers which were analysed by gas chromatography: 2,3,5-tri-*O*-methyl-L-arabinose (7 mol); 2,3-di-*O*-methyl-L-arabinose (0·5 mol); 2,3,4,6-tetra-*O*-methyl-D-galactose (1 mol); 2,4,6-tri-*O*-methyl-D-galactose (2·5 mol) and 2,3,4-tri-*O*-methyl-D-galactose (5·5 mol) and 2,4-di-*O*-methyl-D-galactose (8 mol). A possible structure for the polysaccharide is proposed that is similar, in general, to those for arabinogalactans of coniferous woods.

INTRODUCTION

ALTHOUGH the growth of plant cells in suspension culture has been studied for a decade,[1–5] a limited amount of attention has been devoted to the chemical composition of the polysaccharides that are formed. Polysaccharides of similar compositions to those present in the primary cell wall have been obtained from suspension-cultured cambial cells of sycamore.[4,6] Detailed studies of the chemical structure of three extracellular polysaccharides isolated from the culture medium of suspension-cultured sycamore cells have been made by Aspinall *et al.*[7] In the present work red kidney bean cells (*Phaseolus vulgaris*) grown in suspension culture have provided polysaccharides in the culture medium and detailed information on the composition and structure of an arabinogalactan from this source is reported here.

RESULTS AND DISCUSSION

Red kidney bean root cells grown in suspension culture medium produced a mixture of polysaccharides from which an arabinogalactan was isolated in pure form. The other polysaccharide material contained glucose, xylose and galactose, but was not obtained sufficiently pure to warrant structural studies. It appeared to belong to the class of polysaccharides known as 'amyloids'.[8,9]

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The arabinogalactan was separated from the other polysaccharide material by chromatography on DEAE cellulose and elution with 0·5 M ammonium carbonate. The recovered product was a white powder $[\alpha]_D^{22}$ −50° (*c* 1·0, water) and contained L-arabinose ($[\alpha]_D^{20}$ +104°, equilibrium, water) and D-galactose ($[\alpha]_D^{20}$ +80° equilibrium, water) in a molar ratio of 1·0:2·75. The monosaccharides were identified as their alditol acetates on GLC (column A) by comparison with authentic specimens. Homogeneity of the polysaccharide was established by the presence of a single symmetrical peak in its electrophoretic pattern (μ[electrophoretic mobility] 6·78 × 10^{-5} cm^3 S^{-1} V^{-1}) and also by a single peak on sedimentation analysis in the ultracentrifuge. Molecular weight estimation by gel permeation chromatography on Bio-Gel P 100 gave a value of 25 000 ± 1000.

Methylation analysis showed that the molecule was highly branched. The galactose residues were joined by (1→3) and (1→6) linkages. This result was confirmed by the observation that partial acid hydrolysis yielded 3-*O*-β-D-galactopyranosyl-D-galactose and 6-*O*-β-D-galactopyranosyl-D-galactose. The L-arabinofuranose residues were present almost exclusively as end groups as shown by the high proportion of 2,3,5-tri-*O*-methyl-L-arabinose. Mild acid hydrolysis of the arabinogalactan which removed the L-arabinose residues while leaving a galactan residue intact was additional proof that the arabinose residues were in peripheral positions in the molecule. A small amount of 2,3-di-*O*-methyl-arabinose was found in the hydrolyzate from the methylated arabinogalactan to the extent of one residue in every fifty anhydro sugar residues. The ready removal of the L-arabinose residues by mild acid hydrolysis suggested that the non-terminal L-arabinose residues occupied a penultimate position to the terminal L-arabinose residues and were exterior to the galactan core. A similar arrangement has been suggested in the arabinogalactan of suspension-cultured sycamore cells.[7]

Although the present data do not permit a unique formula to be written the following structure is consistent with the experimental evidence.

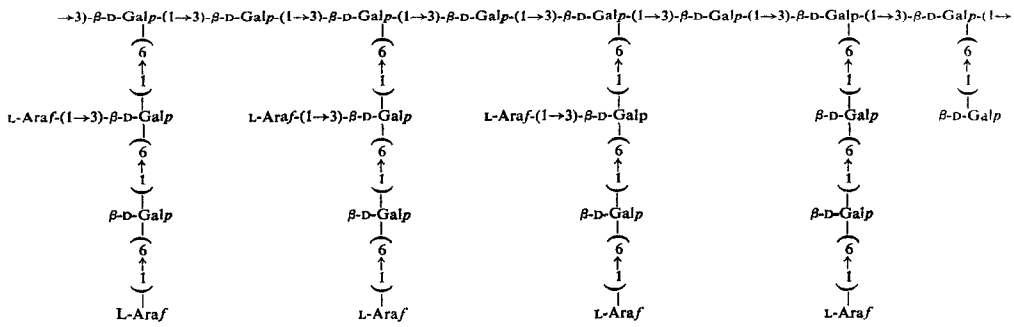

FIG. 1. POSSIBLE STRUCTURE FOR THE ARABINOGALACTAN FROM SUSPENSION-CULTURED BEAN ROOT CELLS.

The glycosidic linkages between the D-galactose residues were determined but the distribution of these linkages is not known at present. The present arabinogalactan is envisaged as having a backbone of (1→3)-β-linked D-galactose residues of which approximately five out of eight residues have a branch of (1→6)-β-linked galactobiose attached to C-6. Single L-arabinofuranosyl residues are attached glycosidically to the galactobiose side chains at C-3 although the methylation data would also permit their attachment to C-6 of the (1→3)

linked D-galactose main chain. The former arbitrary attachment is based on comparison with established structures for wood arabinogalactans.[10] The presence of 2,3,4,6-tetra-*O*-methyl-D-galactose in the hydrolyzate of the methylated polysaccharide showed that one D-galactose residue in 24 sugar residues constituted a non-reducing end group.

The proposed structure is in agreement with the results of periodate oxidation. It requires a periodate consumption of 0·85 mol/mol of anhydro sugar residue; the experimental results gave a periodate consumption of 0·90 mol/mol of anhydro sugar.

The unit structure proposed in Fig. 1 has a molecular weight of 3700 and this unit would be repeated approximately 7 times in the arabinogalactan molecule.

The arabinogalactan from kidney bean culture medium is very similar in constitution to that found by Aspinall *et al.*[7] in the culture medium of suspension-cultured sycamore cells. Only a minor difference in the arabinose:galactose ratio was found. It appears likely that the arabinogalactans isolated from suspension-cultured cells have the same general structure as those commonly occurring in coniferous woods,[10] although the wood arabinogalactans contain in general, an arabinose:galactose ratio of 1:6–7 and have a higher molecular weight (*ca.* 100 000).[11,12] The finding of an arabinogalactan elaborated by non-differentiating plant root cells that is similar to the arabinogalactans found in highly differentiated coniferous wood cells is of considerable biological interest.

The formation site of the extracellular polysaccharides in suspension-cultured cells is not known. The presence of the same monosaccharides in the cell wall polysaccharides of sycamore and in its extracellular polysaccharides suggests that the latter are synthesized within the cell and excreted into the medium.[4] Similar observations have been made on suspension-cultured tobacco cells[13] and in the present study of kidney bean root.

EXPERIMENTAL

The cultures of red kidney bean root cells were grown by Dr. I. Veliky, Division of Biology, on a basal medium in a specially designed glass fermenter, both of which have been described in detail.[14,15]

Chromatography. Descending paper chromatography was effected on Whatman No. 1 paper with the following solvent systems (A) EtOAc–pyridine-H_2O (5:2:5, upper), (B) EtOAc–HOAc–HCOOH–H_2O (18:3:1:4). Reducing sugars were detected by *p*-anisidine hydrochloride[16] and by alkaline silver nitrate.[17]

Gas chromatography (GLC) was carried out on a Hewlett-Packard Model No. 402 with glass columns (1 m × 3 mm) with the following packings. (A) 3% ECNSS-M on Gas Chrom Q, (B) 10% NPGS on Celite 545. Retention times (T_G) are quoted relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

Sugar analysis. Hydrolysis of polysaccharides was with 0·5 M H_2SO_4 in sealed tubes at 100° for 11 hr. The hydrolyzate was neutralized with $BaCO_3$, filtered and concentrated. Total sugar was determined by the phenol-sulfuric acid method[18] using galactose as the reference standard, and individual monosaccharides were analysed as their alditol acetates[19] by GLC on column A.

Electrophoresis. Polysaccharide (150 mg) was dissolved in 0·05 M borate buffer (pH 9·3) (15 ml) and dialysed against the same buffer (2 l.) at 4° for 24 hr. The electrophoretic examination was made in a Spinco Model H apparatus.

Sedimentation. An aliquot (0·7 ml) of the same solution as used for electrophoresis was centrifuged in a Spinco Model E Ultracentrifuge at a rotor speed of 59 780 rpm and photographs were taken at regular intervals during sedimentation.

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Molecular weight. MW measurements were made by gel permeation chromatography on Bio-Gel P 100 using Blue Dextran and dinitrophenyl lysine to determine the void volume and bed volume of the column respectively. The eluting agent was H_2O and the polysaccharide was located in the eluate with phenol-sulfuric acid reagent. The MW was estimated by plotting the elution volume of the arabinogalactan against the log of the MW of the known standards according to the method described by Andrews.[20]

General. All evaporations were carried out under reduced pressure under 40°. Optical rotations were recorded at 25° on a Perkin–Elmer 141 polarimeter and are equilibrium values for aq. solutions unless stated otherwise.

Recovery of crude polysaccharides. After maximum growth had been attained in the suspension cultures, the cellular material was removed from the medium by filtration through Miracloth (Chicopee Mills Inc., N.Y.) and washed with H_2O. The culture medium (10 l.) to which the cell washings were added was concentrated 10-fold and a small precipitate was removed by centrifugation and discarded. The crude polysaccharides were recovered by precipitation with EtOH (3 vol.). The precipitate was washed with 80% EtOH, re-suspended in H_2O and centrifuged to remove a small amount of insoluble material. After dialysis, the polysaccharide was recovered by freeze drying (2·42 g, ash 2·6%). Acid hydrolysis of a small amount of the polysaccharide and paper chromatographic examination of the hydrolyzate showed that galactose, glucose, arabinose and xylose were present, with the hexoses being in excess of the pentoses. Electrophoresis in borate buffer showed three peaks indicating that the preparation was a mixture.

Fractionation of the polysaccharides. The polysaccharide mixture (2·4 g) was dissolved in water (100 ml) and applied to a column (63 × 3·5 cm) of DEAE cellulose (carbonate form). The fractions were eluted consecutively with H_2O (1500 ml), 0·5 M ammonium carbonate (1000 ml) and 0·2 M NaOH (1000 ml) and the fractionation procedure was followed analytically with the phenol-sulfuric acid reagent. Each eluate was dialysed, concentrated and freeze dried. The H_2O-eluted fraction (0·95 g) I on acid hydrolysis and paper chromatographic examination was shown to contain glucose, xylose and smaller amounts of galactose and arabinose. The fraction eluted by ammonium carbonate solution (1·00 g) II contained only galactose and arabinose. The fraction eluted by NaOH (0·24 g) III contained galactose, glucose, arabinose and xylose in approximately the same proportion as in the starting material as judged by paper chromatography. On electrophoresis I showed one major component and two minor components, II gave a single sharp peak indicating that the polysaccharide was homogeneous. Fraction I could not be purified by further chromatography on DEAE-cellulose and on cellulose[7] or by precipitation procedures using Fehling's solution.[21] Since the purity of this polysaccharide was in doubt as shown by electrophoretic and ultracentrifugal examination it was not investigated further at this time. A detailed study of the purified arabinogalactan II was made.

Partial acid hydrolysis. Polysaccharide (20 mg) was heated in 0·025 N H_2SO_4 (5 ml) at 75° for 4·5 hr. Chromatographic examination (solvent A) of the neutralized solution $BaCO_3$ showed that approximately 95% of the arabinose units and 10% of the galactose units had been released as free sugars. In another experiment polysaccharide (40 mg) was heated with 0·25 M sulfuric acid (10 ml) at 100° for 1 hr. The neutralized solution was examined chromatographically (solvent A) and showed the presence of L-arabinose, D-galactose, 6-*O*-β-(D-galactopyranosyl)-D-galactose and 3-*O*-β-(D-galactopyranosyl)-D-galactose by co-chromatography with authentic specimens.

Methylation. Arabinogalactan (600 mg) was methylated by the Hakomori procedure[22] with MeI and NaH in dimethyl sulfoxide. The IR spectrum showed some absorption at 3500 cm^{-1} due to —OH groups

TABLE 1. METHYL ETHERS FROM HYDROLYSATE OF METHYLATED ARABINOGALACTAN

Sugars	T_G* Column A	T_G* Column B	Relative molar proportions
2,3,5-Tri-*O*-methyl-L-arabinose	0·46	0·47	7·0
2,3-Di-*O*-methyl-L-arabinose	1·30	1·19	0·4
2,3,4,6-Tetra-*O*-methyl-D-galactose	1·30	1·06	1·0
2,4,6-Tri-*O*-methyl-D-galactose	2·40	1·94	2·5
2,3,4-Tri-*O*-methyl-D-galactose	3·72	2·58	5·5
2,4-Di-*O*-methyl-D-galactose	7·33	4·29	8·0

* Retention times of the corresponding alditol acetates on the ECNSS-M column (A) at 160° and the NPGS column (B) at 180° relative to 1,5-diacetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

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indicating incomplete methylation. Complete methylation was achieved by five Purdie methylations to yield methylated arabinogalactan (360 mg), $[\alpha]_D^{22}$ −45° (c 1·0 in $CHCl_3$) found: OMe, 41·5%). A portion of the methylated polysaccharide was hydrolyzed with H_2SO_4 according to Garegg and Lindberg.[23] The methyl ethers of the sugars were converted into their acetylated alditols[24] which were identified quantitatively by GLC on columns A and B by comparison with authentic samples. The results are given in Table 1.

Periodate oxidation. Arabinogalactan (7·15 mg) dissolved in 0·015 M $NaIO_4$ solution (10 ml) was oxidized at 20° in the dark. The consumption of periodate was estimated spectrophotometrically[25] at 24 hr intervals until it became constant. The consumption of periodate was 0·90 mol/mol of anhydro sugar based on average MW (calc. 155).

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Key Word Index—Phaseolus vulgaris; Leguminosae; kidney bean; tissue culture; arabinogalactan; arabinose; galactose.