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A GLUCOMANNAN FROM THE TUBERS OF *ARUM ORIENTALE*

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Key Word Index—*Arum orientale*; Araceae; glucomannans.

Abstract—Two glucomannans (*A* and *B*), and an attendant polysaccharide (*C*) have been isolated from the tubers of *Arum orientale* (L.) by gel chromatography. Glucomannan *B* was found to be composed of D-glucose and D-mannose in a molar ratio of 2:3·1, and traces of uronic acid. IR spectra, enzymatic hydrolysis with α- and β-amylase, and periodate oxidation followed by reduction, showed the presence of a β-(1→4) glucoside linkage in this glucomannan, and of hexose residues in a β-pyranose form.

INTRODUCTION

IT HAS been shown recently that some plants of the families Araceae,[1,2] Iridaceae,[3] Amaryllidaceae,[4] Liliaceae,[5,6] and Orchidaceae[7] contain glucomannans. In a previous paper,[8] we reported studies of a glucomannan isolated from the tubers of *Arum orientale* (L.). The classical method used for the isolation of the mannans with Fehling's solution was found to be inconvenient. The glucomannan obtained by this method became insoluble in water, which impeded its further investigation. The present paper describes the isolation and investigation of an electrophoretically homogeneous glucomannan isolated from *Arum orientale* (L.).

RESULTS AND DISCUSSION

The crude polysaccharide was isolated from the *Arum* tubers by extraction with cold water followed by precipitation with ethanol. The polysaccharide obtained was soluble in water and non-reducing.

After electrophoresis on cellulose acetate strips and thin-layer gel chromatography of the crude polysaccharide coloured with Procion blue M 3G, three polysaccharide components (*A*, *B* and *C*), moving to the anode (Fig. 1, upper part) were detected.

The crude polysaccharide was separated into four fractions by chromatography on a DEAE Sephadex A50 column. Fractions I–III were obtained by gradient elution with 0 to 2 M aqueous potassium acetate, and fraction IV with sodium hydroxide. The results are shown in Fig. 1. The main fraction I was electrophoretically homogeneous (Fig. 1,

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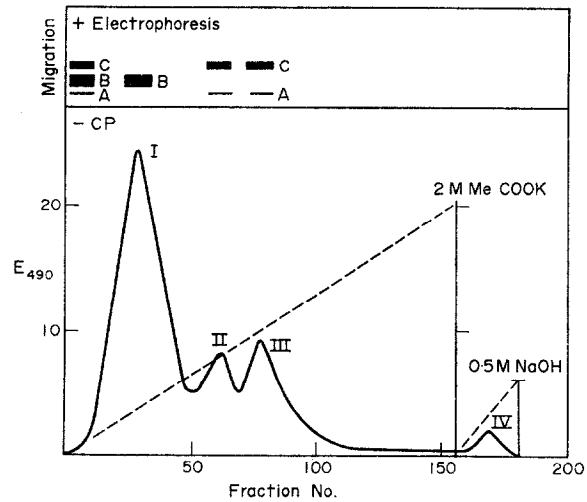

FIG. 1. ION-EXCHANGE GEL CHROMATOGRAPHY ON A DEAE SEPHADEX A-50 COLUMN OF CRUDE POLYSACCHARIDE MIXTURE, ISOLATED FROM TUBERS OF *Arum orientale*
The upper part of the figure shows electrophoresis of the crude polysaccharide (CP), fractions (I), (II) and (III) on cellulose acetate strips.

upper part) and largest in quantity (polysaccharide *B*). Fractions II and III contained the remaining two polysaccharide components (*A* and *C*) Fig. 1, upper part). These were obtained in an electrophoretically homogeneous state by additional separation on a Sephadex G75 column. Fraction IV containing polysaccharides *A* and *C*, as well as traces of polysaccharide *B*, was not studied further for lack of material.

TABLE 1. IR SPECTRA OF THE HOMOGENEOUS GLUCOMANNAN B ISOLATED FROM *Arum orientale*

Absorption bands (cm^{-1})	Interpretation
1730 and 1248	Presence of a carboxyl-containing component
893 ± 2	Presence of a β-glucoside linkage of type 2b after Barker *et al.*[10] absence of absorption in the interval 830–850 cm^{-1} (α-glucoside bond of type a gives rise to absorption at 840 cm^{-1})
890 ± 15	Presence of β-glucopyranose residues
894 ± 3	Presence of β-mannopyranose residues
880 ± 8	Presence of mannopyranose residues of type 2c.[10] This is an additional absorption of the mannopyranose residues in comparison to the glucopyranose residues
800	Presence of hexose residues in a pyranose form

PC and TLC revealed that D-mannose, D-glucose, and traces of uronic acid were the sole products resulting from complete acid hydrolysis of the polysaccharides *A* and *B*. Hydrolysis of the polysaccharide *C* yielded D-glucose, D-galactose, L-arabinose, L-rhamnose and traces of uronic acid.

Of the two isolated glucomannans (*A* and *B*), polysaccharide *B* was predominant (about

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70%) and was therefore chosen for further investigations. After trimethylsilylation, GLC of the monosaccharides obtained on hydrolysis confirmed the composition of polysaccharide *B*. D-Glucose and D-mannose were detected in a molar ratio of 2:3·1. The IR spectra of the homogeneous glucomannan *B* is given in Table 1. The stability of polysaccharide *B* under the conditions of complete hydrolysis indicate sugar units of a pyranose and not a furanose structure. This result is consistent with the data obtained by IR spectroscopy.

Incubation of polysaccharide *B* with α- and β-amylase liberated no free monosaccharides. This result lends further support to the data obtained by IR spectroscopy: the absence of an α-glucoside linkage, and the presence of a β-glucoside linkage in the polysaccharide chain.

Treatment of glucomannan *B* with sodium metaperiodate at 5° resulted in a periodate consumption of 1 mol per hexose unit. The formic acid produced amounted to 0·04 mol per mol of hexose unit. About 80% oxidation of the glucomannan was achieved after one day with complete oxidation on the 6th day. Reduction of the polyaldehyde with borohydride, followed by acid hydrolysis of the polyalcohol mixture gave erythritol, glycerol (in traces), and D-mannose (in traces). The presence of erythritol in the hydrolysate confirms the presence of a 1 → 4 glucoside linkage in the polysaccharide chain. Traces of free mannose and glycerol can be interpreted as originating from 1 → 3 linkages or branching of the mannose residues at C-2 in glucomannan *B*.

EXPERIMENTAL

General methods. PC was performed on Whatman papers No. 1 and 3 MM using: (A) acetone-*n*-BuOH-H_2O (7:2:1); (B) EtOH-*n*-BuOH-H_2O (5:4:1); (C) EtOAc-pyridine-H_2O (8:2:1); and (D) tert-Amyl OH-*n*-PrOH-N_2O (40:10:50). Aniline hydrogen phthalate (G) and ammoniacal $AgNO_3$ (H) were used for detection of monosaccharides. Higher alcohols, glycerol and erythritol were detected with $NaIO_4$–benzidine (I). *TLC* was carried out on silica gel G (Merck) impregnated with 0·2 M Na_2HPO_4 in the system: (E) EtOAc-*iso*-PrOH-H_2O (8:2:1, v/v); and (F) acetone-H_2O-$CHCl_3$-MeOH (8:5:1:1). Monosaccharides were located on TLC with naphthoresorcinol (J). *GLC* was performed on an F & M model 402 apparatus with a flame ionization detector using air as carrier gas. GLC of trimethylsilyl derivatives was carried out using a column of 3·8% SE-30 on Gas-chrom P. *IR* spectra were taken on a Perkin Elmer model 257 instrument in the region 700 to 2000 cm^{-1}. The elution of polysaccharide from columns was monitored by the phenolsulphuric acid method at 490 nm.[11] The tubers of *Arum orientale* (L.) were gathered in 1969 and 1970 (July–August) from different regions of Bulgaria.

Extraction of the crude polysaccharide. The meal of ground tubers from *Arum orientale* (L.) was suspended in EtOH at 95° for 1 hr. The insoluble material was washed with C_6H_6 and dried. The pretreated tubers were extracted with H_2O (1:10) at 4° with stirring for 18 hr. The extract was clarified by centrifugation and precipitated by adding 90% EtOH. The crude polysaccharide was an amorphous light brown powder. Its aq. soln gave an insoluble complex with Fehling's solution but did not reduce it, even on prolonged boiling, indicating the absence of free sugars.[12]

Fractionation experiments. The crude polysaccharide was fractionated on DEAE Sephadex A50. The material (about 2 g) was dissolved in H_2O and poured onto a column (4·7 × 50 cm) of DEAE Sephadex A50 in acetate form. The column was eluted with a gradient of potassium acetate (0–2 M) and finally with 0·5 M NaOH. Additional fractionation of the heterogeneous fractions (II and III) was preformed on a Sephadex G75 column. The separated fractions were dialysed against H_2O and freeze-dried.

Electrophoretic studies. The Procion dye complexes from the crude polysaccharide and the polysaccharides (*A*, *B* and *C*) were analysed for homogeneity on cellulose acetate strips (25 × 140 mm) by the Dudman–Bishop method,[13] using 0·05 M borate buffer and 0·1 M borax-NaCl buffer; (270 V for 4–5 min).

Thin-layer gel chromatography. The Procion dye complexes from the polysaccharides, as stated above, were analysed for homogeneity on Sephadex layers by the sandwich chamber and TLC method[14] (Boeh-

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ringer Mannheim GmbH, Germany), on 20 × 20 cm plates with Sephadex G75 (superfine) and 0·05 M borate buffer.

Hydrolysis of the glucomannan B. The polysaccharide (100 mg) was treated with 70% sulphuric acid (0·5 ml) overnight. The soln was diluted with H_2O (13 parts) and heated at 100° for 24 hr. After neutralization with $BaCO_3$, the filtrate was passed through weak anion (Duolite A-4) and cation (Amberlite IR-120) exchange-resin columns, and the solution evaporated. The syrup was analysed by PC, TLC (using solvents *a*, *b*, *c*, *d*, *e* and *f*, and using reagents *g*, *h* and *j*), and GLC.

Enzymatic hydrolysis of glucomannan B was carried out with α- and β-amylase in phosphate buffer, pH 6·9.[15] The products of the enzymatic hydrolysis were studied by PC and TLC using solvents and reagents, as described above.

Periodate oxidation studies of glucomannan B. To a portion (80 mg) of glucomannan *B* dissolved in H_2O (45 ml) 0·01 M $NaIO_4$ (200 ml) was added. The solution was made up to 250 ml with H_2O and kept at 5° in the dark. The periodate consumption was determined at intervals by the Fleury–Lange method[16] and formic acid was measured by titration with 0·01 N NaOH after reducing excess periodate with ethylene glycol. The resulting polyaldehyde was dialysed against H_2O followed by reduction with $NaBH_4$ (1500 mg) for 2 hr at room temp. with continuous stirring. Finally the polyalcohol was dialysed against H_2O, freeze-dried and hydrolysed with 1 N H_2SO_4 for 10 hr in a sealed tube. After neutralization with $BaCO_3$, the filtrate was treated with resins as described above and the soln evaporated. The syrupy residue was analysed by PC (Whatman No. 1) using solvents *c* and *d*, our reagent I. The presence of D-mannose was proved by PC and TLC using solvents *a*, *b*, *c*, *d*, *e* and *f*, and using reagents *g*, *h* and *j*.

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