Carbohydrate Research, 155 (1986) 290–293
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

A glucomannan isolated from the leaves of *Arum maculatum* L.

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(Received January 20th, 1986; accepted for publication, April 29th, 1986)

We have described[1] the isolation of two acetylated glucomannans (M_1 and M_2) from the tubers of *Arum maculatum* L. of which M_2 preponderated (70%). It was shown that in M_2 there were two D-Glc*p* residues at the most linked to each other, and four D-Man*p* residues at the most linked to each other, in the polysaccharide chain[2]. Some D-Man*p* residues of the main chain carried[3] branches at position 3. We now report on an electrophoretically and chromatographically homogeneous glucomannan isolated from the leaves of *Arum maculatum* L.

The crude polysaccharide, isolated by extraction with cold water followed by precipitation with ethanol, was soluble in water, non-reducing, gave no colour with iodine, contained no proteins, and gave glucose, mannose, galactose, arabinose, xylose, rhamnose, and glucuronic acid on hydrolysis with acid.

Thin-layer gel chromatography (t.l.g.c.) in the first direction and then thin-layer gel electrophoresis (t.l.g.e.) in the second direction of the complex of the crude polysaccharide with Procion Blue M 3G on Sephadex G-100 (superfine) gave four components (AM_1–AM_4).

Precipitation of the crude polysaccharide from its aqueous solution using cetyltrimethylammonium bromide (Cetavlon) also gave four fractions of which AM_1, AM_3, and AM_4 were homogeneous (t.l.g.c.–t.l.g.e.), and AM_2 contained traces of AM_3. Elution of the last fraction from DEAE-cellulose with M potassium acetate gave AM_2, which was homogeneous in t.l.g.c.–t.l.g.e.

On hydrolysis with acid, AM_2 gave glucose, mannose, and traces of glucuronic acid, AM_1 gave glucose, arabinose, rhamnose, glucuronic acid, and traces of xylose, AM_3 gave galactose, mannose, xylose, and glucuronic acid, and AM_4 gave galactose, arabinose, rhamnose, and glucuronic acid.

The preponderant polysaccharide AM_2 (50%) was investigated further. AM_2 was readily soluble in water and aqueous alkali, and it gave no colour with iodine. Its $[\alpha]_D^{22}$ value of $-30°$ (*c* 0.15, 0.5M NaOH) indicated that most of the monosaccharide residues were β. The relatively low viscosity (η_{rel} 1.42) of a 0.15%

*Deceased.

0008-6215/86/$ 03.50

aqueous solution of AM_2, which did not change on increasing the concentration, suggested that the molecule was globular.

Acid hydrolysis of AM_2 gave (p.c.) glucose and mannose in the molar ratio 1:1.8, and the i.r. absorbtions of AM_2 at 1730 and 1248 (COOH), and 894, 875, and 800 cm^{-1} were characteristic[4] of β-D-glucopyranose and β-D-mannopyranose residues. The rate of acid hydrolysis of AM_2 also indicated pyranose sugar units. Incubation of AM_2 with alpha- and beta-amylase[5] liberated no free monosaccharides, indicating the absence of α-D-glucosidic linkages in the polysaccharide chain.

At 4° in the dark, AM_2 consumed 1 mol of periodate per hexose residue and liberated 0.03 mol of formic acid. After 1 day, 75% oxidation had occurred and reaction was complete after 6 days. Borohydride reduction of the product followed by acid hydrolysis gave erythritol, glycerol (trace), and mannose (trace). The formation of erythritol confirmed the presence of a (1→4)-glucosidic linkage in the polysaccharide chain. The traces of mannose and glycerol probably originated from (1→3) linkages or from mannose residues branched at position 2.

EXPERIMENTAL

General methods. — Protein was determined by the biuret method[6], and carbohydrate by the phenol–sulfuric acid method[7] at 490 nm. P.c. was performed on Whatman 3MM and FN_3 papers with *A*, 1-butanol–acetic acid–water (4:1:5); *B*, acetone–1-butanol–water (7:2:1); and *C*, 1-butanol–ethanol–water (4:1:2.2); and detection as appropriate with aniline hydrogen phthalate (*F*) and periodate–benzidine (*G*). T.l.c. was performed on silica gel G, using *D*, ethyl acetate–methanol–acetic acid–water (60:15:15:10), and detection with thymol-sulfuric acid (*H*); and on cellulose, using *E*, formic acid–water-*tert*-butyl alcohol–2-butanone (15:15:40:30), and detection with reagent *F*. I.r . spectra (700–2000 cm^{-1}) were obtained with a Zeiss UR-10 Spectrometer and optical rotations (589 nm) with a Société Jouan polarimeter (0.5-cm cell). Viscosities were determined at 30° using an Ostwald viscometer.

Plant material. — The leaves of *Arum maculatum* L., collected in May 1981 from various regions of Bulgaria, were dried at 40–50°.

Isolation and fractionation of the crude polysaccharide. — The pulp of the leaves was treated thrice with ethanol (1:10, w/v) at 95° for 1 h. The insoluble material was stirred with water (1:25, w/v) at 4° for 18 h, and the extract was centrifuged and treated with 4 vol. of aqueous 96% ethanol. The crude polysaccharide was collected and the precipitation procedure was repeated. The precipitate was collected by centrifugation and was dehydrated with ether to give an amorphous light-brown powder. An aqueous solution gave an insoluble complex with, but did not reduce, Fehling's solution.

To an aqueous 0.7% solution (300 mL) of the crude polysaccharide was added aqueous 5% Cetavlon (20 mL). The resulting precipitate was collected by

centrifugation (4000 r.p.m., 20 min), dissolved in 0.3M hydrochloric acid, and precipitated with 4 vol. of aqueous 96% ethanol. The precipitate was collected by centrifugation, dissolved in water, precipitated with ethanol, collected by centrifugation, and dehydrated with ether to give AM_1 which was homogeneous in t.l.g.c.–t.l.g.e. of the Procion dye complexes on Sephadex G-100 (superfine)[8].

The supernatant solution was treated with aqueous 5% Cetavlon (10 mL), and the precipitate was collected by centrifugation and treated as for AM_1 to give AM_4 which was homogeneous in t.l.g.c.–t.l.g.e.

The supernatant solution was treated with aqueous 5% Cetavlon (10 mL) as described above, to give AM_3 which was homogeneous in t.l.g.c.–t.l.g.e.

The supernatant solution was treated with 0.3M hydrochloric acid and then 4 vol. of aqueous 96% ethanol. The precipitate was collected, dissolved in water, and precipitated with 4 vol. of ethanol to give a mixture of AM_2 and AM_3 as shown by t.l.g.c.–t.l.g.e.

The mixture of AM_2 and AM_3 was eluted from a column (3 × 40 cm) of DEAE-cellulose with a gradient (0→2M) of potassium acetate. The fractions were dialysed against water and precipitated by adding 4 vol. of aqueous 96% ethanol; AM_2 was eluted with M potassium acetate and was homogeneous in t.l.g.c.–t.l.g.e.

Two-dimensional identification of polysaccharides[8]. — Preliminary dyeing of the crude polysaccharide and AM_1–AM_4 with Procion Blue M 3G (I.C.I.) was achieved by the method of Dudman and Bishop[9]. The aqueous solutions of the dyed polysaccharides, after the non-reacted dye and salts had been removed, were freeze-dried. Sephadex G-100 (superfine) and plates 20 × 20 cm and 15 × 15 cm in size were used for t.l.g.c.–t.l.g.e. A sample (30–40 μL) of an aqueous 1% solution of the polysaccharide was applied to each plate. Descending t.l.g.c. was carried out in the first dimension (the plate was fixed at an angle of 15°) in 0.05M borate buffer for 4–5 h. T.l.g.e. was then carried out in the same buffer in the second dimension at 150 V for 2–3 h. The thin-layer gel chromatogram and electrophoregram was copied on Whatman 3MM paper.

Reactions of the polysaccharides. — (*a*) *Acid hydrolysis.* The crude polysaccharide and AM_1–AM_4 were treated severally with aqueous 72% sulfuric acid (1 mL to 40 mg) at 20° for 18 h. Each solution was diluted to ~5% acid, heated at 100° for 26 h, then neutralised ($BaCO_3$), passed through columns of Duolite A-4 (HO^-) and Amberlite IR-120 (H^+) resins, and concentrated. Each syrupy residue was analysed by p.c. (solvents A–C) and t.l.c. (solvents *D* and *E*) with detection by reagents *F* and *H*. AM_2 gave glucose and mannose in the molar ratio 1:1.8.

(*b*) *Enzymic hydrolysis.* AM_2 was hydrolysed with alpha- and beta-amylase in phosphate buffer (pH 6.9)[5] and the products were subjected to p.c. and t.l.c.

(*c*) *Periodate oxidation.* To a solution of AM_2 (80 mg) in water (60 mL) was added 0.25M sodium periodate (10 mL). The solution was made up to 100 mL with water and stored at 5° in the dark, the periodate consumption was determined by the Fleury–Lange method[10], and the formic acid released was titrated with 0.01M KOH after reducing the excess of periodate with ethylene glycol. The resulting polyaldehyde was dialysed against water and then reduced conventionally with sodium borohydride (1.5 g) for 2 h at room temperature. The product was dialysed

against water (18 h), precipitated with 96% ethanol, and hydrolysed with 0.05M sulfuric acid for 10 h at 100° in a sealed tube. The hydrolysate was neutralised with $BaCO_3$, then filtered, treated with ion-exchange resins as described above, and concentrated. The syrupy residue was analysed by p.c. (solvent *A*) and t.l.c. (solvents *D* and *E*) with detection by reagent *G*. The presence of mannose was proved by p.c. and t.l.c., as described above.

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