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STRUCTURE OF A NEW GALACTOMANNAN FROM THE SEEDS OF *Ipomoea fistulosa*

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ABSTRACT

The seeds of *Ipomoea fistulosa* contain a water-soluble galactomannan composed of residues of D-galactose and D-mannose in the ratio 3:10. Hydrolysis of the methylated polysaccharide gave 2,3,4,6-tetra-*O*-methyl-D-mannose (3 mol), 2,3,6-tri-*O*-methyl-D-mannose (7 mol), and 2,3-di-*O*-methyl-D-mannose (3 mol). Periodate-oxidation data indicated 22.1% of end groups, and methylation 22.8%. The above findings, together with data on the oligosaccharides formed on partial hydrolysis with acid, showed the galactomannan to consist of a linear chain of (1→4)-linked β-D-mannosyl residues to which α-D-galactosyl groups are attached by (1→6)-linkages.

INTRODUCTION

We report on a galactomannan isolated from the seeds of *Ipomoea fistulosa* (syn. *Ipomoea carnea*) belonging to the Convolvulaceae family.

RESULTS AND DISCUSSION

The galactomannan was isolated from dried, crushed, and defatted seeds of *Ipomoea fistulosa* by extraction with cold 1.5% acetic acid, and its homogeneity was verified by fractional precipitation, *via* acetylation–deacetylation, and by zone electrophoresis.

The polysaccharide was water-soluble, and had $[\alpha]_D^{25}$ +72.5° (water), an ash content of 0.18%, and a negligible content of methoxyl and acetyl groups and uronic acid.

Complete hydrolysis of the polysaccharide with acid yielded (p.c.) D-galactose and D-mannose in the molar ratio 3:10. When graded, acid hydrolysis of the galactomannan was monitored by p.c., galactose was found to be released first and mannose could be detected only after 20 min. This finding indicates that the polysaccharide consists of a main chain of mannose residues with D-galactose occupying terminal positions.

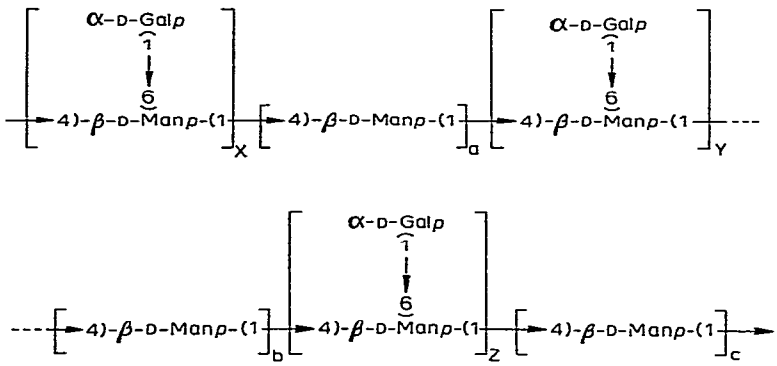
The galactomannan was exhaustively methylated, first by the method of Parikh

et al.[1] and then by the Purdie method[2]. Hydrolysis of the methylated polysaccharide yielded 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,6-tri-*O*-methyl-D-mannose, and 2,3-di-*O*-methyl-D-mannose in the ratios 3:7:3. The identity of the partially methylated monosaccharides was established on the basis of crystalline derivatives. The formation of 2,3,4,6-tetra-*O*-methyl-D-galactose confirmed the conclusion made from the results of graded hydrolysis of the polysaccharide and indicated that the galactose residues occupy the terminal positions. The formation of the partially methylated D-mannoses showed that D-mannose residues form the main chain of the galactomannan which is branched at positions 1, 4, and 6.

Oxidation of the galactomannan with periodate for 60 h liberated formic acid corresponding to 22.1% of end groups (*cf.* 22.8% from the methylation studies), and destroyed all of the D-galactose residues and most of the D-mannose residues. Prolonged oxidation destroyed both the hexoses.

From the foregoing results, it is postulated that the galactomannan consists of a linear chain of (1→4)-linked D-mannopyranosyl residues to which D-galactopyranosyl groups are attached by (1→6)-linkages. The galactomannan showed i.r. absorptions at 810 and 875 cm^{-1} indicating[3] the presence of both α and β linkages.

Partial hydrolysis of the galactomannan with acid yielded D-mannose and D-galactose along with three (1→4)-linked D-mannosaccharides (biose, triose, and tetraose), epimelibiose [α D-Gal*p*-(1→6)-D-Man*p*], and galactosylmannobiose [α-D-Gal*p*-(1→6)-β-D-Man*p*-(1→4)-D-Man*p*]. Mannotriose, epimelibose, and galactosylmannobiose were the major products The foregoing data accord with the following structure:



where X + Y + Z = 3, a + b + c = 7, and possibly a = b = 3 and c = 1.

A polysaccharide of this structure should consume 1.23 mol of periodate, with the liberation of 0.23 mol of formic acid, per mol of hexosyl residue; the observed values were 1.27 and 0.21 mol, respectively.

EXPERIMENTAL

Solutions were concentrated at reduced pressure and low temperature unless otherwise specified. All products were dried *in vacuo* over calcium chloride. Melting

points are uncorrected and $[\alpha]_D$ values are for equilibria. Fractionation of hydrolysates was carried out by descending p.c. at room temperature by using the non-aqueous phases of *A*, 1-butanol–acetic acid–water (4:1:5); *B*, 1-butanol–ethanol–water (5:1:4); and *C*, ethyl acetate–pyridine–water (2:1:2); and the homogeneous solvent *D*, ethyl acetate–pyridine–water (10:4:3); and detection with aniline hydrogen phthalate.

Galactomannan. — (*a*) *Isolation.* Defatted, dried, and crushed seeds of *Ipomoea fistulosa* (1 kg) were extracted successively with light petroleum (b.p. 60–80°) and ethanol, and then stirred with 1.5% aqueous acetic acid at room temperature for 12 h. Extraction with acid was repeated until no precipitate was obtained on addition of excess of ethanol to the extract. The acid extract was added slowly with stirring to ethanol (2 vol.). The crude polysaccharide was collected, washed with ethanol, and dried (17 g). The dry product (17 g) was reprecipitated from solution in distilled water by using ethanol, to yield a white, amorphous mass (15 g; ash, 0.18%), $[\alpha]_D^{25}$ +72.5° (water).

(*b*) *Homogeneity.* A solution of the polysaccharide (2 g) in water (250 ml) was poured into ethanol (400 ml) with continuous stirring. The resulting precipitate (*A*) was collected, and the filtrate was diluted with ethanol (800 ml) to give precipitate *B*. Quantitative analysis of the products of hydrolysis of *A* and *B* revealed D-galactose and D-mannose in the molar ratio 3:10.

The polysaccharide (1 g) was treated with anhydrous sodium acetate (5 g) and acetic anhydride (10 ml) for 20 h at ~100°. The cooled mixture was poured onto crushed ice, and the crude product was dissolved in chloroform. Insoluble impurities were removed by filtration, the solvent was evaporated, and the resulting residue was recrystallised from aqueous acetone. The acetate (0.7 g) had $[\alpha]_D^{25}$ +24.5° (chloroform) (Found: AcO, 41.1%). On deacetylation, it gave a product having $[\alpha]_D^{25}$ +71° (water).

The polysaccharide (50 mg) was subjected to zone electrophoresis on Whatman No. 1 paper in borate buffer (pH 9.5). The paper was cut into segments (1 cm wide) each of which was extracted with water (10 ml). Each extract (5 ml) was treated with 8% aqueous phenol (1 ml) followed by conc. sulphuric acid (15 ml). The absorbance of the characteristic yellow–orange colour was measured in a Klett–Summerson photoelectric colorimeter using filter No. 50. A plot of absorbance against segment number showed only a single, sharp peak.

(*c*) *Hydrolysis.* The galactomannan (1.5 g) was hydrolysed at 100° with M sulphuric acid (50 ml) for 15 h. P.c. (solvents *A* and *B*) of the hydrolysate revealed galactose and mannose only. The syrupy hydrolysis product was fractionated by elution from a cellulose column with solvent *B* to give, first, D-mannose, which, after crystallisation from aqueous methanol, had m.p. and mixture m.p. 130–132°, $[\alpha]_D^{25}$ +13° (water); the derived phenylhydrazone had m.p. 192–194°. Eluted second was D-galactose which, after crystallisation from aqueous methanol, had m.p. and mixture m.p. 163–165°, $[\alpha]_D^{25}$ +77.5° (water); the derived phenylhydrazone had m.p. 154–156°.

After separation by p.c. (solvent *B*), quantification of the D-mannose and D-galactose by periodate oxidation[4] indicated a molar ratio of 3:10.

(*d*) *Graded hydrolysis.* The galactomannan (100 mg) was hydrolysed with 0.25M H_2SO_4 (30 ml) and the hydrolysate was monitored by p.c. (solvent *B*) during 3 h. Galactose was detected after 10 min and mannose after 20 min.

(*e*) *Periodate oxidation.* To a solution[5] of the galactomannan (300 mg) in water (25 ml) were added KCl (3.5 g) and 0.25M sodium metaperiodate (25 ml). The volume was made up to 100 ml with water. The oxidation was conducted in the dark at room temperature. Aliquots (5 ml) were withdrawn at intervals and titrated with 7.1mM sodium hydroxide after reduction of the excess of periodate with ethylene glycol. The amount of formic acid liberated (after 60 h) corresponded to 22.1% of hexosyl end-groups.

After 60 h, excess (30 ml) of ethylene glycol was added and the solution was concentrated. The concentrate was hydrolysed with M H_2SO_4 for 15 h at 100°. P.c. of the hydrolysate revealed mannose but not galactose.

To a solution[6] of the galactomannan (300 mg) in water (25 ml) was added 0.25M sodium metaperiodate (25 ml). The volume was made up to 100 ml with water. Aliquots (5 ml) were withdrawn at intervals and titrated against 75mM sodium thiosulphate. The amount of metaperiodate consumed (25.3 mg in 80 h) corresponded to the consumption of 1.27 mol of metaperiodate per mol of hexosyl residue. After 84 h, the oxidised polysaccharide was examined for the presence of mannose and galactose as described above; neither was detected.

(*f*) *Methylation analysis.* The methylated product (1.76 g; OMe, 43.4%), $[\alpha]_D^{25}$ +51° (chloroform), was obtained by two consecutive applications of the methods of Parikh *et al.*[1] and Purdie[2] to the galactomannan (3 g).

The methylated galactomannan (1.5 g) was hydrolysed with boiling 0.5M H_2SO_4 for 14 h. The hydrolysis product was fractionated on Whatman No. 3 paper with solvent *B*, to give the following products

2,3,4,6-Tetra-*O*-methyl-D-galactose (200 mg), m.p. 70–71° (from light petroleum), $[\alpha]_D^{25}$ +110.5° (water), R_{TMG} (mobility relative to that of 2,3,4,6-tetra-*O*-methyl-D-glucose) 0.89; lit.[7,8] m.p. 72–75°, R_{TMG} 0.88 (solvent *B*) (Found: OMe, 51.5. $C_{10}H_{20}O_6$ calc.: OMe, 52.5%). The derived anilide had m.p. 184–186° (from ethanol); lit.[9] m.p. 186–197°.

2,3,6-Tri-*O*-methyl-D-mannose (550 mg), syrup, $[\alpha]_D^{25}$ −11.5° (water), R_{TMG} 0.81 (solvent *B*) (Found: OMe, 42.1. $C_9H_{18}O_6$ calc.: OMe, 41.8%). The derived 1,4-di-*p*-nitrobenzoate had m.p. 186–187°, $[\alpha]_D^{25}$ +32.5° (chloroform).

2,3-Di-*O*-methyl-D-mannose (175 mg), syrup, $[\alpha]_D^{25}$ −15° (water), R_{TMG} 0.55; lit.[8] R_{TMG} 0.54 (solvent *B*) (Found: OMe, 29.5. $C_8H_{16}O_6$ calc.: OMe, 29.8%). The derived 1,4,6-tri-*p*-nitrobenzoate had m.p. 191–193°; lit.[10] m.p. 194°.

The methylated galactomannan (100 mg) was hydrolysed with 0.5M H_2SO_4, and D-glucose (25 mg) was then added. After neutralisation of the mixture, the methylated sugars were isolated by p.c. (solvent *B*) and quantified by using alkaline hypoiodite[11]. The results (in triplicate), expressed as ml of 0.01M iodine consumed,

were as follows: tetra-*O*-methylhexose, 1.02, 1.38, 1.21; tri-*O*-methylhexose, 2.38, 3.32, 2.82; di-*O*-methylhexose, 1.08, 1.36, 1.19; and glucose, 1.14, 1.62, 1.40. These data correspond to average molar ratios for tetra-, tri-, and di-*O*-methylhexoses of 3:7:3.

(*g*) *Partial, acid hydrolysis.* — A solution of the galactomannan (5 g) in water (500 ml) and conc. HCl (16 ml) was heated for 4 h at 80 ±1°, and then neutralised, filtered, and concentrated. P.c. of the resulting syrup revealed seven products which were isolated by preparative p.c.

Mannotetraose {β-D-Man*p*-(1—[→4)-β-D-Man*p*-(1—]$_2$→4)-D-Man*p*} (300 mg), R_{Man} 0.10 (solvent *D*), R_{Glc} 0.16 (solvent *C*), m.p 231–233° (from ethanol), $[\alpha]_D^{25}$ −30° (water), lit.[12,13] m.p. 232–234°, $[\alpha]_D$ −31°. Hydrolysis with M H_2SO_4 gave (p.c.) only mannose, and partial hydrolysis with 0.4M HCl yielded (p.c.) mannotriose, mannobiose, and mannose. During 48 h, the compound consumed 6.8 mol of periodate with the liberation of 3.1 mol of formic acid.

Mannotriose [β-D-Man*p*-(1→4)-β-D-Man*p*-(1→4)-D-Man*p*] (750 mg), R_{Man} 0.23 (solvent *D*), R_{Glc} 0.33 (solvent *C*), m.p. 211–213° (from ethanol), $[\alpha]_D^{25}$ −18° (water); lit.[12,13] m.p. 214–215°, $[\alpha]_D$ −15 to 26°. Hydrolysis gave (p.c.) only mannose, and partial hydrolysis with acid afforded (p.c.) mannobiose and mannose. During 48 h, the compound consumed 6 3 mol of periodate with the liberation of 2.8 mol of formic acid.

Mannobiose [β-D-Man*p*-(1→4)-D-Man*p*] (350 mg), R_{Man} 0.52 (solvent *D*), R_{Glc} 0.64 (solvent *C*), m.p. 206° (from ethanol), $[\alpha]_D^{25}$ −9° (water); lit.[12,13] m.p. 193–210°, $[\alpha]_D$ −5 to −9°. The derived phenylosazone had m.p. 204–206°; lit.[13] m.p. 203–206°. Acid hydrolysis of the disaccharide gave (p.c.) mannose only. During 48 h, the disaccharide consumed 5.2 mol of periodate with the liberation of 2.3 mol of formic acid.

Epimelibiose [α-D-Gal*p*-(1→6)-D-Man*p*] (640 mg), R_{Glc} 0.59 (solvent *C*), m.p. 204–205° (from ethanol), $[\alpha]_D^{25}$ +121–125° (water); lit.[14,15] m.p. 201–203°, $[\alpha]_D$ +120.9–124°. The derived phenylosazone had m.p. 178–179°; lit.[16] m.p. 175–176°. Acid hydrolysis gave (p.c.) galactose and mannose. During 48 h, the disaccharide consumed 5.8 mol of metaperiodate with the liberation of 4 2 mol of formic acid. The disaccharide was not hydrolysed by almond emulsin.

Galactosylmannobiose [α-D-Gal*p*-(1→6)-β-D-Man*p*-(1→4)-D-Man*p*] (550 mg), R_{Glc} 0.31 (solvent *C*), m.p. 227–230° (from ethanol), $[\alpha]_D^{25}$ +93° (water); lit.[14] m.p. 228–229°, $[\alpha]_D$ +93.3–94.4°. Hydrolysis gave (p.c.) galactose and mannose, and partial hydrolysis with acid afforded (p.c.) epimelibiose and mannose. During 48 h, the trisaccharide consumed 6.9 mol of metaperiodate with the liberation of 4.3 mol of formic acid.

D-Galactose and D-mannose were also isolated and identified.

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