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Research Note

A neutral seed gum from *Abutilon indicum*

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A water soluble galactomannan has been isolated from the seeds of *Abutilon indicum* containing D-galactose and D-mannose in 2:3 molar ratio. Acid catalysed fragmentation, periodate oxidation and methylation showed that the seed-gum has branched structure consisting of linear chain β-D(1→4) linked mannopyranosyl units, some of which are substituted at O-6 by two α-D(1→6) galactopyranosyl units mutually linked glycosidically as end groups. © 1997 Elsevier Science Ltd

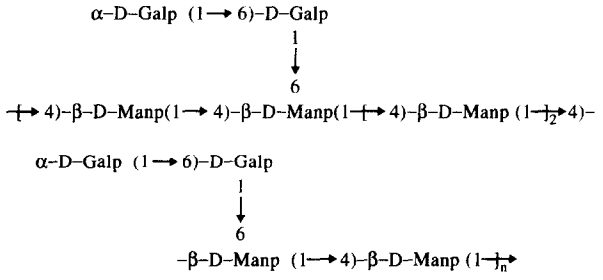
The plant *Abutilon indicum* is reported to have many medicinal and industrial uses (Kirtikar and Basu, 1932; Chopra *et al.*, 1956). For this reason the seed mucilage from the plant was subjected to an extensive structural study.

The polysaccharide was extracted from defatted seeds with 1.5% aqueous acetic acid and by repeated precipitation with 95% ethanol. It was purified by repeated deproteinization using chloroform and by complexation with Fehling's solution. The homogeneity of the polysaccharide was verified by fractional precipitation and zone electrophoresis. The pure polysaccharide had $[\alpha]_D^{25} = +83°$(water), ash content 0.3% and a negligible percentage of methoxyl, acetyl and uronic acid.

Complete acid hydrolysis of the polysaccharide yielded D-galactose and D-mannose in molar ratio 2:3 respectively. On sequential hydrolysis, galactose was liberated first followed by mannose, suggesting the presence of α-linked D-galactose units on the periphery as end groups.

Completely methylated seed gum, $[\alpha]_D^{28} = +54°$ (C 1.5, chloroform) on hydrolysis gave 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,4-tri-*O*-methyl-D-galactose, 2,3,6-tri-*O*-methyl-D-mannose and 2,3-di-*O*-methyl-D-mannose in the molar ratio 1:1:2:1 respectively. The identity of these methylated monosaccharides was established on the basis of R_{TMG} values, optical rotations, and their crystalline derivatives. The percentage of terminal groups, calculated from methylation studies was 39.4%. The presence of 2,3,4,6-tetra-*O*-methyl-D-galactose must have arisen from D-galactosyl end groups on the side chain (cf. the earlier conclusion from sequential hydrolysis). The galactose units yielding 2,3,4-tri-*O*-methyl-D-galactose must be linked through O-1 and O-6. Similarly the mannosyl residues that yield 2,3-di-*O*-methyl-D-mannose must be linked through O-1, O-4 and O-6 and they also constitute the branching points in the main chain of the polysaccharide molecule. The formation of 2,3,6-tri-*O*-methyl-D-mannose indicates the presence of (1→4) linked D-mannosyl residues forming the backbone. Oxidation of the seed gum with sodium metaperiodate consumed 0.86 mol of oxidant with the liberation of 0.247 mol of formic acid per 100 g of the polysaccharide, indicating ~40% end groups. The galactose and mannose residues were completely oxidized within 96 h. These results accord with those of the methylation study. Acid catalysed partial hydrolysis of the purified galactomannan gave: mannobiose [β-D-Manp (1→4)-D-Manp], epimelibiose [α-D-Galp(1→6)-D-Manp], mannotriose [β-D-Manp(1→4)-β-D-Manp (1→4)-D-Manp], galactosyl-epimelibiose [α-D-Galp (1→6)-α-D-Galp (1→6)-D-Manp] and galactobiose [α-D-Galp (1→6)-D-Galp] along with the component monosaccharides.

The above results indicate that the polysaccharide has the following structure:



Structure of seed-gum *Abutilon indicum*

EXPERIMENTAL

Solutions were concentrated under diminished pressure at 60–62°C. Paper chromatography was carried out at room temperature with solvent systems A, 1-butanol–ethanol–water (Hirst and Jones, 1949) (5:1:4); B, 1-butanol–2-propanol–water (Rizvi *et al.*, 1971) (11:6:3); C, ethyl-acetate–pyridine–water (Aspinall *et al.*, 1962) (10:4:3); D, ethyl-acetate–pyridine–water (Meir, 1960) (2:1:2), with detection using aniline hydrogen phthalate.

Isolation of the polysaccharide

Dried crushed seeds were extracted successively with light petroleum and ethanol to defat and decolorise respectively. The defatted and decolorised seeds were extracted with 1.5% aqueous acetic acid, and the extract was added slowly, with stirring, to large excess of ethanol. The crude polysaccharide was collected, washed with ethanol and dried, (yield 3.0 g per 100 g).

Purification of the polysaccharide

The dried polysaccharide was redissolved in water and the solution was shaken well with chloroform, whereupon denatured proteins formed a gel at the water–chloroform interface (Staub, 1965), which was removed. This treatment was repeated five times to remove all of the proteins. An excess of Fehling's solution was added to deproteinized aqueous solution of the polysaccharide and a copper complex was precipitated (Srivastava and Singh, 1967). The complex was centrifuged, washed thoroughly with dilute Fehling's solution and suspended in cold water. The complex was decomposed with M hydrochloric acid. The polysaccharide was regenerated by slowly adding the solution to ethanol (5 vol.) with stirring. The pure product was precipitated from its solution in 1% acetic acid by adding excess of ethanol, to yield a nonreducing, white, amorphous material (ash content 0.3%, $[\alpha]_D^{25} = +83°$ (water)).

Homogeneity of the polysaccharide

The polysaccharide (1.5 g) was fractionally (Khanna and Gupta, 1967) precipitated from the aqueous solution (300 ml) by the addition of ethanol (400 and 800 ml). The fractions (**a** and **b**) were collected by centrifugation, washed with ethanol and dried. Hydrolysis of fractions **a** and **b** gave D-galactose and D-mannose in molar ratio of 2:3 and both the fractions retained their original specific rotation, $[\alpha]_D^{25} + 83°$ (water).

The polysaccharide (50 mg) was subjected to conventional zone electrophoresis (Khanna and Gupta, 1967; Foster, 1957) on Whatman No. 1 MM paper in 0.05 M sodium tetraborate (pH 9.2) for 6 h at 320 V and 3.7 mA. A plot of the absorbance against segment number showed only a single sharp peak, which confirms homogeneity.

Investigation of the structure of the polysaccharide

The purified polysaccharide was completely hydrolysed with M H_2SO_4 at 100°C for 36 h. Paper chromatography (solvent B) of the hydrolysate revealed galactose (R_F 0.15) and mannose (R_F 0.21). Identities and configuration were confirmed by co-chromatography with authentic samples and prepared derivatives: D-galactose, m.p. 164°C, $[\alpha]_D^{30} + 80°$ (water); D-galactose phenyl hydrazone, m.p. 153°C; D-mannose, m.p. 131°C, $[\alpha]_D^{30} + 14°$ (water); D-mannose phenyl hydrazone, m.p. 198°C.

The polysaccharide (300 mg) together with D-ribose (30 mg) as reference, was treated with M H_2SO_4 at 100°C for 20 h. A portion (1 ml) of the hydrolysate was subjected to paper chromatography (solvent B) on Whatman No. 3 MM paper and the individual monosaccharides were quantified (Khanna and Gupta, 1967) by periodate oxidation. Assuming 100% recovery of D-ribose, the molar ratio of D-galactose to D-mannose was found to be 2:3.

The polysaccharide was hydrolysed (Smith and Montgomery, 1959) with 25 mM H_2SO_4 at 100°C for 6 h. Pc (solvent B) of the hydrolysate showed that the galactose was liberated first.

To the solution of the polysaccharide (300 mg) in water (30 ml) were added KCl (2.5 g) and 0.25 M sodium metaperiodate (Brown *et al.*, 1948) (25 ml). The volume was made up to 100 ml with water, and the mixture was stored in the dark at room temperature. Aliquots (2 ml) were withdrawn at intervals, and, after the excess of periodate had been reduced with ethylene glycol, titrated with 0.01 M sodium hydroxide; the formic acid liberated was 0.226 mol per 100 g (84 h), corresponding to 36.4% of end groups.

The polysaccharide was subjected to four Haworth's methylations (Haworth, 1915) followed by four Purdie's methylations (Purdie and Irvine, 1903). The

product $[\alpha]_D^{25}+54°$ (C 1.2, chloroform) was hydrolysed (Brown *et al.*, 1948) with 90% aqueous formic acid at 100°C for 6 h, then with M H_2SO_4 for 14 h at 100°C, and the products were fractionated on Whatman No. 3 MM paper (solvent A) to give the following compounds: (i) 2,3,4-tri-*O*-methyl-D-galactose, R_{TMG} 0.64, m.p. 85–86°C, $[\alpha]_D^{25}+152°$ (water) (cf. literature (Onuki, 1933) values, m.p. 85°C, $[\alpha]_D^{25}+154°$ (water)); (ii) 2,3,4,6-tetra-*O*-methyl-D-galactose, m.p. 72–73°C, $[\alpha]_D^{32}+120°$ (C 1, water) (cf. literature (Robertson, 1934) values, m.p. 74°C, $[\alpha]_D^{32}+121°$ (water)); (iii) 2,3-di-*O*-methyl-D-mannose, m.p. 107–108°C, $[\alpha]_D^{25}-16°$ (C 1.5, water) (cf. literature (Robertson, 1934) values, m.p. 106°C, $[\alpha]_D^{25}-15.8°$). The anilide (Hirst and Jones, 1949) had m.p. 136°C; (iv) 2,3,6-tri-*O*-methyl-D-mannose, $[\alpha]_D^{25}-11°$ (water) (cf. literature (Hirst *et al.*, 1949) value $[\alpha]_D^{25}-10°$ (water)); the hydrazide (Hirst *et al.*, 1949) had m.p. 121–131°C.

The methylated polysaccharide (300 mg), together with D-glucose (30 mg) as reference, was treated with 0.75 M sulfuric acid for 18 h at 100°C. The resulting methylated sugars were separated by paper chromatography (solvent A) and quantified by titration with alkaline hypoiodite (Robertson, 1934). The molar ratios of the methylated sugars were found to be 1:1:1:2 respectively.

The polysaccharide was hydrolysed with 0.05 M sulphuric acid for 12 h at 100°C. The hydrolysate was subjected to preparative paper chromatography (solvent D), and elution of different fractions with distilled water gave D-galactose, D-mannose and the following oligosaccharides: (i) swietenose [α-D-Galp(1→6)-D-Galp], m.p. 128°C, $[\alpha]_D^{25}+150°$ (water) (cf. literature (De Grand Champ Chaudun *et al.*, 1960) values, m.p. 230°C, $[\alpha]_D^{25}+154°$); (ii) epimelibiose [α-D-Galp(1→6)-D-Manp], m.p. 199°C, $[\alpha]_D^{32}+120.5°$ (C 1.2, water) (cf. literature (Bailey, 1965) values, m.p. 200°C, $[\alpha]_D^{32}+121°$ (water)); (iii) mannobiose [β-Manp(1→4)-D-Manp], m.p. 203–205°C (from ethanol), $[\alpha]_D^{25}-9°$ (water) (cf. literature (Aspinall *et al.*, 1958) values, m.p. 202–203°C, $[\alpha]_D^{25}-5.2$ to 8.2°); derivative phenyl osazone had m.p. 204°C (cf. literature (Srivastava and Singh, 1967) value 203–206°C); (iv) 6^2-*O*-α-D-galactosyl-6-*O*-α-D-galactosyl-D-mannose [α-D-Galp (1→6)-α-D-Galp (1→6)-D-Manp], m.p. 122°C, $[\alpha]_D^{25}+129°$ (water) (cf. literature (De Grand Champ Chaudun *et al.*, 1960) values, m.p. 124°C, $[\alpha]_D^{25}+131°$), R_{6lu} (solvent C) 0.33 (cf. literature (Hirst *et al.*, 1949) value 0.32); (v) mannotriose [β-D-Manp-(1→4)-β-D-Manp (1→4)-D-Manp], m.p. 211–213°C (from ethanol), $[\alpha]_D^{25}-12°$ (water) (cf. literature (Tyminski and Timell, 1960) values, m.p. 214–215°C, $[\alpha]_D^{25}-15$ to $-26°$).

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