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A Non-ionic Seed Gum from *Ipomoea palmata*

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A polysaccharide has been extracted from *Ipomoea palmata* seeds with cold acidulated water, and purified to give a water soluble product containing D-galactose and D-mannose in 2 : 3 molar ratio. Acid-catalysed fragmentation, periodate oxidation, methylation and enzymic hydrolysis showed that the seed gum has a branched structure consisting of a linear chain of β-D-(1→4) linked mannopyranosyl units, some of which are substituted at 0–6 by two α-D-(1→6) galactopyranosyl units mutually linked glycosidically Methylation analysis of the galactomannan afforded 2,3,4-tri- and 2,3,4,6-tetra-*O*-methylgalactose, along with 2,3-di- and 2,3,6-tri-*O*-methylmannose, in the molar ratio of 1 : 1: 1 : 2 Both the methylation and periodate oxidation studies showed 39 7% of end-groups The significance of these results, together with the findings of partial hydrolysis with acid are discussed in relation to ascertaining the structure of the repeating unit of the polysaccharide.

THE plants of *Ipomoea palmata*[1] (Convolvulaceae) have been described as being highly medicinal and a rich source of polysaccharides. The seed of *I. palmata* was locally collected (Allahabad) and identified by Dr. O. P. Mishra, Dy. Director, Botanical Survey of India, Allahabad. Owing to the highly medicinal and increasing industrial acceptance of plant mucilages, the seed mucilage from this plant has now been subjected to extensive structural study.

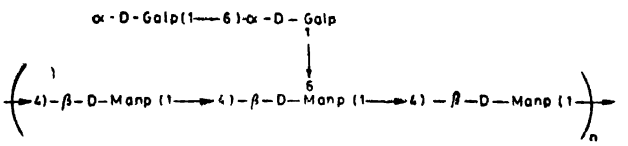
The polysaccharide was conveniently extracted from the crushed, defatted and decolourised seeds with 1% acetic acid, and by repeated precipitation from its solution therein with ethanol. It was purified by repeated deproteinisation using chloroform and by complexation with Fehling's solution. The homogeneity of the polysaccharide was verified by fractional precipitation and zone electrophoresis, and via acetylation and deacetylation. The white, amorphous polysaccharide was water-soluble, and had $[\alpha]_D^{25}$ +65° (water), an ash content of 0.28% and a negligible percentage of methoxyl and acetyl groups, and uronic acid. Upon complete acid hydrolysis, the polysaccharide yielded D-galactose and D-mannose in molar ratio of 2 : 3. Graded hydrolysis with 25 m*M* sulphuric acid resulted in the favoured removal of galactose, suggesting the presence of α-linked D-galactose units on the periphery as end-groups.

To determine the positions of linkage between the monosaccharide units of the galactomannan, it was exhaustively methylated first by the Haworth method and then by the Purdie method to afford a brown, semisolid, glassy mass that had $[\alpha]_D^{30}$ +58° (chloroform) and was devoid of an absorption band in the hydroxyl region of its ir spectrum. Hydrolysis of the methylated polysaccharide gave 2,3,4-tri- and 2,3,4-tetra-*O*-methyl-D-galactose, as well as 2,3-di- and 2,3,6-tri-*O*-methyl-D-mannose in the molar ratios of 1 : 1 : 1 : 2. The identity of these methylated monosaccharides was established on the basis of their R_{TMG} values, optical rotations and crystalline derivatives. The percentage of terminal groups calculated from methylation studies was 39.9%. The 2,3,4,6-tetra-*O*-methyl-D-galactose present must have arisen from D-galactosyl end-groups on the side chain (confirming the earlier conclusion from graded hydrolysis), and the galactose units yielding 2,3,4-tri-*O*-methyl-D-galactose must be linked through O-1 and O-6. The mannosyl residues that yield 2,3-di-*O*-methyl-D-mannose are linked through O-1, O-4 and O-6, and constitute the branching point in the main chain of the molecule The formation of 2,3,6-tri-*O*-methyl-D-mannose indicates the presence (1→4)linked D-mannosyl residues, forming the main chain. Oxidation of the seed polysaccharide with sodium metaperiodate consumed 0.38 mol of oxidant with the liberation of 245 mmol of formic acid per 100 g of polysaccharide indicating 39.7% end-groups.

Examination of the oxopolysaccharide (obtained after 84 h) showed the absence of D-galactose, but a small proportion of D-mannose was detected. However, prolonged oxidation (96 h) decomposed both the hexoses. These results indicated that D-galactopyranosyl end-groups are present in the polysaccharide, and that these protect some of the D-mannopyranosyl units from oxidation.

Acid-catalysed partial hydrolysis of the purified galactomannan gave three disaccharides, identified as α-D-Galp-(1→6)-D-Galp, α-D-Galp-(1→6)-D-Manp and β-D-Manp-(1→4)-D-Manp, and two trisaccharides, α-D-Galp-(1→6)-α-D-Galp(1→6)-D-Manp and β-D-Manp-(1→4)-β-D-Manp-(1→4)-D-Manp, along with the component sugars, mannose and galactose. All of the oligosaccharides were characterised (see

Experimental). These results corroborated the earlier findings of the methylation studies. The foregoing evidence accords with the following structure.



Experimental

Paper chromatography was conducted at room temperature by the descending technique using the following solvent mixtures (v/v): (A) 5 : 1 : 4, 1-butanol – ethanol – water[2], (B) 11:6:3, 1-butanol – 2-propanol – water[3], (C) 10:4:3, ethyl acetate – pyridine – water[4] and (D) 2 : 1 : 2, ethyl acetate – pyridine – water[5], and detection with aniline hydrogen phthalate. All evaporations were conducted under diminished pressure at 35 – 40°. Melting points are uncorrected and all specific rotations were measured at equilibrium. Amberlite 1R-120 (H^+) ion-exchange resin was used for decationising the hydrolytes, and all residues were dried under reduced pressure over anhydrous calcium chloride. The emulsin used was extracted from almonds.

Isolation of the polysaccharide : Dried, crushed seeds (1 kg) were successively extracted with light petroleum (b.p. 60 – 80°) and ethanol, and then stirred with 1% acetic acid for 24 h at room temperature. The acid extract was slowly added, with stirring, to ethanol (5 vol) to give a crude product. Dissolution and reprecipitation were repeated, and the resulting polysaccharide was washed with ethanol and dried.

Purification of the polysaccharide : The dried polysaccharide was redissolved in water and the solution shaken well with chloroform and then centrifuged whereupon the denaturated proteins formed, at the water – chloroform interface[6], a gel that was removed. This treatment was repeated 5 times to remove all of the proteins. An excess of Fehling's solution was added to the deproteinized aqueous solution of the polysaccharide and a copper complex was precipitated[7]. 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 reprecipitated from its solution in 1% acetic acid by ethanol, to yield a non-reducing, white, amorphous material (ash content, 0.28%), $[\alpha]_D^{25}$ +65° (water).

Homogeneity of the polysaccharide : The polysaccharide (1.5 g) was fractionally[8] precipitated from the aqueous solution (300 ml) by adding 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 the original specific rotation, $[\alpha]_D^{25}$ +65° (water).

The polysaccharide (1 g) was treated with acetic anhydride – sodium acetate[9] and the resulting acetate (600 mg) had $[\alpha]_D^{25}$ +27.2° (chloroform) ; deacetylation regenerated material having $[\alpha]_D^{25}$ +65° (water). The polysaccharide (50 mg) was subjected to conventional zone electrophoresis[8,10] on Whatman (no. 1) 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 peak.

Investigation of the polysaccharide :

Hydrolysis : The purified polysaccharide (1 g) was hydrolysed with *M*-sulphuric acid for 36 h at 100°. Pc (solvent C) of the hydrolysate then revealed galactose (R_f 0.15) and mannose (R_f 0.21). Identities were confirmed by co-chromatography with authentic samples and preparation of derivatives, and the absolute configuration from the specific rotations : D-galactose, m.p. 164°, $[\alpha]_D^{25}$ +80° (water) ; D-galactose phenylhydrazone, m.p. 155° ; D-mannose, m.p. 131°, $[\alpha]_D^{25}$ +12.6° (water) ; D-mannose phenylhydrazone, m.p. 196°.

The polysaccharide (300 mg), together with D-ribose (30 mg) as reference sugar, was treated with *M*-sulphuric acid for 36 h at 100°. A portion (1 ml) of the hydrolysate was subjected to pc (solvent B) on Whatman (no. 3) paper, and the individual monosaccharides were quantified[8] by periodate oxidation. Assuming 100% recovery of ribose the molar ratio of galactose to mannose was 2 : 3.

Graded hydrolysis : The polysaccharide (200 mg) was hydrolysed with 25 m*M* sulphuric acid (40 ml) for 4 h at 100°. The hydrolysate was subjected to pc (solvent B) after 5, 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min, and galactose was found to be liberated first followed by mannose.

Periodate oxidation[11] *:* To a solution of the polysaccharide (300 mg) in water (30 ml) were added KCl (2.5 g) and 0.25 *M*-sodium metaperiodate (25 ml). The volume was made upto 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 being reduced with ethylene glycol, titrated with 0.01 *M* sodium hydroxide ; formic acid liberated was 245 mmol per 100 g.

After 84 h, an excess (30 ml) of ethylene glycol was added, the solution was evaporated and the residue was hydrolysed. Pc (solvent B) then revealed only mannose.

To a solution of the polysaccharide (300 mg) in water (30 ml) was added 0.25 *M* sodium metaperiodate (25 ml), and the volume was made upto 100 ml with water. The uptake of periodate was determined at intervals by the procedure of Andrews *et al.*[12]. The uptake became constant in 96 h and

corresponds to 0.88 g per 100 g of the polysaccharide. After 96 h, the oxopolysaccharide was hydrolysed as already described ; neither galactose nor mannose was detected.

Methylation : The polysaccharide (5 g) was subjected to Haworth methylations[13], followed by Purdie methylations[14]. The completely methylated polysaccharide had $[\alpha]_D^{28}+58°$ (chloroform). The methylated derivative (1.5 g) was hydrolysed with 90% formic acid for 6 h at 100° and then with 0.75 *M*-sulphuric acid for 10 h at 100°, and the products were fractionated on Whatman (no. 3) paper (solvent A) with 2,3,4,6-tetra-*O*-methyl-D-galactose (TMG) as the reference, to give the following products. *2,3,4-Tri-O-methyl-D-galactose :* R_{TMG} 0.64, m.p. 85–6°, $[\alpha]_D^{28}+154°$ (water) (lit.[15] 85°, +154°) ; it was oxidised with barium iodide-iodine solution-barium hydroxide by Goebel method and tri-*O*-methyl-D-galactono-1,4-lactone was obtained, which spontaneously changed into tri-*O*-methyl-D-galactonic acid, needles, m.p. 107–08°. *2,3,4,6-Tetra-O-methyl-D-galactose :* R_{TMG} 0 87, m.p. 70–2°, $[\alpha]_D^{28}+120°$ (water) (lit.[16] 74°, +121°) ; the anilide, m.p. 192–93°, $[\alpha]_D^{28}+43°$ (acetone) (lit.[16] 194°, +45°). *2,3-Di-O-methyl-D-mannose :* R_{TMG} 0.55, m.p. 107–08°, $[\alpha]_D^{28}-16°$ (water) (lit.[17] 108°, −15.8°) ; the anilide, m.p. 136° (lit.[16] 138°). *2.3.6-Tri-O-methyl-D-mannose :* R_{TMG} 0.81, syrup, $[\alpha]_D^{28}$ −12° (water) (lit.[18] −10°) ; the hydrazide, m.p. 121–31° (lit.[19] 131°).

The methylated polysaccharide (300 mg) together with added D-glucose as a reference, was treated with 0.75*M* sulphuric acid for 16 h at 100°. The resulting methylated sugars were separated by pc (solvent A) and quantified by titration with alkaline hypoiodite. The molar ratios of fractions 1 to 4 were 1 : 1 : 1 : 2.

Partial hydrolysis with acid : The polysaccharide (4 g) was hydrolysed with 0.05*M* sulphuric acid for 14 h at 100°. The hydrolysate was subjected to preparative pc (solvent D) and elution of the fractions with distilled water gave D-galactose, D-mannose and the following oligosaccharides. *Swietenose* (α-D-*Galp*-(*1→6*)-D-*Galp*)[20] *:* M.p. 128°, $[\alpha]_D^{28}+144°$ (water) (lit.[20] 130, +154°) ; it reduced Fehlings solutions and the Tollens reagent, and afforded only D-galactose on hydrolysis ; methylation and hydrolysis gave 2,3,4,6-tetra- and 2,3,4-tri-*O*-methyl-D-galactose ; it was resistant to emulsin, indicating α-linkage ; the octacetate, m.p. 222–25°, $[\alpha]_D^{28}+180°$ (chloroform) (lit.[20] 224°, +180°). *Epimelibiose* (α-D-*Galp*-(*1→6*)-D-*Manp*)[21] *:* M.p. 202°, $[\alpha]_D^{28}+120°$ (water) (lit.[21] 201–02°, +123–24° ; acid hydrolysis gave galactose and mannose and methylation followed by hydrolysis gave 2,3,4,6-tetra-*O*-methylgalactose and 2,3,4-tri-*O*-methyl-D-mannose. *Mannobiose* (β-D-β*Manp*-(*1→4*)-D-*Manp*)[22,23] *:* M.p. 203–04° (ethanol), $[\alpha]_D^{28}-9°$ (water) (lit.[22] 202–03°, −5.2 to −8.2°) ; the phenylosazone, m.p. 204° (lit.[21] 203–06°) ; acid hydrolysis gave mannose only (pc) and emulsin hydrolysed the disaccharide, indicating a β-linkage ; methylation followed by hydrolysis gave 2,3,4,6-tetra- and 2,3.6-tri-*O*-methyl-D-mannose. *6²-O-α-D-Galactosyl-6-O-α-D-galactosyl-D-mannose* (α-D-*Galp*-(*1→6*)-α-D-*Galp*-*1*(*1→6*)-D-*Manp*)[20] *:* M.p. 122°, $[\alpha]_D^{28}+129°$ (water) (lit.[20] 124°, +131°) ; R_{GIU} (solvent C) 0.33 (lit.[20] 0.32) ; acid hydrolysis gave galactose and mannose in molar ratio of 2 : 1 ; could not be hydrolysed by emulsin, showing α-linkages ; methylation followed by hydrolysis, afforded 2,3,4-tri- and 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3,4-tri-*O*-methyl-D-mannose. *Mannotriose* (β-D-*Manp*-(*1→4*)-D-*Manp-1*(*1→4*)-D-*Manp*)[22,23] ; M.p. 166° (ethanol), $[\alpha]_D^{28}-17°$ (water) (lit.[23] 169.5°, −15 to −20°) ; acid hydrolysis gave (pc) mannose only, and partial hydrolysis with acid gave mannobiose and mannose ; methylation followed by hydrolysis gave 2,3,4,6-tetra- and 2,3,6-tri-*O*-methyl-D-Mannose ; it was cleaved by emulsin, showing the presence of β-linkages.

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