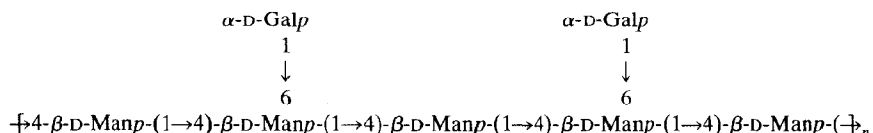


A water-soluble polysaccharide isolated from seeds of *Cassia ovata*

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These results indicate that the polysaccharide is a (1→4)-β-D-mannan substituted at positions 6 by D-galactosyl groups. A possible repeating unit of the polysaccharide is shown in **1**.



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EXPERIMENTAL

Solutions were concentrated under diminished pressure at 60–62°. All residues were dried *in vacuo* over anhydrous calcium chloride. Melting points are uncorrected and $[\alpha]_D$ values are for equilibria. P.c. was carried out at room temperature with A, 1-butanol–ethanol–water (5:1:4); B, 1-butanol–ethanol–water (4:1:5); C, 1-butanol–2-propanol–water (11:6:3); D, ethyl acetate–pyridine–water (10:4:3); and E, ethyl acetate–pyridine–water (2:1:2); with detection using aniline hydrogenphthalate.

Isolation of the polysaccharide. — Dried crushed seeds were extracted successively with light petroleum and ethanol to defat and decolorise. The defatted and decolorised seeds were extracted with aq. 2% acetic acid, and the extract was added slowly with stirring to a large excess of ethanol. The crude polysaccharide was collected, washed with ethanol, dried and precipitated from solution in aq. 1% acetic acid with ethanol. The product (yield, ~3.2 g/100 g) had $[\alpha]_D^{25} +66^\circ$ (c 1, water), and gave 0.35% of ash.

The homogeneity of the polysaccharide was tested by fractional precipitation from its aqueous solution with ethanol. Each fraction had $[\alpha]_D^{25} +68^\circ$ (water) and on hydrolysis with M sulphuric acid at 100° for 20 h, gave D-galactose and D-mannose in the molar ratio 2:5. The polysaccharide was subjected to zone electrophoresis^{2,3} on Whatman No. 1 paper in borate buffer (pH 9.2) at 320 V and 3.7 mA for 6 h. The paper was cut into 31 equal segments and each was eluted with distilled water. The intensity of the characteristic yellow-orange colour developed in each eluate by adding aq. 8% phenol (1 mL) and conc. H₂SO₄ (6 mL) was measured in a Klett–Summerson photoelectric colorimeter (filter No. 50). A plot of absorbance against segment number showed a single sharp peak.

The polysaccharide was treated with sodium acetate–acetic anhydride⁴ and the resulting acetate had $[\alpha]_D^{28} +27.5^\circ$ (c 1.2, chloroform). Deacetylation⁴ generated material with $[\alpha]_D^{28} +57.5^\circ$ (c 1.3, water).

Investigation of the structure of the polysaccharide. — The purified polysaccharide was completely hydrolysed with M H₂SO₄ at 100° for 20 h. P.c. (solvent D) of the hydrolysate revealed galactose (*R_F* 0.15) and mannose (*R_F* 0.21). The absolute configurations were confirmed by the preparation of D-galactose phenyl-osazone, m.p. 164°, $[\alpha]_D^{30} +80^\circ$ (c 1.5, water), and the isolation of D-mannose, m.p. 131°, $[\alpha]_D +14^\circ$ (c 1.8, water).

The polysaccharide (300 mg), together with D-ribose (30 mg) as reference, was treated with M H₂SO₄ at 100° for 20 h. P.c. and quantification² of the components in the hydrolysate revealed the molar ratio of galactose and mannose to be 2:5.

The polysaccharide was hydrolysed⁵ with 25mM H₂SO₄ at 100° for 6 h. P.c. (solvent C) of the hydrolysate showed that the galactose was liberated first.

To a solution of the polysaccharide were added KCl and 0.25M sodium metaperiodate⁶. The amount of formic acid liberated was 0.230 mol/100 g (72 h), corresponding to 28.7% of end groups.

The polysaccharide was methylated (Haworth⁷ then Purdie⁸). The product, $[\alpha]_D^{25} +11^\circ$ (c 1.2, chloroform), was hydrolysed⁶ with aq. 90% formic acid at 100° for 6 h, then with M H₂SO₄ for 14 h at 100°, and the products were fractionated on Whatman No. 3 paper (solvent A) to give the following compounds. 2,3,4,6-Tetra-*O*-methyl-D-galactose, m.p. 72–73°, $[\alpha]_D^{32} +120^\circ$ (c 1, water); lit.⁹ m.p. 74°, $[\alpha]_D^{32} +121^\circ$ (water). 2,3-Di-*O*-methyl-D-mannose, m.p. 107–108°, $[\alpha]_D^{25} -16^\circ$ (c 1.5, water); lit.⁹ m.p. 106°, $[\alpha]_D^{25} -15.8^\circ$; the anilide¹⁰ had m.p. 136°. 2,3,6-Tri-*O*-methyl-D-mannose, $[\alpha]_D^{25} -11^\circ$ (water); lit.¹¹ $[\alpha]_D^{25} -10^\circ$ (water); the hydrazide¹² had m.p. 121–131°.

The methylated polysaccharide together with D-glucose as reference was treated with M H₂SO₄ at 100° for 18 h. The resulting methylated sugars were subjected to p.c. (solvent A) and their molar ratios were determined by alkaline hypoiodite¹¹. The molar ratios of the three methylated sugars were 2:2:3.

The polysaccharide was hydrolysed with 0.25M H₂SO₄ at 100° for 12 h. Preparative p.c. (solvents D and E) of the hydrolysate gave D-galactose, D-mannose, and the following oligosaccharides. Mannobiose [β -D-Manp-(1→4)-D-Manp], m.p. 203–205° (from ethanol), $[\alpha]_D^{25} -9^\circ$ (c 1.2, water); lit.^{12,13} m.p. 193–210°, $[\alpha]_D^{25} -5$ to -9° (water). Epimelibiose [α -D-Galp-(1→6)-D-Manp], m.p. 199°, $[\alpha]_D^{32} +120.5^\circ$ (c 1.3, water); lit.¹⁴ m.p. 200°, $[\alpha]_D^{32} +121^\circ$ (water). Mannotriose [β -D-Manp-(1→4)- β -D-Manp-(1→4)-D-Manp], m.p. 211–213° (from ethanol), $[\alpha]_D^{25} -13^\circ$ (c 1.2, water); lit.^{12,13} m.p. 214–215°, $[\alpha]_D -15$ to -26° (water). Galactosylmannobiose [α -D-Galp-(1→6)- β -D-Manp-(1→4)-D-Manp], m.p. 225–227°, $[\alpha]_D^{25} +93^\circ$ (c 1.5, water); lit.¹⁵ m.p. 228–229°, $[\alpha]_D^{25} +93.3^\circ$ (water).

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