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## Note

# Structure of a water-soluble galactomannan from the seeds of *Teramnus labialis*

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The high medicinal value of *Teramnus* species and the industrial importance of plant mucilages prompted this detailed chemical study of the polysaccharide from the seeds of *Teramnus labialis*[1].

## RESULTS AND DISCUSSION

A galactomannan extracted from the defatted seeds with 1% acetic acid was fractionated to purify it by (*i*) repeated precipitation from a large excess of 95% ethanol, (*ii*) via its copper complex (Fehling's solution method)[2], and (*iii*) by deproteinisation. Its homogeneity was tested by fractional precipitation, by zone electrophoresis, and by p.c. in different solvent systems. It had $[\alpha]_D^{30}$ +54° (water), was soluble in water, and had an ash content of 0.25%. The content of methoxyl, acetyl, carboxyl, and glycuronan were negligible, and also nitrogen, sulphur, phosphorus, and halogens were absent.

Complete hydrolysis with M sulphuric acid liberated D-galactose and D-mannose in 2:3 ratio, and graded hydrolysis with 25mM sulphuric acid released D-galactose first, showing galactose to be terminal, probably $\alpha$-linked to a mannan main-chain.

Haworth methylation followed by Purdie methylation gave the completely methylated polysaccharide, $[\alpha]_D^{30}$ +48° (chloroform), hydrolysis of which afforded 2,3-di-*O*-methyl-D-mannose, 2,3,6-tri-*O*-methyl-D-mannose, and 2,3,4,6-tetra-*O*-methyl-D-galactose in 2:1:2 molar ratio.

The 2,3,4,6-tetra-*O*-methyl-D-galactose arises from nonreducing, terminal D-galactosyl groups. The production of 2,3-di- and 2,3,6-tri-*O*-methyl-D-mannose is consistent only with a mannan backbone having O-1, O-4, and O-6 in one, and O-1 and O-4 in the other mannose residue engaged in glycosidic linkages. I.r.

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absorptions at 810 and 875 $cm^{-1}$ suggested the presence of both $\alpha$ and $\beta$ linkages.

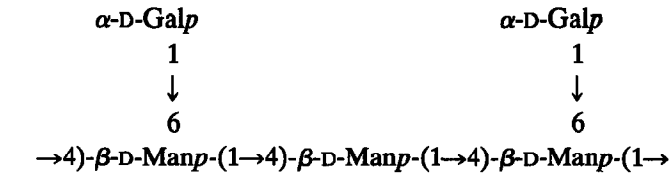
The methylation results indicate the polysaccharide to consist of five monosaccharide units, with two galactopyranosyl groups as nonreducing end-groups and three mannose residues in the main chain.

Periodate oxidation liberated 246 mmol of formic acid with the consumption of 874 mmol of oxidant per 100 g of polysaccharide, which indicated 39.8% of terminal groups. Examination of the oxidation product after 72 h showed the complete absence of galactose, whereas mannose disappeared only after 96 h.

Borohydride reduction of the periodate-oxidised polysaccharide followed by hydrolysis with 0.5M sulphuric acid gave glycerol and erythritol. These two products clearly indicate the involvement of position 1,2 or 1,6 and 1,4 or 1,4,6 of the hexose units in glycosidic linkages.

Partial acid hydrolysis of the polysaccharide afforded $\alpha$-D-Gal*p*-(1→6)-D-Man*p*, $\beta$-D-Man*p*-(1→4)-D-Man*p*, $\beta$-D-Man*p*-(1→4)-$\beta$-D-Man*p*-(1→4)-D-Man*p*, $\alpha$-D-Gal*p*-(1→6)-$\beta$-D-Man*p*-(1→4)-D-Man*p* in addition to D-galactose and D-mannose.

On the basis of the foregoing data, the polysaccharide is assigned the following structure.




## EXPERIMENTAL

*General methods.* — Evaporations were performed under diminished pressure, melting points are uncorrected, and all optical rotations are equilibrium values. I.r. spectra were recorded as Nujol mulls or KBr pellets with a Perkin–Elmer Model 337 i.r. spectrophotometer. The identities of the compounds were confirmed by mixed m.p. Paper chromatography (p.c.) was conducted by the descending technique on Whatman no. 1 filter paper with the following solvent mixtures: *A*, 5:1:4 1-butanol–ethanol–water[3]; *B*, 11:6:3 1-butanol–2-propanol–water[4]; *C*, 10:4:3 ethyl acetate–pyridine–water[5]; *D*, 2:1:2 ethyl acetate–pyridine–water[6]; and *E*, 31:11:9 1-butanol–ethanol–water[7].

Spots in p.c. were located by use of aniline hydrogenphthalate. Emulsin for enzymic hydrolysis was extracted from almonds. Plant material was self-collected and authenticated by Dr. B. K. Verma, Department of Botany, University of Allahabad.

*Extraction and purification of the polysaccharide.* — The crushed and dried seeds of *T. labialis* were treated with light petroleum and ethanol, respectively, and then suspended in 1% acetic acid overnight. The polysaccharide was precipitated

by pouring the mucilagenous extract into a large excess of 95% ethanol and the collected polysaccharide was dried over $CaCl_2$ under diminished pressure.

The dried polysaccharide was redissolved in water and shaken well with chloroform, and the denatured protein that collected as a gel at the water–chloroform interface[8] was removed. The procedure was repeated four times to free the polysaccharide from protein. It was further purified by complexation with Fehling's solution[2]. The complex was centrifuged, washed with dil. Fehling's solution, and decomposed with M HCl. The polysaccharide was regenerated by pouring the solution with stirring into a large excess of ethanol. Finally, the pure polysaccharide, $[\alpha]_D^{30}$ +54° (water), ash content 0.25%, was obtained by dissolving in water and reprecipitating from ethanol.

*Homogeneity.* — A solution of the polysaccharide (2 g) in distilled water (300 mL) was fractionally precipitated[9] first with 100 mL of ethanol (fraction A), and then 500 mL of ethanol (fraction B), and finally with 1 L of ethanol (fraction C). The three fractions had the same $[\alpha]_D$ values, and p.c. of their hydrolysates and quantification showed the presence of galactose and mannose in the ratio 2:3.

Conventional zone electrophoretic[10] separation of the polysaccharide (1.0 g) in 0.05M sodium tetraborate for 6.5 h at 360 V and 12.5 mA and a plot of the absorbance against segment numbers showed a single, sharp peak, indicating the presence of only one polysaccharide.

Acetylation[11] of the polysaccharide (1.0 g) with fused sodium acetate and acetic anhydride gave the peracetate $[\alpha]_D^{30}$ +34.2° (chloroform). Deacetylation gave a product having the same $[\alpha]_D$ as the original polysaccharide.

*Structural analysis. Hydrolysis.* Complete hydrolysis of the polysaccharide (1.0 g) by M sulphuric acid for 30 h at 100° yielded D-galactose and D-mannose in 2:3 molar ratio (separation by p.c., solvent *B*, and quantification by the sodium metaperiodate method). These were identified by co-chromatography with authentic samples: D-galactose, m.p. 164°, $[\alpha]_D^{30}$ +80° (water), phenylhydrazone m.p. 153°; and D-mannose, m.p. 131°, $[\alpha]_D^{30}$ +14° (water), phenylhydrazone m.p. 196°.

*Graded hydrolysis*[12]. Hydrolysis of the polysaccharide (300 mg) with 25mM sulphuric acid for 4 h at 100° and p.c. of the hydrolysates after 5, 15, 30, 45, 60, and 90 min indicated the liberation of D-galactose first, followed by D-mannose.

*Periodate oxidation*[13]. To a solution of the polysaccharide (300 mg) in water (30 mL) was added potassium chloride (2.5 g) and 25 mL of 0.25M sodium metaperiodate, and the volume was made up to 100 mL with water. It was kept in the dark and 2-mL aliquots were withdrawn after 6, 12, 24, 36, 48, 72, 96, and 120 h. The excess of periodate was reduced with ethylene glycol and the formic acid liberated was titrated against 0.01M sodium hydroxide, giving 246 mmol per 100 g of polysaccharide. The uptake[13] of sodium metaperiodate became constant within 96 h, consuming 874 mmol of oxidant per 100 g of polysaccharide.

*Borohydride reduction*[14]. The periodate-oxidised polysaccharide was reduced

with sodium borohydride and then hydrolysed with 0.5M sulphuric acid for 16 h at 100°. The hydrolysate was separated preparatively by p.c. (solvent *E*) to yield: glycerol, syrup, trinitrobenzoate, m.p. 185°, lit. m.p. 186°, and erythritol, m.p. 120°, lit. m.p. 120–122°.

*Methylation*[15,16]. The polysaccharide (8 g) was completely methylated by Haworth's[15] and Purdie's[16] methods by repeating both procedures four times. Completion of methylation was confirmed by the absence of i.r. absorption at 3200–3600 $cm^{-1}$.

The methylated product (2 g) was successively hydrolysed with 90% formic acid for 6 h at 100° and 0.75M sulphuric acid for 10 h at 100°. The hydrolysate was separated on Whatman No. 3 paper (solvent *A*), using 2,3,4,6-tetra-*O*-methyl-D-glucose (TMG) as the reference sugar. The following compounds were isolated in the molar ratio 2:1:2 as quantified by the hypoiodite method: 2,3-di-*O*-methyl-D-mannose, $R_{TMG}$ 0.53, m.p. 107–108°, $[\alpha]_D^{25}$ −16° (water) [lit.[17] m.p. 108°, $[\alpha]_D^{25}$ −15° (water)]. The "anilide" had m.p. 137°, (lit.[18] m.p. 138°); 2,3,6-tri-*O*-methyl-D-mannose, $R_{TMG}$ 0.81, syrup, $[\alpha]_D^{25}$ −11° (water), ['it.[19], $[\alpha]_D^{25}$ −10° (water)]. The derived hydrazide had m.p. 125–131° (lit.[20] m.p. 131°); and 2,3,4,6-tetra-*O*-methyl-D-galactose, $R_{TMG}$ 0.87, m.p. 73°, $[\alpha]_D^{32}$ +120.3° (water) [lit.[18] m.p. 74°, $[\alpha]_D^{32}$ +121° (water)]. The "anilide" had m.p. 193°, $[\alpha]_D^{32}$ +44° (acetone) [lit.[18] m.p. 194°, $[\alpha]_D^{32}$ +45° (acetone)].

*Partial acid hydrolysis.* — The polysaccharide (4.5 g) was hydrolysed with 0.05M sulphuric acid for 14 h at 100° and the hydrolysate was separated by p.c. on a preparative scale (solvent *D*) giving D-galactose, D-mannose, and the following oligosaccharides:

Epimelibiose, α-D-Gal*p*-(1→6)-D-Man*p*[21], m.p. 200°, $[\alpha]_D^{32}$ +122° (water) [lit.[21], m.p. 201–202°, $[\alpha]_d^{32}$ +121–124° (water)]. Acid hydrolysis gave galactose and mannose in equal proportion. Methylation and hydrolysis gave 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3,4-tri-*O*-methyl-D-mannose. It was not hydrolysed by emulsin, showing absence of the β linkage.

Mannobiose, β-D-Man*p*-(1→4)-D-Man*p*[22,23], m.p. 204°, $[\alpha]_D^{25}$ −10.1° (water) [lit.[22] m.p. 202–203°, $[\alpha]_D^{25}$ −5.2 to −8.2°]. Its phenylosazone had m.p. 203° (lit.[23] m.p. 203–206°); acid hydrolysis gave D-mannose only. It was hydrolysed by emulsin, indicating the presence of a β linkage. Methylation and subsequent hydrolysis afforded 2,3,4,6-tetra-*O*-methyl-D-mannose and 2,3,6-tri-*O*-methyl-D-mannose.

Mannotriose, β-D-Man*p*-(1→4)-β-D-Man*p*-(1→4)-D-Man*p*[22,23], m.p. 211–213°, $[\alpha]_D^{25}$ −14° (water) [lit.[23] m.p. 214–215°, $[\alpha]_D^{25}$ −15° to −26° (water)]. Acid hydrolysis indicated the presence of mannose only, whereas partial acid hydrolysis afforded mannose and mannobiose. It was cleaved by emulsin, showing the presence of a β linkage. Methylation and subsequent hydrolysis afforded 2,3,4,6-tetra-*O*-methyl-D-mannose and 2,3,6-tri-*O*-methyl-D-mannose.

Galactosylmannobiose, α-D-Gal*p*-(1→6)-β-D-Man*p*-(1→4)-D-Man*p*[24], m.p. 227°, $[\alpha]_D^{25}$ +98.9° (water) [lit.[24] m.p. 228–229°, $[\alpha]_D^{25}$ +93–98.9° (water)]. Hydrolysis and quantification gave D-galactose and D-mannose in the molar ratio

1:2. Methylation and subsequent hydrolysis gave 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,4-tri-*O*-methyl-D-mannose, and 2,3,6-tri-*O*-methyl-D-mannose. Hydrolysis with emulsin gave mannose and epimelibiose, indicating one $\alpha$ and one $\beta$ linkage.

REFERENCES

1 K. R. KIRTIKAR AND B. D. BASU, in L. M. BASU (Ed.), *Indian Medicinal Plants*, 2nd ed., vol. 1, Allahabad, 1933, p. 774.
2 H. C. SRIVASTAVA AND P. P. SINGH, *Carbohydr. Res.*, 4 (1967) 326–331.
3 E. L. HIRST AND J. K. N. JONES, *Disc. Faraday Soc.*, 7 (1949) 268–271.
4 S. A. I. RIZVI, P. C. GUPTA, AND R. K. KAUL, *Planta Med.*, 19 (1971) 222–233.
5 G. O. ASPINALL, R. BEGBIE, AND J. E. MCKAY, *J. Chem. Soc.*, (1962) 214–219.
6 H. MEIER, *Acta Chem. Scand.*, 14 (1960) 749–752.
7 V. P. KAPOOR AND S. MUKHARJEE, *Can. J. Chem.*, 47 (1969) 2883.
8 A. M. STAUB, *Methods Carbohydr. Chem.*, 5 (1965) 5–6.
9 S. N. KHANNA AND P. C. GUPTA, *Phytochemistry*, 6 (1967) 605–609.
10 A. B. FOSTER, *Adv. Carbohydr. Chem.*, 12 (1957) 81–115; see pp. 86–90.
11 A. S. CEREZO, *J. Org. Chem.*, 30 (1965) 924–927.
12 F. SMITH AND R. MONTGOMERY, *Chemistry of Plant Gums and Mucilages*, Reinhold, New York, 1959, pp. 134–135.
13 P. ANDREWS, L. HOUGH, AND J. K. N. JONES, *J. Am. Chem. Soc.*, 74 (1952) 4029–4032.
14 J. Y. MORIMTO, I. RENE, C. J. UNRAU, AND A. M. UNRAU, *J. Agric. Food Chem.*, 10 (1962) 134–137.
15 W. N. HAWORTH, *J. Chem. Soc.*, 107 (1915) 8–12.
16 T. PURDIE AND J. C. IRVINE, *J. Chem. Soc.*, 83 (1903) 1021–1026.
17 G. J. ROBERTSON, *J. Chem. Soc.*, (1934) 330–336.
18 W. CHARLTON, W. N. HAWORTH, AND W. J. H. HICKINBOTTOM, *J. Chem. Soc.*, (1927) 1527–1536.
19 E. L. HIRST AND J. K. N. JONES, *J. Chem. Soc.*, (1948) 1278–1282.
20 F. SMITH, *J. Am. Chem. Soc.*, 70 (1948) 3249–3253.
21 R. W. BAILEY, *Oligosaccharides*, Vol. 4, Pergamon, Oxford, 1965, pp. 97–101.
22 G. O. ASPINALL, R. B. RASHBROOK, AND G. KESSLER, *J. Chem. Soc.*, (1958) 215–221.
23 A. TYMINSKI AND T. E. TIMELL, *J. Am. Chem. Soc.*, 82 (1960) 2823–2827.
24 R. W. BAILEY, ref. 21, p. 51.