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A GALACTOMANNAN FROM THE SEEDS OF *DELONIX REGIA*

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Abstract—A galactomannan composed of D-galactose (1 mol) and D-mannose (2 mol) was isolated from the seed of *Delonix regia*. Hydrolysis of methylated galactomannan yielded 2,3,4,6-tetra-*O*-methyl-D-galactose (1·02 mol), 2,3,6-tri-*O*-methyl-D-mannose (1·05 mol) and 2,3-di-*O*-methyl-D-mannose (1 mol). The periodate consumption was 1·30 mol for each hexose unit with concomitant liberation of 0·31 mol of formic acid. Hydrolysis of the reduced oxopolysaccharide gave only glycerol (1 mol) and erythritol (1·04 mol). It is established that galactomannan is a highly branched polysaccharide consisting of the main chain of mannose united linked through β (1→4) and side chain of single galactose units linked through α (1→6).

INTRODUCTION

Delonix regia (Bojer) Raf. (Flame tree, Gul mohar; N.O: Leguminosae) is a medium sized tree that grows in all the warmer and damper parts of India. The seeds are known for their use in indigenous medicine and recommended for various diseases. The seed polysaccharide has not been studied.

RESULTS AND DISCUSSION

Isolation of the galactomannan from the seeds was carried out by the usual procedure of extraction with hot water and precipitation with ethanol. The crude polysaccharide was purified by the barium complex method.[1,2] Complete acid hydrolysis of the purified polysaccharide afforded D-galactose (1 mol) and D-mannose (2 mol). The purified polysaccharide was converted into its fully methylated derivative, fractionated and hydrolysed. Paper chromatographic separation of the sugar mixture furnished three fractions namely 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. The identity of the methylated sugars was established from their optical rotations and melting points of their crystalline derivatives. The three sugars were in the molar ratio 1·02: 1·05:1·00.

On periodate oxidation, the polysaccharide consumed 1·30 mol of periodate for each hexose unit, with the liberation of 0·31 mol of formic acid. The periodate oxidized polysaccharide was reduced with sodium borohydride and hydrolysed according to the method of Abdel-Aker *et al.*[3] Paper chromatography of the hydrolysate revealed the presence of glycerol and erythritol. Traces of galactose and mannose were also detected. The glycerol and erythritol were in molar ratio 1:1·94.

[1] V. P. KAPOOR and S. MUKHERJEE, *Current Sci.* **38**, 38 (1969).
[2] V. P. KAPOOR and S. MUKHERJEE, *Can. J. Chem.* **47**, 2883 (1969).
[3] M. ABDEL-AKHER, J. K. HAMILTON, R. MONTGOMERY and F. SMITH, *J. Am. Chem. Soc.* **74**, 4970 (1952).

Isolation of 2,3,6-tri-*O*-methyl-D-mannose shows that the main chain must be composed of 1→4 linkages. The mannose units giving rise to 2,3-di-*O*-methyl-D-mannose would also be 1→4 linked, the galactose end units being linked to it at C-6. The larger proportion of erythritol released upon acid hydrolysis of polyalcohol serves as evidence that the main polymeric linkage was of 1→4 type and the ratio of erythritol to glycerol indicated a branching point, on the average, every two units in the backbone. The fact that only traces of galactose and mannose survived periodate treatment indicate that no significant amounts of 1→3 linkages are present. The molar proportion of periodate consumed and formic acid produced corroborate these findings.

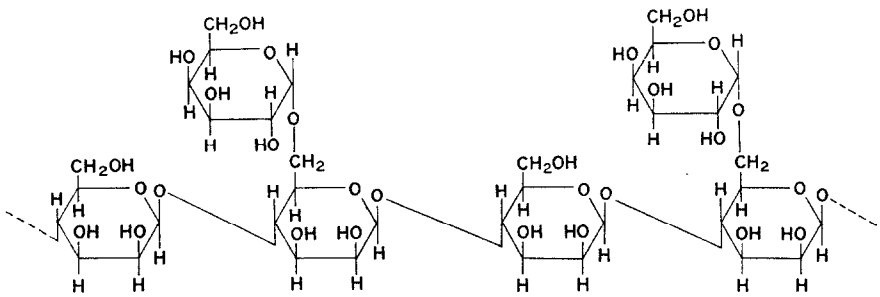

FIG. 1. PROBABLE STRUCTURE OF *Delonix regia* SEED GALACTOMANNAN.

The IR spectrum of galactomannan showed absorption bands at 815 and 872 cm^{-1} indicating the presence of α-linked D-galactopyranose units and β-linked D-mannopyranose units, respectively.[4] Such glycosidic configuration is reported in most seed galactomannans.[5,6]

From the above observations it is postulated that the galactomannan is a highly branched polysaccharide in which mannose units form the main chain and the galactose units are present as branches, the former being attached through β (1→4) and branches of single galactose units through α (1→6). The simplest symmetrical structure which can accommodate the above findings is shown in Fig. 1.

EXPERIMENTAL

All specific rotations are equilibrium values and m.ps. are uncorrected. Descending paper chromatography was on Whatman No. 1 paper using non-aq. phases of the following solvents: (a) *n*-BuOH–HOAc–H_2O (4:1:5); (b) Butanone–EtOAc–H_2O–NH_3 (80:20:8:1) and (c) *n*-BuOH–EtOH–H_2O (31:11:9). *p*-Anisidine phosphate[6,7] was used as spray reagent. Amberlite IR-120 (H^+) and IR-45 (OH^-) were used for deionization.

Isolation and purification. Powdered seeds (200 g) of *Delonix regia* were suspended in H_2O (2 l.) overnight, then heated at 100° for 4 hr. The swollen material was blended with further H_2O. The viscous solution (5·5 l.) was filtered through muslin and centrifuged at 25 000 rpm. Addition of EtOH (12 l.) to this solution precipitated the polysaccharide in the form of coarse powder (37·2 g) which was redissolved in H_2O (6 l.). To this solution satd. $Ba(OH)_2$ solution was added when the polysaccharide precipitated as the Ba complex. The Ba complex was made into paste with H_2O (100 ml) and decomposed by dropwise addition of 2 N HOAc (3 l.) with vigorous stirring. The polysaccharide was regenerated from this solution with EtOH (6·5 l.). The polysaccharide was successively washed with 50, 80, 90 and 95% EtOH and deionized (yield,

[4] S. A. BARKER E. J. BOURNE and D. H. WHIFFEN, in *Method of Biochemical Analysis* (edited by D. GLICK), Vol. 3, p. 213, Interscience, New York (1956).
[5] F. SMITH and R. MONTGOMERY, in *The Chemistry of Plant Gums and Mucilages*, p. 330, Reinhold, New York (1959).
[6] V. P. KAPOOR and S. MUKHERJEE, *Phytochem.* **10**, 655 (1971).
[7] S. MUKHERJEE and H. C. SRIVASTAVA, *Nature, Lond.* **169**, 330 (1952).

24·6 g). The polysaccharide preparation was a light brown amorphous powder and did not reduce Fehling's solution. N, S, OCH_3, halogens and uronic acid were absent. Preliminary analysis gave sulphated ash 0·15% and pentosans 3·7%.

Hydrolysis of the galactomannan. The polysaccharide (1·2 g) was hydrolysed with N H_2SO_4 (125 ml) at 95° for 17 hr. The resulting solution was neutralized ($BaCO_3$), filtered and concentrated to a thin syrup. Chromatographic examination (solvent a) revealed D-galactose and D-mannose. The resolution of sugar mixture on cellulose column using *n*-BuOH half-satd with H_2O as eluent yielded, (i) D-galactose, m.p. 165°; $[\alpha]_D^{26}$ +81·2° (H_2O); *p*-nitro-*N*-phenyl-D-galactosylamine, m.p. 218–219°; $[\alpha]_D^{26}$ −246° (pyridine) and (ii) D-mannose, m.p. 132°; $[\alpha]_D^{27}$ +14° (H_2O); *p*-nitro-*N*-phenyl-D-mannosylamine, m.p. 219°; $[\alpha]_D^{26}$ −327° (pyridine). The molar ratio of D-galactose and D-mannose in the hydrolysate of the polysaccharide determined by phenol-sulphuric acid method,[8] was 1:2.

Methylation of the galactomannan. The polysaccharide (5 g) was subjected to six methylation treatments by Haworth's method followed by two treatments with DMSO method,[9] when a light yellow glassy solid (4·2 g, $[\alpha]_D^{25}$ +45·6° ($CHCl_3$)) was obtained (Found: OCH_3, 43·5). Fractional extractions of the methylated galactomannan with the mixtures of $CHCl_3$–light petroleum (40–60°) furnished a main fraction (yield, 76%) which had $[\alpha]_D^{25}$ +46·5° ($CHCl_3$) (Found: OCH_3, 43·8).

Hydrolysis of the methylated galactomannan. The purified methylated polysaccharide (1·5 g) on methanolysis (2% methanolic HCl at 95° for 7 hr) followed by subsequent hydrolysis (N H_2SO_4 at 95° for 10 hr) was found to contain a mixture of three methylated sugars. The sugar mixture was separated on Whatman No. 3MM Sheets using solvent b.

Fraction I. (302 mg). R_G^* in solvent b, 0·92 (Found: OCH_3, 52·3. Calc. for $C_{10}H_{20}O_6$: OCH_3, 52·5). $[\alpha]_D^{24}$ +110·5° (H_2O). Demethylation showed that parent sugar was galactose. The sugar was characterized as 2,3,4,6-tetra-*O*-methyl-D-galactose by conversion into *N*-phenyl-2,3,4,6-tetra-*O*-methyl-D-galactosylamine, m.p. and m.m.p. 192°; $[\alpha]_D^{23}$ +41° (acetone) (lit. m.p. 191–192° and $[\alpha]_D$ +40°).[10]

Fraction II. (321 mg). R_G^* in solvent b, 0·67 (Found: OCH_3, 41·5. Calc. for $C_9H_{18}O_6$: OCH_3, 41·8). $[\alpha]_D^{23}$ +8·5° (H_2O). On demethylation it gave mannose with traces of galactose. On oxidation, 2,3,6-tri-*O*-methyl-D-mannose with Br_2 afforded 2,3,6-tri-*O*-methyl-D-manno-γ-lactone, m.p. and m.m.p. 84°; $[\alpha]_D^{22}$ +64° (H_2O) (lit. m.p. 84–85° and $[\alpha]_D$ +65·6°).[11] The lactone was converted to 2,3,6-tri-methyl-D-mannoic acid phenyl hydrazide, m.p. and m.m.p. 128–130°; $[\alpha]_D^{22}$ −19·5° (H_2O) (lit. m.p. 131° and $[\alpha]_D$ −21°).[11]

Fraction III. (296 mg). R_G^* in solvent b, 0·34 (Found: OCH_3, 29·6. Calc. for $C_8H_{16}O_6$: OCH_3, 29·8). $[\alpha]_D^{24}$ +15·5° (H_2O) and $[\alpha]_D^{24}$ −6·2° (MeOH). Upon demethylation this fraction gave mannose only. It was identified as 2,3-di-*O*-methyl-D-mannose by conversion into 2,3-di-*O*-methyl-D-mannose-1,4,6-tri-*p*-nitrobenzoate, m.p. and m.m.p. 192°; $[\alpha]_D^{23}$ +66° ($CHCl_3$) (lit. m.p. 192–194° and $[\alpha]_D$ +66°).[12] The molecular proportion of the methylated sugars determined by hypoiodite titrations,[13] after separation in solvent b, was 1·02 (Fraction I): 1·05 (Fraction II): 1·00 (Fraction III).

Periodate oxidation of the galactomannan. The polysaccharide (81 mg) was dissolved in H_2O (25 ml) and the solution was cooled to 0°. A cold solution of sodium metaperiodate (0·15 M, 30 ml) was added to solution and volume was made up to 100 ml. The reaction was conducted at 5° and amounts of periodate consumed and formic acid produced were estimated[14] at different time intervals. The periodate oxidation was completed in 81 hr corresponding to 1·30 mol of periodate consumed and 0·31 mol of formic acid liberated per mol of hexose unit.

Periodate degradation of the galactomannan. The polysaccharide (1 g) was oxidized with sodium metaperiodate as mentioned above. After removing the excess of periodate, the solution was reduced with $NaBH_4$ (0·9 g) at room temp. for 20 hr. The solution was neutralized (HOAc), deionized and evaporated to dryness. After removing boric acid by repeated evaporations with MeOH, the residue was hydrolysed with N H_2SO_4 (100 ml) for 15 hr at 95°. The hydrolysate was neutralized ($BaCO_3$), filtered, deionized and evaporated. Chromatography (solvent c) revealed glycerol, erythritol and traces of galactose and mannose. The mixture was separated on Whatman No. 3 MM sheets (solvent c). *Fraction* I was identified as glycerol by conversion into glycerol-tri-*p*-nitrobenzoate, m.p. and m.m.p. 184–186° (lit. 186–188°).[10] *Fraction* II which was erythritol had m.p. and m.m.p. 121° (lit. m.p. 120–122°).[15] It was converted into tetra-*O*-tosyl-erythritol, m.p. and m.m.p. 164–165° (lit. m.p. 164–165°)[15] and erythritol-tetra-*p*-nitrobenzoate, m.p. and m.m.p.

* R_G values are with reference to 2,3,4,6-tetra-*O*-methyl-D-glucose.

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248–249° (lit. 247–249°).[16] The ratio of glycerol and erythritol as determined by periodate chromotropic acid method,[17] was 1·00:1·94.

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Key Word Index—Delonix regia; Leguminosae; seed; polysaccharide; galactomannan.