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Structural Elucidation of Oligosaccharides from *Grewia oppositifolia* Seeds Galactomannan

R.B. SINGH
Department of Zoology, Social Forestry and Environment
Institute of Basic Sciences, Khandari Campus
Agra University, Agra-282 002, India

Partial acid hydrolysis of *Grewia oppositifolia* seeds galactomannan afforded O-α-D-galactopyranosyl-(1→6)-O-α-D-mannopyranose, O-β-D-mannopyranosyl-(1→4)-*o*-β-D-galactopyranose and *o*-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose.

INTRODUCTION

In our earlier communications[1,2] of *Grewia oppositifolia* (Tiliaceae) seeds galactomannan afforded D-galactose and D-mannose in 2:5 molar ratio. Present manuscript deals with isolation and structure elucidation of oligosaccharides. Partial acid hydrolysis of galactomannan followed by column[3] over charcoal-celite and paper chromatography[4] of hydrolyzate produced two disaccharides as O-α-D-galactopyranosyl-(1→6)-O-α-D-mannopyranose, O-β-D-mannopyranosyl-(1→4)-O-β-D-galactopyranose and one trisaccharide as O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose. Each oligosac-charide was purified separately and characterized by its optical rotation, formation of crystalline derivative (disaccharide), degree of polymerization[5], reduction with sodium borohydride[6] and periodate oxidation[7].

EXPERIMENTAL

Paper chromatography was carried out by descending technique[8] on Whateman No. 3MM filter paper using following solvent mixtures (v/v): (S_1), N-butanol-ethanol-water (4:1:5-upper phase)[9], (S_2) ethyl acetate-acetic acid-water (9:2:2-upper phase)[10], (S_3) ethyl acetate-pyridine-water (10:4:3-upper phase)[11] and used (R_1) *p*-anisidine phosphate[12] as spray reagent. Deionization was done with freshly regenerated amberlite ion exchange resins[13] IR-120 (H^+) and IR-45 (OH^-). Sugar mixtures were separated on charcoal-celit (1:1, w/w) column using water followed by 2.5, 5, 7.5 and 10% aqueous ethanol (v/v) as eluants. Obtained fractions were futher separated by paper chromatography and degree of polymerization was determined by Timell's method[5].

Partial acid hydrolysis: After a long series of trial experiments, the following method was carried out for partial acid hydrolysis[14] to give maximum yield of oligosaccharides. Galactomannan (20 gm) was dissolved in H_2SO_4 (1.5 N,

*For correspondence: Dr. R.B. Singh, 8/53-B, Kaushalpur, Agra-282 005, India.

720 mL) at 18–20°C (24 h) then heated on water-bath (1/2 h). Hydrolyzate was cooled, filtered, neutralized ($BaCO_3$), filtered and then concentrated to a small volume (40 mL), added ethanol (400 mL), obtained degraded polysaccharide as white coarse power, filtered and dried. Paper chromatograph of syrup obtained after ethanolic extract concentration showed the presence of D-galactose, and D-mannose and a number of oligosaccharides.

Separation of oligosaccharides: Oligosaccharides were separated by chromatographic adsorption on charcoal-celite (1:1, w/w) column (60 × 2.5 cm) using graded elution method[15]. Column was first eluted with water (2 L) under 7 lbs/sq. inch pressure to remove monosaccharides, then successively with 3 L each of 2.5, 5, 7.5 and 10% aq. ethanol (v/v) and examined by paper chromatography with solvent (S_1) and used (R_1) as spray reagent. All fractions of gradient elution were found to contain a mixture of three oligosaccharides.

Appropriate fractions of eluates were mixed together and suspended impurities removed by dissolving them in aq. methanol, filtered and concentrated to syrup. Constituent oligosaccharides were separated on Whatman No. 3MM paper using solvent (S_2) and corresponding sugar strips cut out with the help of guide spot, then eluted with water[16] and finally concentrated to syrup. This led to the isolation of two disaccharides[17] and one trisaccharide[17] in pure form, which were characterized as follows:

I. *O-α-D-Galactopyranosyl-(1→6)-O-α-D-mannopyranose*: It (250 mg) was dissolved in water-ethanol mixture (1:1, v/v), filtered and added *n*-butanol (4 mL) in filtrate, obtained crystalline product (m.p. 200–202°C, reported 201–202°C)[18]. It had R_{gal} 0.52 in solvent (S_2), R_{glu} 0.45 in solvent (S_3), $[\alpha]_D^{25} = +121°$ (H_2O), Lit. $[\alpha]_D = +120°$[19] and DP 1.70. Acid hydrolysis (H_2SO_4, 1 N) afforded D-galactose, D-mannose in equimolecular proportion as determined by phenol-sulphuric acid method[20]. Phenyl hydrazone derivative[21] was prepared by usual method, had m.p. 176–178°C (reported 175–176°C)[22]. Periodate oxidation[7] results showed the consumption of 6.15 moles of periodate with simultaneous liberation of 4.75 moles of formic acid per mole of disaccharide (50 h). Reduction with sodium borohydride followed by hyrolysis gave D-galactose, indicating reducing end to be D-mannose.

II. *O-α-D-Mannopyranosyl-(1→4)-O-β-galactopyranose*: It (230 mg) was dissolved in aq. methanol (50 mL), filtered and concentrated to syrup. It had R_{gal} 0.62 in solvent (S_2), R_{glu} 0.50 in solvent (S_3), $[\alpha]_D^{25} = (H^2O) + 16°$, Lit $[\alpha]_D = +17°$[23]. Acid hydrolysis (H_2SO_4, 1 N) afforded D-galactose and D-mannose by phenol-sulphuric acid method[20] in equal amount and degree of polymerization was found to be 1.88. Phenyl hydrazone derivative[21] was prepared by usual manner, had m.p. 190–192°C (reported 194–195°C)[24]. Periodate oxidation[7] showed the consumption of 5.95 moles of periodate and liberation of 3.80 moles of formic acid per mole of disaccharide (55 h).

III. *O-β-D-Mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose*: It (220 mg) had R_{gal} 0.31 in solvent (S_2), R_{glu} 0.17 in solvent (S_3). $[\alpha]_D^{25} = -21.5°$ (H_2O), Lit $[\alpha]_D = -22°$.[25] Acid hydrolysis (H_2SO_4, 1 N) showed the presence of D-mannose and degree of polymerization 2.96. Periodate

oxidation studies[7] showed the consumption of 6.55 moles of periodate and liberated 4.60 moles of formic acid per mole of trisaccharide (60 h).

RESULTS AND DISCUSSION

Grewia oppositifolia seeds galactomannan, upon partial acid hydrolysis (H_2SO_4, 1 N), followed by charcoal-celite column and paper chromatography of hydrolyzate afforded two disaccharides and one trisaccharide. Each oligosaccharide was purified separately and characterized by its optical rotations, degree of polymerization, formation of crystalline derivatives (disaccharide), reduction with sodium borohydride, complete acid hydrolysis and periodate oxidation studies. Oligosaccharides were identified as O-α-D-galactopyranosyl-(1→6)-O-α-D-mannopyranose (I), O-β-D-mannopyranosyl-(1→4)-O-β-D-galactopyranose (II) and *o*-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose (III).

Isolation of oligosaccharides (II) and (III) clearly indicates that the main polymer chain of galactomannan is made up of D-galactopyranose and D-mannopyranose units with (1→4)-β-type linkages. Oligosaccharide (II) also supports that D-mannose units are joined together with (1→4)-β-type linkages. Isolation of (I) supports the fact that branches of main chain consist of single non-reducing D-galactopyranose residue which is glycosidically attached to (1→6)-α-type linkages with D-mannopyranose unit of main chain. The earlier proposed structure of *Grewia oppositifolia* seeds polysaccharide (Fig. 1) is favoured by the above results.

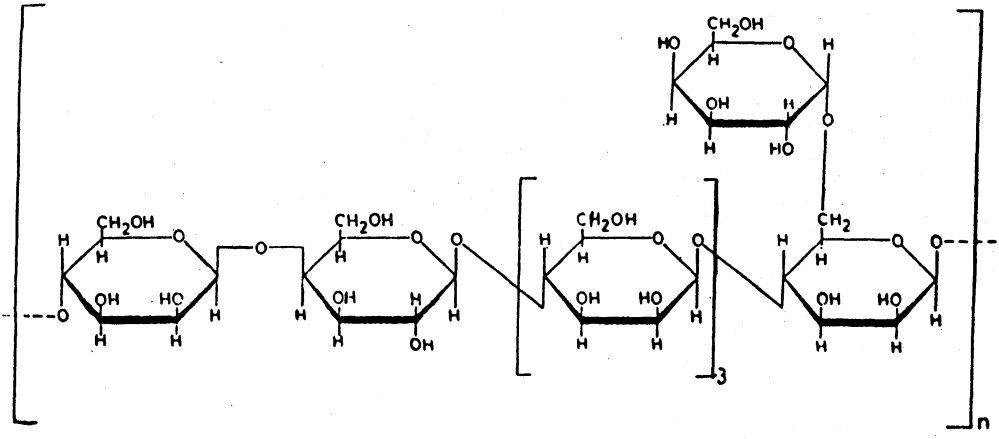

Fig. 1. Structure of *Grewia oppositifolia* seeds polysaccharide

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