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Galactomannan from *Grewia oppositifolia* Seeds

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Grewia oppositifolia seeds yielded water-soluble sugars, as D-galactose and D-mannose, in 2 : 5 moles. It produced methylated sugars as 2,3,4,6-tetra-O-methyl-D-galacose, 2,3,6-tri-O-methyl-D-mannose; 2,3,6-tri-O-methyl-D-galactose and 2, 3-di-O-methyl-D-mannose in 1 : 4 : 1 : 1 molar ratio. Periodate oxidation results showed the consumption of 1.2 moles of periodate with liberation of 0.25 moles of formic acid.

INTRODUCTION

Grewia oppositifolia[1] (Tiliaceae) occurs in Northern India and its seeds are used in Ayurvedic system of medicine. The isolation, purification and nature of sugars have been reported.[2] The present manuscript deals with methylation and periodate oxidation of seeds which was carried out for polysaccharide structure.

EXPERIMENTAL

All evaporations were carried out at 40–50°C under reduced pressure, unless otherwise stated. The specific rotations are in equilibrium values and melting points are uncorrected. Paper chromatography was carried out on Whatman No. 3MM paper by descendng technique[3] with solvent mixtures (v/v): (A) *n*-butanol-ethanol-water (4 : 1 : 5, upper phase)[4]; (B) *n*-butanol-acetic acid-water (4 : 1 : 5, upper phase)[4]; (C) benzene-ethanol-water (169 : 47 : 15, upper phase)[5] and (D) butanone-water (azeotropic mixture)[6]. The spray reagent (R_1) *p*-anisidine phosphate[7] was used for detecting the methyl sugars.

Methylation and characterization of polysaccharide

Polysaccharide (8 g) was methylated by Hakomari's method[8] three times with dimethyl sulphate (90 mL) and sodium hydroxide (45%, 150 mL) to get a brownish yellow product. Found: OCH_3, 44.52%. It was again methylated by Purdie's reagent[9] (acetone, methyl iodide and silver oxide) to furnish a fully methylated product (6.2 g). Found: OCH_3, 44.98% which gave no hydroxyl group in the absorption band at 3600–3500 cm^{-1} in IR-spectrum (KBr)[10].

Methylated sugar (6 g) was purified by fractional dissolution method[11] with pet. ether (40–60°C) and chloroform mixture being increased from 0–25% on water-bath (2 h). Each fraction was concentrated, dried under vacuum (15 mm, over P_2O_5) for constant weight. The specific rotation and methoxyl contents were determined as usual and results are given in Table-1.

TABLE-1
FRACTIONATION OF METHYLATED SUGARS

Fraction No.	State	Solvent composition (%)		Yield (g)	—OCH_3 (%)	$[\alpha]_D^{25}$ ($CHCl_3$)
		Pet. ether	Chloroform			
1.	Oily liquid	100	00	0.4246	—	—
2.	Oily liquid	95	05	0.4022	—	—
3.	Cryspy liquid	90	10	0.5864	51.8	+15.8°
4.	Cryspy solid	85	15	0.4250	41.5	−10.8°
5.	Crypsy solid	80	20	1.9824	40.2	+79.8°
6.	Crypsy solid	75	25	1.6646	29.0	−15.2°

Methylated sugar (4.6 g) was hydrolyzed with sulphuric acid[12] (72%, 50 mL), then kept at 18–20°C (1 h); afterwards the content was diluted with water to have 12% concentration with respect to H_2SO_4, then heated on water-bath (4 h). Hydrolysate was neutralized ($BaCO_3$), filtered and evaporated to syrup. Paper chromatography of syrup in solvent (C) and using (R_1) as spray reagent revealed the presence of four methylated sugars which were cut out with the help of guide spot; then sugar strips were eluted with water[13]. The eluted sugar components were evaporated separately which were characterized and identified as follows:

Fraction (I): *2,3,4,6-tetra-O-methyl-D-galactose*: It (760 mg) moved as a single spot on paper chromatogram, had R_f 0.74 (in solvent D) and R_g 0.92 (in solvent A), $[\alpha]_D^{25}$ +102° (H_2O) (Lit. $[\alpha]_D$ +100°)[14]. It gave D-galactose on demethylation[15]. Found: —OCH_3, 51.8%, calcd: $C_{10}H_{20}O_6$: —OCH_3, 52.6%. Aniline derivative was prepared by usual manner as N-phenyl-2,3,4,6-tetra-O-methyl-D-galactopyranosyl amine, having m.p. and mixed m.p. 191–192°C (Lit. 190–191°C and 192°C)[16, 17].

Fraction (II): *2,3,6-tri-O-methyl-D-mannose*: Sugar syrup (1.30 g) had R_f 0.48 (in solvent D) and R_g 0.84 (in solvent A), $[\alpha]_D^{25}$ +10.8° ($CHCl_3$), −5.8° (H_2O) (Lit. $[\alpha]_D$ −6.5° [18] and −15.7°)[19]. It gave D-mannose on demethylation, Found: —OCH_3, 41.4%; Calcd. $C_9H_{18}O_6$ requires: —OCH_3, 41.9%. The derivative was prepared as 2,3,6-tri-O-methyl-D-mannoic acid phenylhydrazide having m.p. and mixed m.p. 128–130°C (Lit. 130–132°C)[17].

Fraction (III): *2,3,6-tri-O-methyl-D-galactose*: It (720 mg) had R_f 0.69 (in solvent D) and R_g 0.95 (in solvent A), $[\alpha]_D^{25}$ +79.8° ($CHCl_3$) (Lit. $[\alpha]_D$ +82°)[20]. It gave D-galactose on demethylation. Found: —OCH_3, 40.2%, Calcd. $C_9H_{18}O_6$ requires: —OCH_3, 41%. Derivative was prepared as 2,3,6–tri-O-methyl-N-phenyl-D-galactopyranosyl amine, having m.p. and mixed m.p. 177–178°C.

Fraction (IV): *2,3-di-O-methyl-D-mannose*: It (730 mg) moved as single spot in solvent (D) on paper chromatogram, had R_f 0.21 (in solvent D) and R_g 0.58 (in solvent A), $[\alpha]_D^{25}$ −15.2° (H_2O) and +4.6° (MeOH) (Lit $[\alpha]_D$ −17° (H_2O) and +6° (MeOH)[21]. It gave D-mannose on demethylation Found: —OCH_3, 29%; Calcd. $C_8H_{16}O_6$ requires: —OCH_3, 28.8%. Derivative was prepared as 2,3-di-O-methyl-γ-D-mannolactone, having m.p. and mixed m.p. 105–107°C (Lit. 106–108°C)[21].

Quantitative estimation of methylated sugar: The methylated sugar (400 mg) was quantitatively separated by paper chromatography on Whatman No. 3MM paper in solvent (B) and different methylated sugar zones were cut out with the help of guide spot, then eluted with water[13]. The eluted sugars were estimated by alkaline hypoiodite method[22]. It was found that 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,6-tri-O-methyl-D-mannose, 2,3,6-tri-O-methyl-D-galactose and 2,3-di-O-methyl-D-mannose were present in 1 : 4 : 1 : 1 molar ratio.

Periodate Oxidation of Polysaccharide: The pearіodate oxidation[23] was carried out by dissolving polysaccharide (600 mg) in water (100 mL) and sodium metaperiodate (0.125 M, 100 mL) at 5–8°C in refrigerator (40 h). The periodate consumption[24] and formic acid liberation[25] per mole of anhydrohexose sugar at various time intervals are given in Table-2.

TABLE-2
PERIODATE OXIDATION OF POLYSACCHARIDE

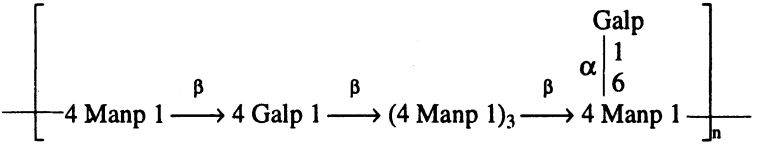
S. No.	Sugar fraction	Time (h)							
		5	10	15	20	25	30	35	40
1.	Periodate consumption (moles/mole)	0.30	0.60	0.85	1.05	1.15	1.20	1.20	1.20
2.	Formic acid liberation (moles/mole)	0.80	0.14	0.19	0.22	0.24	0.25	0.25	0.25

RESULTS AND DISCUSSION

Grewia oppositifolia seeds' polysaccharide was methylated by Hakomari and Purdie's method, then the methyl sugars were separated on paper chromatogram which were identified as 2,3.4, 6-tetra-O-methyl-D-galactose, 2,3,6-tri-O-methyl-D-mannose, 2,3,6-tri-O-methyl-D-galactose and 2,3-di-O-methyl-D-mannose in 1:4:1:1 molar ratio. Formation of 2,3,4,6-tetra-O-methyl-D-galactose indicates that the D-galactose is at the non-reducing end of the polymer chain of repeating unit and attached through (1→6)-α-type linkages with D-mannose unit. The 2,3-di-O-methyl-D-mannose reveals the branching point in galactomannose polymer on D-mannose unit at C_1, C_4, and C_6 position. The 2,3,6-tri-O-methyl-D-galactose and 2,3,6-tri-O-methyl-D-mannose indicate that the main chain of polysaccharide is composed of D-galactose units which are joined through (1→4)-β-type glycosidic linkages at C_1 and C_4. It proved that the branching must take place at C_6 position of D-mannose unit which was confirmed by 2,3-di-O-methyl-D-mannose. The D-galactose unit is constructing the non-reducing ends of the polymer chain which are joined through C_1 to C_6 of D-mannose unit by (1→6)-α-type linkages.

The ratio between tetra-, tri- and di-O-methyl hexose clearly indicates that there is one branch point in the repeating unit of polymer chain. The main chain length of polymer is constituted of six hexose units, since the ratio of D-galactose and D-mannose was found to be 2 : 5 moles. Periodate oxidation results showed that it consumed 1.2 moles of periodate and liberated 0.25 moles of formic acid per mole

of anhydrohexose unit (40 h). Structure of *Grewia oppositifolia* seeds' polysaccharide is proposed for the galactomannan (Fig. 1) on the basis of above finding results.



where Manp = D-Mannopyranose; Galp = D-Galactopyranose

Fig. 1 Structure of *Grewia oppositifolia* seeds polysaccharide

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