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Polysaccharide from *Jacaranda mimosaefolia* Seeds

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

Jacaranda mimosaefolia seeds extract yielded water soluble sugars as D-galactose and D-mannose in 2 : 7 moles. It gave methyl sugars, 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 in 2 : 5 : 2 moles. It consumed 2.30 moles of periodate and liberated 1.05 moles of formic acid per mole of anhydrohexose unit by periodate oxidation.

INTRODUCTION

Jacaranda mimosaefolia[1] (Bignoniaceae) occurs in hilly region of Northern India, Brazil, Agrentina, Tropical America and Philippines. Its seeds are used in Ayurvedic system of medicine[2] and contain water-soluble sugars[3]. The present manuscript deals with methylation and periodate oxidation for polysaccharide structure.

EXPERIMENTAL

Paper chromatographic analysis was carried out on Whatman No. 3MM filter paper by descending technique[4] for identification of methylated sugars. The solvent mixtures were applied in paper chromatogram for determination of sugars as (A) *n*-butanol-ethanol-water (4 : 1 : 5, upper layer)[5], (B) *n*-butanol-acetic acid-water (4 : 1 : 5, upper layer)[5], (C) benzene-ethanol water (169 : 47 : 15, upper layer)[6] and (D) butanone-water (azeotropic mixture)[7]. The spray reagent (R_1) *p*-anisidine phosphate[8] was used for detecting the sugars.

Methylation of polysaccharide

Polysaccharide (10 g) was methylated by Srivastava method[9] for four times with dimethyl sulphate (80 mL) and sodium hydroxide (45%, 150 mL) to get a brownish yellow product. It was again remethylated by Purdie's reagent[10] (acetone, methyl iodide and silver oxide) to obtain a fully methylated product (7.64 g). It found —OCH_3, 44.95%, giving no hydroxyl peak at 3600–3500 cm^{-1} region absorption band in IR-spectrum (KBr)[11].

Fractionation of methylated sugar

Methyl sugar (7.5 g) was fractionated by fractional dissolution method[12] with pet. ether (40–60°C) and chloroform; then the latter solvent being increased from

0–25% in water-bath (2 h), the fraction obtained was concentrated and dried under high vacuum (15 mm, over P_2O_5) for constant weight. The specific rotations and methoxyl contents of sugar fractions 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 (%)	$[\alpha]_D^{22}$ ($CHCl_3$)
		Pet. ether	Chloroform			
1.	Oily liquid	100	00	0.4246	—	—
2.	Oily liquid	95	05	0.3996	—	—
3.	Oily liquid	90	10	0.5828	—	—
4.	Cryspy solid	85	15	0.4062	54.1	+15.6°
5.	Crypsy solid	80	20	1.9824	44.2	+17.0°
6.	Crypsy solid	75	25	1.6608	29.2	+14.9°

Hydrolysis and characterization of methylated sugar

Methylated sugar (4 g) was hydrolysed with sulphuric acid[13] (72%, 10 mL); afterwards the solution was left at room temperature (1 h). Contents were diluted with water to have 12% concentration with respect to H_2SO_4; then the reaction mixture heated (4 h) on water-bath. Hydrolysate was neutralized ($BaCO_3$), filtered and evaporated to a syrup. Paper chromatographic analysis of syrup using (C) as solvent (C) and (R_1) as spray reagent revealed the presence of three methyl sugars. These sugars were seprated by paper chromatography on Whatman No. 3MM filter paper and corresponding sugar strips were cut out with the help of guide spots and then eluted with water[14]. The eluted fractions were evaporated separately which were characterised and identified as follows:

(I) *2,3,4,6-tetra-O-methyl-D-galactose*: It (400 mg) gave a single spot of D-galactose on paper chromatogram in solvent (D). Found: —OCH_3, 54.1%; calcd. for $C_{10}H_{20}O_6$ required: —OCH_3, 55.2% which gave D-galactose on demethylation[15]. The anilide derivative was prepared by usual manner as N-phenyl-2,3,4,6-tetra-O-methyl-D-galactopyranosyl amine, having m.p. 189–190°C (Lit. 190–191°C)[18]. It has R_f 0.71 in solvent (D) and R_g 0.93 in solvent (A), $[\alpha]_D^{22}$ +150° (H_2O) (Lit. $[\alpha]_D$ +160°).[17]

(II) *2,3,6-tri-O-methyl-D-mannose*: It (800 mg) was used in paper chromatography in solvent (D), Found: —OCH_3, 44.2%; calcd. for $C_9H_{18}O_6$ required: —OCH_3, 42% which gave D-mannose on demethylation. Derivative was prepared as 2,3,6-tri-O-methyl-D-mannonic acid phenylhydrazide having m.p. 129–130°C (Lit. 130–132°C)[18]. It had R_f 0.47 (D) and R_g 0.82 (A), $[\alpha]_D^{22}$ −14° ($CHCl_3$) and −6° (H_2O) (Lit. $[\alpha]_D$ −6.5°(H_2O)[19] and −15.7° ($CHCl_3$)[19]).

(III) *2,3-di-O-methyl-D-mannose*: It (750 mg) gave a single spot on paper chromatogram, Found: —OCH_3, 29.2%; calcd. for $C_8H_{16}O_6$ required: —OCH_3, 30.1% which gave D-mannose on demethylation[15]. Derivative was prepared by

usual manner as 2,3-di-O-methyl-γ-D-mannolactone, having m.p. 104–106°C (Lit. 106–108°C)[17]. It had R_f 0.22 (D) and R_g 0.57 (A), $[\alpha]_D^{21}$ −14° (H_2O) and + 5.5° (MeOH) (Lit. $[\alpha]_D$ −16° (H_2O) and +6° (MeOH))[17].

Quantitative estimation of methylated sugars: Methyl sugar mixture (300 mg) was quantitatively separated by paper chromatography on Whatman No. 3MM paper using solvent (B) and sugar zones containing tetra-, tri- and di-O-methyl sugars were cut out with the help of guide spots; then it was eluted with water[14]. It was found that 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 were present in 2 : 5 : 2 molar ratio by alkaline hypoiodite method[21].

Periodate oxidation of polysaccharide: Polysaccharide (500 mg) on periodate oxidation[22] was oxidized with water (50 mL) and sodium metaperiodate (0.125 M, 100 mL) at 4.8°C (55 h). Periodate consumption[23] (2.30 moles) and formic acid liberation[24] (1.05 moles) per mole of anhydrohexose unit at various time intervals are given in Table-2.

TABLE-2
PERIODATE OXIDATION OF POLYSACCHARIDE

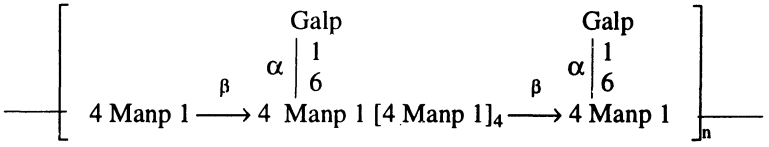
S. No.	Sugar fraction	Time (h)						
		10	20	30	40	45	50	55
1.	Periodate consumption (moles/mole)	0.75	1.50	1.95	2.20	2.30	2.30	2.30
2.	Formic acid liberation (moles/mole)	0.40	0.70	0.90	1.00	1.05	1.05	1.05

RESULTS AND DISCUSSION

Jacaranda mimosaefolia seeds' polysaccharide was methylated by Srivastava and Purdie's method to give 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 in 2 : 5 : 2 molar ratio on paper chromatogram. Formation of 2,3,4,6-tetra-O-methyl-D-galactose indicates that D-galactose is attached at non-reducing end of polymer chain through (1→6)-α-type with D-mannose while 2, 3, -di-O-methyl-D-mannose reveals a branching point in galactomannan polymer on D-mannose and D-galactose unit at C_1, C_4 and C_6. The 2,3,6-tri-O-methyl-D-mannose indicating the main chain or backbone of polymer are composed of D-mannose units, which are joined by (1→4)-β-type glycosidic linkages. The D-mannose units are occupied to form backbone of galactomannan at C_1 and C_4 position. It is evident that the branching takes place at C_6 of D-mannose, which is confirmed by the formation of 2,3-di-methyl-D-mannose. The D-galactose are constructing the non-reducing end of polymer chain which is joined through C_1 and C_6 of D-mannose unit.

Molar ratio between tetra-, tri- and di-O-methyl hexoses clearly indicates that there are two branch points in the repeating unit of the polymer chain. The main chain length of polymer is constituted of seven hexose units, since the molar ratio of D-galactose and D-mannose was found to be 2 : 7 moles.

Periodate oxidation showed the liberation of 1.05 moles of formic acid for 4 anhydrohexose units with consumption of 2.30 moles of periodate for each



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

Fig. 1 Polysaccharide structure of *Jacaranda mimosaefolia* seeds.

anhydrohexose unit of the polymer chain (55 h). Polysaccharide structure of *Jacaranda mimosaefolia* seeds was obtained on the basis of these results (Fig. 1).

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