



Water soluble seeds polysaccharide structure obtained after methylation studies confirmed by periodate oxidation method from *Cassia laevigata* Linn. plant

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Abstract

Cassia laevigata Linn. seeds yielded a water soluble polysaccharide on acid hydrolysis as D-galactose and D-mannose in 5 : 2 molar ratio by TLC, column and paper chromatographic analysis. Upon periodate oxidation of seed polysaccharide with sodium metaperiodate, (0.125 M). It consumed 2.55 moles of periodate with simultaneous liberation of 1.00 moles of formic acid per mole of anhydrohexose unit after 60 hrs. On the basis of methylation results, the proposed water soluble seeds polysaccharide structure has been confirmed by periodate oxidation method.

Keywords: periodate oxidation, periodate consumption, formic acid liberation of *Cassia laevigata* Linn. seed polysaccharide

Introduction

Cassia laevigata Linn. Plant ^[1] belongs to the family – Caesalpiniaceae is an large shrub and occurs in Himalayan region of Northern India. Seeds are medically used in indigenous system of medicine for the treatment of diarrhoea, asthma and other human disease. Seeds contain a water soluble polysaccharide on acid hydrolysis yielded D-galactose and D-mannose in 5:2 molar ratio by TLC, column and paper chromatographic analysis. Present manuscript mainly deals with the periodate oxidation method for the confirmation of water soluble seeds polysaccharide structure of *Cassia laevigata* Linn. plant which was obtained by methylation results. Periodate oxidation reaction is used in the carbohydrate chemistry and it also applicable in the seeds polysaccharide structure. It was first discovered by Malaprade ^[2] and Fluery & Lange ^[3], have given a better method for more extensive used of periodic acid for the oxidation of glycol group. Periodate oxidation reaction is considered to be a dialdehyde type of oxidation. Perlin ^[4] observed that the periodic acid and lead tetra acetate showed that the glycol groups undergoes cyclic ester formation with oxidants. The nature of seeds polysaccharide ^[5] methylation studies ^[6] and structural evolution of oligosaccharides ^[7] have already been studied. Recently the investigation of periodate oxidation studies on some medicinal plants of *Madhuca longifolia* Linn ^[8], *Moringa oleifera* Lam. ^[9] *Cassia alata* ^[10] *Quercus incana* Roxb. ^[11] *Jacaranda mimosaeifolia* Lin. ^[12], *Grewia*

Oppositifolia Roxb ^[13], *Cassia glauca* Linn. ^[14] etc plant have been studies.

Materials and Methods

Periodate oxidation of seeds polysaccharide

Seeds polysaccharide (500 mg) was oxidized ^[15] with water (50ml) and sodium metaperiodate (0.125M, 100 ml) and volume made upto 250 ml with water at 4-8^oC in refrigerator for 60 hrs. Reaction mixture (5 ml) was taken in a conical flask and added sodium bicarbonate solution (0.1N, 5 ml), sodium arsenite solution (0.01 N, 25 ml) and potassium iodide solution (0.01N, 5ml). Reaction mixture was left for 2 hrs and added iodine solution (0.01N, 5 ml). It was titrated against sodium thiosulphate solution (0.01 N) using starch as an indicator. A blank reading was also carried out in a similar way. The difference between blank and experimental values gives of periodate consumption ^[3] of 2.55 moles of anhydrohexose sugars unit after 60 hrs. Formic acid liberation was determined by taken the reaction mixture (5 ml) in a conical flask and added ethylene glycol (100ml) to destroy the excess of periodate ions present in the reaction mixture for 2 hrs. Formic acid liberation ^[16, 17] was titrated against sodium hydroxide solution (0.01N) using methyl red dye as an indicator. A blank titration was also carried out in a similar way for the estimation of formic acid. It liberated 1.00 moles of formic acid per mole of anhydrohexose sugar unit after 60 hrs and results are given in Table-1.

Table 1: Periodate oxidation of *Cassia laevigata* Linn. seeds polysaccharide.

S. No.	Sugar Fraction	Time (hrs)							
		10	20	30	40	45	50	55	60
1.	Periodate consumption per anhydrohexose sugar unit (moles/mole)	0.65	1.20	1.80	2.20	2.40	2.55	2.55	2.55
	Formic acid liberation per anhydrohexose sugar unit (moles/mole)	0.25	0.45	0.70	0.90	0.95	1.00	1.00	1.00

Periodate oxidized compound was again oxidized in a separate lot under condition similar to the above experiments was hydrolysed with sulphuric acid (1N) and worked upto a syrup. This hydrolysed sugar syrup on paper chromatographic examination ^[18] with solvent mixture (v/v), (A) n –butanol : ethanol : water (4 : 1 : 5) ^[19] using (R) p-

anisidine phosphate ^[20] as spray reagent gave sugar spots as D-galactose and D-mannose in 5 : 2 molar ratio.

Results and Discussion

Cassia laevigata Linn. water soluble seeds polysaccharide was oxidized with sodium metaperiodate (0.125 M) by usual

manner. It liberated 1.00 moles of formic acid per mole equivalent of seed polysaccharide with simultaneous consumption of 2.55 moles of periodate for anhydrohexose sugar units of the polymer chain after 60 hrs. Presence of $(1 \rightarrow 6)$ - α -type and $(1 \rightarrow 4)$ - β -type linkage are also confirmed by the periodate oxidation results. The glycol groups undergoes to the cyclic ester formation with oxidant and reaction is considered to be dialdehyde type of oxidation. Polysaccharide containing free hydroxyl groups resulted in the consumption of periodate ions during periodate oxidation reaction. It is concluded from the above facts that the probably there is two branching point from the repeating unit of the polysaccharide structure. Formic acid appears is to be originating from reducing as well as non-reducing terminal point of seed polysaccharide structure.

Probable reaction showed that the D-galactose and D-mannose units were containing adjacent free hydroxyl groups resulting in the consumption of periodate ions. The every second repeating unit of the seed polysaccharide structure of *Cassia laevigata* Linn. plant is of two D-galactose unit are at non reducing end and three D-galactose and two D-mannose units are in the main polymer chains. It is concluded from the above facts that probably two branching points occurs seven repeating units of the polysaccharide structure constituting the non-ionic polysaccharide. Water soluble seeds polysaccharide structure of *Cassia laevigata* Linn. plant was obtained from methylation studies was also confirmed by the periodate oxidation results as shown in Figure - I.

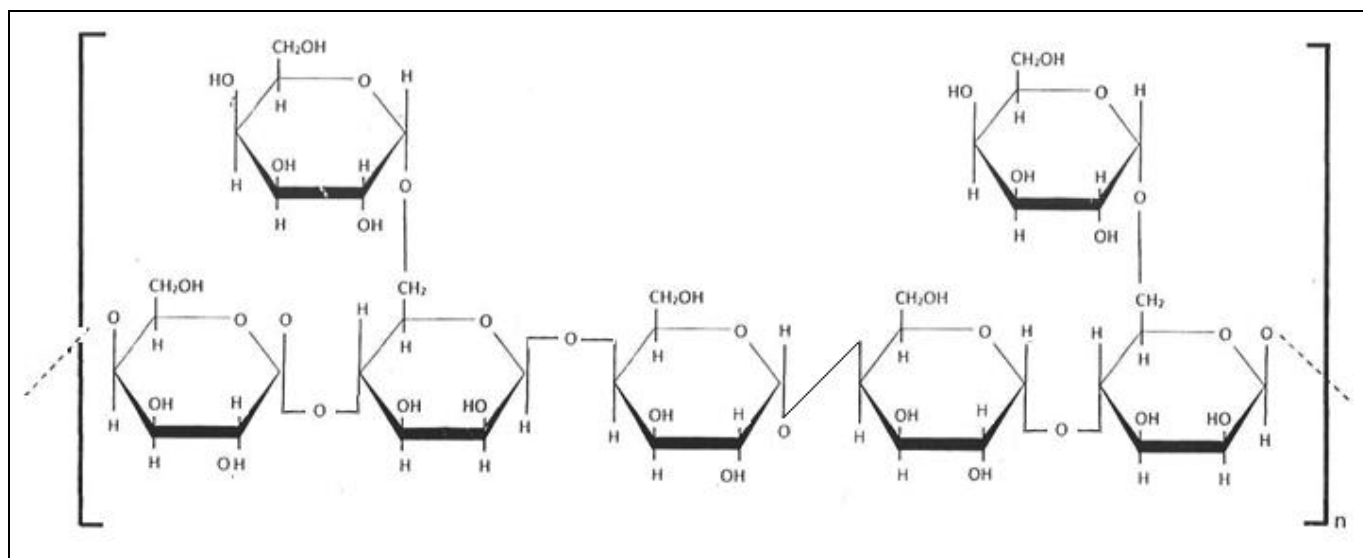


Fig 1: Seeds polysaccharide structures of *Cassia laevigata* Linn. plant

Conclusion

Cassia laevigata Linn. plant belongs to the family – Caesalpiniaceae and occurs in Himalayan region of Northern India. Its seeds yielded a water soluble seeds polysaccharide by usual manner on acid hydrolysis as D-galactose and D-mannose in molar ratio of 5:2 by TLC column and paper chromatographic analysis. The Periodate oxidation of seed polysaccharide with sodium metaperiodate (0.125 M). It liberated 1.00 moles of formic acid per equivalent of seeds polysaccharide with simultaneous liberation of 2.55 moles of periodate for anhydrohexose sugar units of polymer chain after 60 hrs. The presence of linkages of $(1 \rightarrow 6)$ - α -type and $(1 \rightarrow 4)$ - β -type linkage are also confirmed by the periodate oxidation results. It showed that the above finding results probably indicated the two branching point which occurs seven repeating units of the seeds polysaccharide structure. Seed Polysaccharide structure showed that the D-galactose and D-mannose sugar units containing free hydroxyl groups in periodate consumption. Every second repeating unit of the seed polysaccharide structure is showed two D-galactose sugar units are at non-reducing end while three D-galactose and two D-mannose sugar units are at the main polymer chain. It is also concluded that the water soluble seeds polysaccharide structure of *Cassia laevigata* Linn. plant obtained from methylation results was also confirmed by the periodate oxidation method.

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