



Confirmation of water soluble seeds polysaccharide structure from *Cassia auriculata* Linn. plant by periodate oxidation method

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Abstract

Cassia auriculata Linn. seeds yielded a water-soluble polysaccharide on acid hydrolysis as D-galactose and D-mannose in 1:3 molar ratio. Upon periodate oxidation of seeds polysaccharide with sodium metaperiodate, it consumed 1.25 moles of periodate with simultaneous liberation of 0.35 moles of formic acid per mole of anhydro hexose sugar unit after 50 hours. On the basis of methylation results, the proposed seeds polysaccharide structure of *Cassia auriculata* Linn. plant has been confirmed by periodate oxidation method.

Keywords: periodate oxidation, periodate consumption, formic acid liberation of *cassia auriculata* Linn. seeds polysaccharide

Introduction

Cassia auriculata Linn. Plant ^[1] belongs to the family-Caesalpinaceae is a small perennial shrub and commonly called as *Tarwar* or *Avaram*. It occurs in Northern and Southern India and Western Peninsula. Seeds are medically used in indigenous system of medicine ^[2] for the treatment of diarrhoea, asthma and other human diseases and vary in color from brown to dull olive. The bark is extensively used for tanning and dyeing purposes as buff colours. Bark is highly astringent and is employed in place of oak and bark for gargles, enemas, leaves and fruits are anthelmintic, Root is used in the treatment of skin disease. Plant is well known for its medicinal and economic values. It has been studied for its flavonoids, tannins, anthraquinones and proteolytic enzymes ^[3]. The nature of seeds polysaccharide ^[4], methylation studies ^[5] and structural elucidation of oligosaccharides ^[6] have already been studied. Seeds contain a water-soluble polysaccharide on acid hydrolysis and yielded D-galactose and D-mannose in 1:3 molar ratio on paper chromatogram. Present manuscript mainly deals with the periodate oxidation method for the confirmation of seeds polysaccharide structure of *Cassia auriculata* Linn. plant which was obtained by methylation results. Periodate oxidation reaction is used in carbohydrate chemistry and it is also applicable in the seed's polysaccharide structure. It was first discovered by Malaprade ^[7] and Fluery & Lange ^[8], have given a better method for more extensive use of periodic acid for the oxidation of glycol group. Periodate oxidation reaction is considered to be a dialdehyde type of oxidation. Perlin ^[9] observed that the periodic acid and lead tetra acetate showed that the glycol groups undergo cyclic ester formation with oxidants. Recently the investigation has already been studied the periodate oxidation studies on

some medicinal plants as *Madhuca longifolia* Linn ^[10], *Moringa oleifera* Lam ^[11], *Cassia alata* Linn ^[12], *Quercus incana* Roxb ^[13], *Jacaranda mimosaeifolia* Linn ^[14], *Grewia oppositifolia* Roxb ^[15], *Cassia glauca* Lam ^[16], *Cassia laevigata* Linn ^[17], etc. plant.

Materials and methods

Periodate oxidation of *Cassia auriculata* Linn. seeds polysaccharide

Seeds polysaccharide (1 gm) was oxidised ^[18] with water (100 ml) and sodium metaperiodate (0.125M, 100 ml) and volume made up to 250 ml with water at 4-8 °C in refrigerator for 50 hrs. Reaction mixture (5 ml) was taken in a conical flask and added sodium bicarbonate solution (0.1N, 5 ml), sodium arsenite solution (0.01N, 25 ml) and potassium iodide solution (0.01N, 5 ml). Reaction mixture was left for 2 hrs and added iodine solution (0.01N, 5 ml). It was titrated against sodium thiosulphate solution (0.01N) 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 ^[8] of 1.25 moles of anhydrohexose sugars unit after 50 hrs. Formic acid liberation was determined by taking the reaction mixture (5 ml) in a conical flask and added ethylene glycol (100 ml) to destroy the excess of periodate ions present in the reaction mixture for 2 hrs. Formic acid liberation ^[19, 20] 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 ^[21]. It liberated 0.35 moles of formic acid per mole of anhydrohexose sugar units after 50 hrs and results are given in Table-1.

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

S. No.	Sugar Fraction	Time (hrs)					
		10	20	30	40	45	50
1.	Periodate consumption per anhydrohexose sugar unit (moles/mole)	0.95	1.10	1.20	1.25	1.25	1.25
2.	Formic acid liberation per anhydrohexose sugar unit (moles/mole)	0.20	0.25	0.30	0.35	0.35	0.35

Periodate oxidised compound was again oxidised 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 ^[22] with solvent mixture (v/v), (A) n-butanol: ethanol: water (4:1:5) ^[23] using (R) *p*-anisidine phosphate ^[24] as spray reagent gave sugar spots as D-galactose and D-mannose in 1:3 molar ratio.

Results and discussion

Cassia auriculata Linn. water soluble seeds polysaccharide was oxidised with sodium metaperiodate (0.125M) by usual manner. It liberated 0.35 moles of formic acid per mole equivalent of polysaccharide with simultaneous consumption of 1.25 moles of periodate for anhydrohexose sugar units of the polymer chain after 50 hrs. Presence of (1→6)- α -type and (1→4)- β -type linkages 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 one 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 by which the periodate oxidation of polysaccharide occurs. The periodate oxidation reaction showed that the D-galactose and D-mannose units were containing adjacent free hydroxyl groups resulting in the consumption of periodate ions. It is concluded from the above facts that probably one branching point occurs four repeating units of the polysaccharide structure constituting the non-ionic polysaccharide. Water soluble seeds polysaccharide structure of *Cassia auriculata* Linn. plant was obtained from methylation studies was also confirmed by the periodate oxidation results and seeds poly saccharide structure was shown in Figure-1.

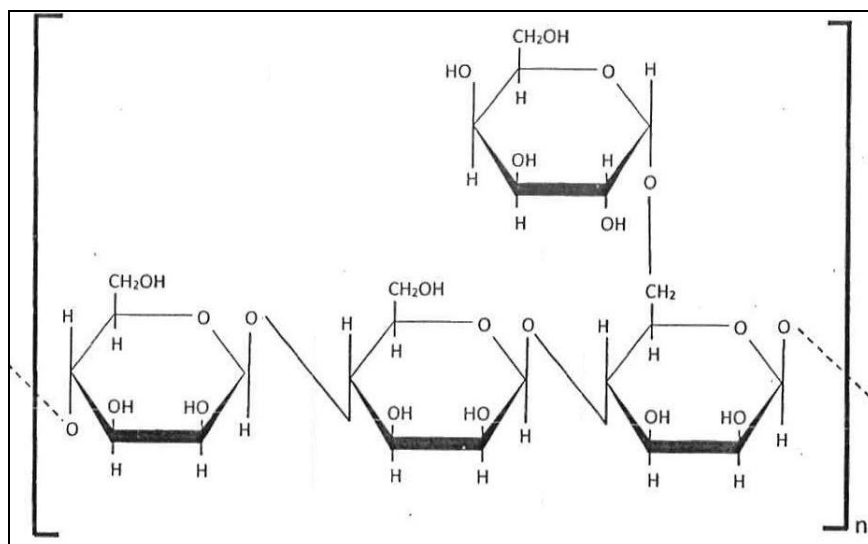


Fig 1: Seeds polysaccharide structure of *Cassia auriculata* Linn. plants.

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