



Periodate oxidation method used for the confirmation of water soluble seeds polysaccharide structure from *Cassia glauca* Lam. plant

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Abstract

Cassia glauca Lam. seeds yielded a water soluble polysaccharide on acid hydrolysis as D-galactose and D-mannose in 1:4 molar ratio. Upon periodate oxidation of seed polysaccharide with sodium metaperiodate, it consumed 1.15 moles of periodate with simultaneous liberation of 0.23 moles of formic acid per mole of anhydrohexose unit after 60 hrs. On the basis of methylation results, the proposed seeds polysaccharide structure has been confirmed by periodate oxidation method.

Keywords: Periodate oxidation, periodate consumption, formic acid liberation of *Cassia glauca* Lam. seed polysaccharide

Introduction

Cassia glauca Lam. Plant ^[1, 2] belongs to the family-Caesalpiniaceae, is a large shrub up to 10m in height. It occurs in Himalayan region of Northern India, Malaysia, Peninsula, North Australia, Pakistan, Sri Lanka, China South America and Tropical Asia. Bark and leaves are medically used in medicine ^[2] for the treatment of diarrhoea, skin infection, diabetes, asthma and other human diseases. Seeds oil are used in indigenous system of medicine for skin and leucoderma diseases. Seeds contain a water soluble polysaccharide on acid hydrolysis and yielded D-galactose and D-mannose in 1:4 molar ratio on paper chromatogram. In our earlier communications, the nature of seeds polysaccharide ^[3], methylation studies ^[4], Smith degradation studies of periodate oxidised product for the confirmation of seeds polysaccharide structure and structure elucidation of oligosaccharides ^[5] have already been studied. Present manuscript mainly deals with the periodate oxidation method for the confirmation of seeds polysaccharide structure of *Cassia glauca* Lam. 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 ^[6] and Fluery & Lange ^[7], 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 ^[8] observed that the periodic acid and lead tetra acetate showed that the glycol groups undergoes cyclic ester formation with oxidants. Recently the investigation have already been studies the periodate oxidation studies on some medicinal plant as *Madhuca longifolia* Linn ^[9], *Moringa oleifera* Lam ^[10], *Cassia alata* Linn ^[11], *Quercus incana* Roxb ^[12], *Grewia oppositifolia* Roxb ^[13], *Jacaranda mimosaeifolia* Linn ^[14], etc.

Materials and Methods

Periodate oxidation of seeds polysaccharide

Seeds polysaccharide (500mg) was oxidised ^[15] with water (50ml) and sodium metaperiodate (0.125M, 100ml) and

volume made up to 250ml with water at 4-8°C in refrigerator for 60 hrs. Reaction mixture (5ml) was taken in a conical flask and added sodium bicarbonate solution (0.1N, 5ml), sodium arsenite solution (0.01N, 25ml) 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 (0.01N) 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 ^[7] of 1.15 moles of anhydrohexose sugars unit after 60 hrs. Formic acid liberation was determined by taken the reaction mixture (5ml) 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 ^[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 ^[18]. It liberated 0.23 moles of formic acid per mole of anhydrohexose sugar units after 60 hrs and results are given in Table-1.

Table 1: Periodate oxidation of *Cassia glauca* Lam. 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)	0.25	0.45	0.70	1.00	1.12	1.15	1.15	1.15
2.	Formic acid liberation per anhydrohexose sugar unit (moles/mole)	0.06	0.10	0.16	0.20	0.22	0.23	0.23	0.23

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 up to a syrup. This hydrolysed sugar syrup on paper chromatographic examination ^[19] with solvent mixture (v/v), (A) *n*-butanol: ethanol: water (4:1:5) ^[20] and using (R) *p*-anisidine phosphate ^[21] as spray reagent gave sugar spots as

D-galactose and D-mannose in 1:4 molar ratio.

Results and Discussion

Cassia glauca Lam. water soluble seeds polysaccharide was oxidised with sodium metaperiodate (0.125M) by usual manner. It liberated 0.23 moles of formic acid per mole equivalent of polysaccharide with simultaneous consumption of 1:15 moles of periodate for anhydrohexose sugar units of the polymer chain after 60 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 five repeating units of the polysaccharide structure constituting the non-ionic polysaccharide. Water soluble seeds polysaccharide structure of *Cassia glauca* Lam. (Figure-1) was obtained from methylation studies was also confirmed by the periodate oxidation results.

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