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Confirmation of seeds polysaccharide structure from *Cassia alata* Linn. plant by periodate oxidation method

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ABSTRACT

Periodate oxidation is one of the most important reaction in structural study of non-ionic polysaccharide. Periodate oxidation was done using sodium metaperiodate as oxidant proposed by Fluery & Lange's method. It consumed 1.16 moles of periodate and liberated 0.23 moles of formic acid per mole of anhydrohexose unit after 50 hrs. The isolated water soluble seeds extract yielded sugars as D-galactose and D-mannose in 2:3 molar ratio. The probable polysaccharide structure from Cassia alata Linn. seeds have been elucidated with methylation results was confirmed by the periodate oxidation results.

Key words: Periodate oxidation, *Cassia alata* seeds polysaccharide

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INTRODUCTION

Cassia alata Linn. plant[1] belongs to family- Caesalpiniaceae and commonly known as *Dadmurdan.* It occurs in Garhwal region of Northern India. Plant is a large shrub and grown as an ornamental purposes. Its seeds are used in Ayurvedic system of medicine such as leaves are used for the treatment of ringworm, skin diseases and also in snakebite. Present manuscript mainly deals with the periodate oxidation study for the confirmation of seeds polysaccharide structure which obtained from the methylation results. Seeds contain a water soluble polysaccharide containing D-galactose and D-mannose in 2:3 molar ratio confirmed by GLC, TLC, Column and Paper chromatography. Reaction of periodate oxidation in polysaccharide was discovered by Malaprade[2], Fluery & Lange[3] have given periodic acid for the oxidation of glycol. Perlin[4] observed that the periodic acid and lead tetracetate showed that the glycol groups undergoes cyclic ester formation with oxidants.

MATERIALS AND METHODS

For periodate oxidation studies[5] of *Cassia alata* Linn, seeds polysaccharide (600 mg) was dissolved in water (50 ml) and added cold solution of sodium metaperiodate (0.125 M, 100 ml), then volume was made upto 250 ml with water. The reaction mixture was kept in dark at 4-8^{0}C in refrigerator for 50 hrs. Aliquot (5 ml) was pipette out at different intervals of time then added sodium bicarbonate solution (0.1 N, 5 ml), sodium arsenite solution (0.01 N, 25 ml) and potassium iodide solution (0.01 N, 5 ml). The reaction mixture was left for 1 hr and added iodine solution (0.01 N, 5 ml). It was titrated against sodium thiosulphate solution (0.1 N) using starch as an indicator. A blank titration was also carried out in a similar way. The difference between blank and experiment value gives the value of periodate consumption[3] (1.16 moles) per anhydrohexose sugar units after 50 hrs and results are shown in Table-1.

The formic acid released[6], [7], [8] from periodate oxidation studies was determined by taking the aliquot (5 ml) in a conical flask and added ethylene glycol (100 ml) to destroy the excess of periodate present in the reaction mixture for 50 min. Formic acid evolved was titrated against sodium hydroxide solution (0.01 N) 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 0.23 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 alata* Linn. seeds polysaccharide

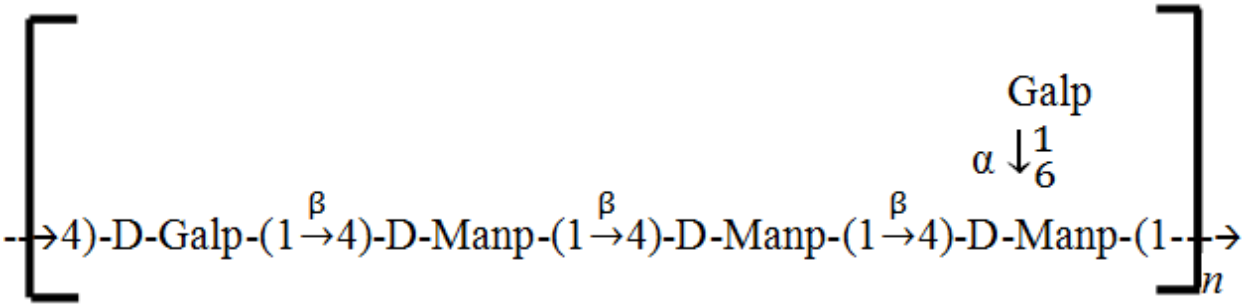
S. No.	Sugar Unit	Time (hrs)					
		10	20	30	40	45	50
1.	Periodate consumption per anhydrohexose sugar unit (moles/mole)	0.52	0.96	1.12	1.16	1.16	1.16
2.	Formic acid liberation per anhydrohexose sugar unit (moles/mole)	0.12	0.19	0.21	0.23	0.23	0.23

RESULTS AND DISCUSSION

Water soluble *Cassia alata* Linn. seeds mucilages yielded polysaccharides as D-galactose and D-mannose in 2:3 molar ratio. Purified seeds polysaccharides was oxidised with sodium metaperiodate by usual manner. It liberated 0.23 moles of formic acid per equivalent of polysaccharide with simultaneous consumption of 1.16 moles of periodate for each anhydrohexose sugar units of the polymer in 50 hrs. Presence of (1→4)-β-type and (1→6)-α-type linkages are also confirmed by periodate oxidation results. Seeds polysaccharide containing free hydroxyl groups resulted in the consumption of periodate ions during periodate oxidation reaction. It is concluded from the above facts that probably there is one branching point from repeating unit of the galactomannan. The formic acid appears is to be originating from reducing as well as non-reducing terminal point unit at the polysaccharide structure.

Periodate oxidation study showed the consumption of 1.16 moles of periodate ions per anhydrohexose sugar units as determined by volumetrically. The probable reaction by which the periodate oxidation of the seeds polysaccharide occurs. Reaction showed that the D-galactopyranose units were containing adjacent free hydroxyl groups resulting in the consumption of periodate ions during periodate oxidation reaction. It is concluded from the above facts that probably one branching point occurs 5 repeating units of galactomannan, constituting the non-ionic polysaccharide. The periodate consumption indicates that on increasing the time from 40-50 hrs, the consumption of moles periodate becomes constant (1.16 moles) after 50 hrs. The formic acid appears is to be originating from reducing as well as non-reducing terminal units of the D-galactopyranose.

From the above facts it may be concluded that the terminal D-galactopyranose units of the polysaccharide are not substituted. The amount of released formic acid increases from 0.12 to 0.23 moles with increases in time from 40 to 50 hrs. The amount of released formic acid becomes constant (0.23 moles). Recently the periodate oxidation studies were carried out from *Cassia hirsuta* Linn. plant[9]. The polysaccharide structure of *Cassia alata* Linn. seeds was obtained from methylation studies (Figure-1) was also confirmed by the periodate oxidation results.



Where : Galp = D-galctopyranose; Manp= D-mannopyranose

Figure-1 : Seeds polysaccharide structure of *Cassia alata* Linn. plant

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