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Archives of Applied Science Research, 2011, 3 (4):7-10
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Confirmation of seeds polysaccharide structure of *Cassia hirsuta* Linn. plant by periodate oxidation studies

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ABSTRACT

Cassia hirsuta Linn. seeds yielded a water soluble polysaccharide as D-galactose and D-mannose in 1:4 molar ratio by alkaline hypoiodite method on paper chromatogram. Reactions of the periodate oxidation is one of the most important in the structure determination of seeds polysaccharide in carbohydrate chemistry. Periodate oxidation method was done with sodium metaperiodate as oxidant and it was proposed by Malaprade and Flurry & Lange's method. The mole of periodate consumption and formic acid liberation during periodate oxidation reaction of seeds polysaccharide was determined volumetrically. Composition and probable polysaccharide structures have also been elucidated with the information obtained from periodate oxidation of seeds polysaccharide. After complete periodate oxidation reaction, it consumed 1.13 moles of oxidant with simultaneous liberation of 0.23 moles of formic acid per mole of anhydrohexose sugar unit of the polymer chain after 30 hrs. Formic acid appear is to be originating from the reducing as well as non - reducing terminal unit of D-galactose and D-mannose are of (1→6)-α-type at non-reducing end while (1→4)-β-type at main polymer chain between D-mannose residues.

Key words: Periodate oxidation, formic acid liberation, peroidate consumption, *Cassia hirsuta* seeds polysaccharide.

INTRODUCTION

Cassia hirsuta Linn.[1] plant belongs to family- Caesalpiniaceae and commonly called as *Senna, hairy senna, khmer*. It is a native of Tropical America and distributed in Thailand, Malaysia, Indo- China, Asian & African Topics, California, Brazil, Mexico, Peninsula, North Australia and India. In India, it occurs in Garhwal region of Northern Himalayas. It is 150 cm in tall, rounded stem, flowers are sulphur colour. It has many medicinal properties like leaves used as herpes,

skin diseases and plant parts used as healing illness in man[2]. Plants are as a gifts of nature have many therapeutic properties and in chemotheraphy as valuable as the synthetic drugs, Stomach troubles, dysentery, rheumatism, fever etc. Seeds yielded a water soluble sugars extract[3] by usual manner as D-galactose and D-mannose in 1:4 molar ratio on paper chromatogram. Present manuscript mainly deals with the periodate oxidation studies of seeds galactomannan for the confirmation of polysaccharide structure which was obtained after methylation results. Peroidate oxidation reaction was done with sodium metaperiodate as oxidant and it was prepared by Malaprade[4] , Fluery & Lange's[5] method has been given a better method for more extensive use of periodic acid for the oxidation of glycols. Perlin[6] has given two important reagents as periodic acid and lead tetra acetate showed that the glycol groups under go cyclic ester formation with periodate oxidation and reaction considered to be dialdehyde type of the oxidation. Chatterjee[7], Kumar[8] and Sarkar[9] have used the periodate oxidation to determine the polysaccharide structure. The central atom of the oxidation reagent must be able to coordinate at least two hydroxyl group. After complete periodate oxidation reaction, polysaccharide consumed 1.13 moles of periodate oxidant and liberated 0.23 moles of formic acid per mole of anhydrohexose unit after 30 hrs.

MATERIALS AND METHODS

Periodate oxidation of galactomannnan were carried out by Abdel & Smith method[10] Seeds polysaccharide (600 mg) was oxidised with water (50 ml) and added sodium metaperiodate (0.124 M, 100 ml) then volume of reaction flasks was made upto 250 ml with water. Reaction flask was kept at 5-8 ^{0}C in refrigerator for 30 hrs. The periodate consumption and formic acid liberation were carried out by Fluery & Lange's methods[5]

The reaction of periodate consumption of polysaccharide were followed by Fluery & Lange's method. Aliquot (5 ml) was taken in iodine flask containing saturated borax solution (5 ml) then mixture diluted with water (50 ml). Boric acid (25 gm) and potassium iodide (40%, 20 ml) was added to the reaction mixture. Excess iodine liberated was titrated against sodium arsenite solution (0.12 N) using starch as an indicator. A blank titration was also carried out in a similar way. Difference between experiments and blank showed the periodate consumption of 1.13 moles after 30 hrs.

Formic acid liberated was carried out by Brown[11] and Halsall *et.al.*[12] Method. Aliquot (5 ml) were taken out from each flask in conical flask and ethylene glycol (5 ml) was added to destroy the excess of periodate present in the reaction mixture. Formic acid involved was titrated against sodium hydroxide solution (0.1 N) free from carbon dioxide using methyl red dye as an indicator near the end point. A blank titration was also carried out in a similar way. It liberated 0.23 mole of formic acid per mole of an hydrohexose unit after 30 hrs.

These two experiments were done at different time intervals. The titre values became constant after 30 hrs which corresponded to 1.13 mole of periodate consumption and 0.23 moles of formic acid liberation per mole of anhydrohexose sugars units and results are given in Table-1.

Table.1. Periodate oxidation of *Cassia hirsuta* Linn. seeds polysaccharide

S. No.	Sugars Present	Time (hrs)					
		5	10	15	20	25	30
1	Periodate consumption (moles/mole)	0.25	0.45	0.70	1.00	1.13	1.13
2-	Formic acid liberation (moles/mole)	0.06	0.10	0.16	0.20	0.23	0.23

RESULTS AND DISCUSSION

Seeds of *Cassia hirsuta* Linn. yielded a water soluble sugars extract as D-galactose and D-mannose in 1:4 molar ratio as determined by alkaline hypoiodite method and monosaccharides identified by paper chromatographic analysis. Periodate oxidation reaction of seeds polysaccharide were carried out for the confirmation of polysaccharide structure which was obtained after methylation studies. Periodate oxidation of polysaccharide was oxidised with sodium metaperiodate as oxidant by usual manner. It liberated 0.23 moles of formic acid per equivalent of polysaccharide with consumption of 1.13 moles of periodate for each anhydrohexose sugar units of the polymer after 30 hrs. Formic acid appear is to be originating from the reducing as well as non - reducing terminal units of the D-galactose and D-mannose are of (1→6)-α-type at non-reducing end while (1→4)-β-type at the main polymer chain between D-mannose residues. These linkages are also confirmed by free hydroxyl groups resulting in the consumption of periodate oxidation results found to be in a good agreement with the polysaccharide structure of *Cassia hirsuta* Linn. seeds. It is concluded from the above facts that probably one branch points occurs 5 repeating units of the galactomannan in the polysaccharide structure obtained after methylation results as shown in Figure- 1

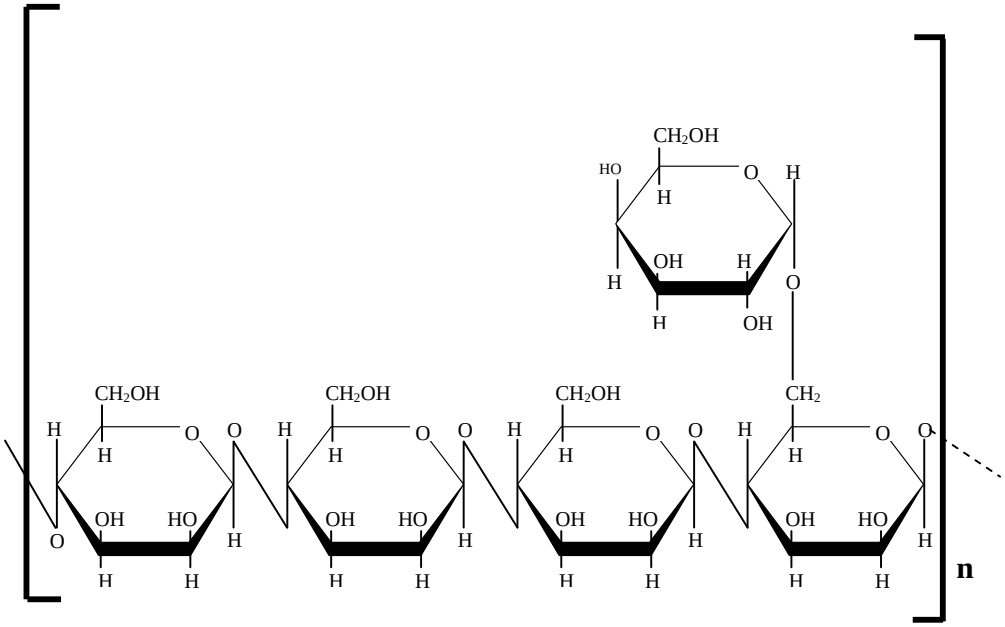

Fig.1. Polysaccharide structure of *Cassia hirsuta* Linn seeds galactomannan

Galactomannan of *Cassia hirsuta* Linn. seeds are commercially used in textile, sugars, backery, cosmetics, food, ice-cream, pudding industries and it is also explored in environment for air pollution minimizing capacity in the environment. Young pods and leaves are used for eaten purpose usually steamed or cooked in vegetable or in salads. Leaves are medically used for the treatment of herpes and a decoction of leaves used against irritation of skin in Thailand. Seeds are used as a substitute for coffee in Laos.

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