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SMITH DEGRADATION TECHNIQUE USED FOR THE IDENTIFICATION OF POLYALCOHOLS FROM PERIODATE OXIDIZED SEEDS POLYSACCHARIDE OF *CASSIA HIRSUTA* LINN. PLANT

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ABSTRACT

Polysaccharides was extracted from *Cassia hirsuta* Linn. seeds with water and precipitated with ethanol. On acid hydrolysis of polysaccharide with sulphuric acid and obtained hydrolysed compound was characterized by column and paper chromatographic analysis was found D-galactose and D-mannose in 1 : 4 molar ratio. Periodate oxidized polysaccharide was carried out on reduction with sodium borohydride by Smith degradation method followed by acid hydrolysis with sulphuric acid yielded polyalcohols as glycerol and erythritol in 1.02 : 3.65 molar ratio with traces of D-mannose on paper chromatogram. Derivatives of glycerol and erythritol were prepared by usual manner as: glycerol-tri-O-*p*-nitrobenzoate m.p. 187.2^{0}C and tetra-O-tosyl-erythritol, m.p. 163-164^{0}C. Absorbance of polyalcohols were recorded at 540 mμ in photoelectrocolorimeter for glycerol and erythritol, it indicated on branch point on the average of four sugar hexoses are in the main polymer chain while one hexose at the non-reducing end for the support of earlier proposed seeds polysaccharide structure of *Cassia hirsuta* Linn. plant.

Key words: Polyalcohols, Glycerol, Erythritol, Polysaccharides of *Cassia hirsute*.

INTRODUCTION

Cassia hirsuta Linn. plant[1,2] belongs to Caesalpiniaceae family and called as *Senna, Stiking, Cassia, Hairy Senna* or *Khmer*. It is native of Tropical America and occurs in foothills of Northern Himalayas, Garhwal region of Northern India, Malaysia, Peninsula, North & South America, Indo-China, Thailand, Asian & African Tropics, Brazil, California, New Mexico and North Australia. It is a perennial erect shrub upto 150 cm in height and its flowering periods from September to December and fruiting from November to January.

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Plant is used as green manure in Africa and planted as a shade plant in coffee plantation. Leaves are used for eaten purposes and medically used for treating herpes in Java and decoction of leaves used against irritation of skin diseases in Thailand. Plant paste are phytomedically used to healing illness in man[3]. It has many therapeutic properties and also in chemotherapy used as synthetic drugs. Medically it is used in stomach troubles, dysentery, rheumatisms, fever and skin diseases. In our earlier investigation, the nature of sugars are obtained from the water soluble seeds polysaccharide as D-galactose and D-mannose in the molar ratio of 1:4 moles, methylation studies to obtained the methyl sugars for the determination of tentative polysaccharide structure and then periodate oxidation studies for the confirmation of polysaccharide structure of *Cassia hirsuta* Linn. seeds. Present investigation mainly deals with the identification of polyalcohols of periodate oxidized polysaccharide by Smith degradation techniques[4] for the confirmation of proposed seeds polysaccharide structure of *Cassia hirsuta* Linn. plants.

EXPERIMENTAL

Polyalcohols sugars were separated from periodate oxidized compound by paper chromatographic analysis[5] on Whatman No. 3 MM filter paper with solvent mixture (v/v): (A) *n*-butanol, ethanol, water (4 : 1 : 5, upper phase)[6] and (B) ethyl acetate, pyridine, water (2 : 1 : 2, upper phase)[7] and used (R) acetonical silver nitrate, alcoholic sodium hydroxide as spray reagent for the detection of polyalcohols[8]. All evaporations were carried out under reduced pressure at 45-50^{0}C.

Identification of polyalcohols

Seeds polysaccharide (1.250 g) was oxidised[9] with sodium metaperiodate (0.125 M, 250 mL) at 5-8^{0}C in refrigerator for 48 hrs. It was further reduced[10] with sodium borohydride (1 g) at room temperature for 24 hrs. The excess periodate was destroyed by ethylene glycol (5 mL) to decompose the excess of periodate ions and reaction mixture was dialysed against running water for 60 hrs and concentrated to a syrup (100 mL). It was hydrolysed with sulphuric acid (1N, 100 mL) for 12 hrs at 100^{0}C on water-bath. The obtained hydrolysate was neutralized with barium carbonate slurry, filtered and filtrate was deionized by passing through the column of Amberlite ion-exchange resin[11] IR-120 (H^+) and IR-45 (OH^-) and finally concentrated to a syrup.

Characterization of polyalcohols

Hydrolysed compound was resolved into its components by paper chromatography on Whatman No. 3 MM filter paper sheet in solvent mixture (A) and used (R) as spray

reagent to revealed the presence of three spots of polyalcohols corresponding to glycerol, erythritol and D-mannose respectively. Component of sugars corresponding to the individual polyalcohols components were cut out with the help of guide spots and eluted with water according to the Dent's method[12]. The resulting solution was evaporated to obtained glycerol, erythritol and D-mannose which were characterized and identified as follows:

I. Glycerol: Syrup (400 mg) was dissolved in ethanol (50 mL) and decolourized with animal charcoal solution and then it filtered off. Filtrate was concentrated to a syrup and it moved single spot parallel to the authentic sample of glycerol on paper chromatogram. Residue (100 mg) was dissolved in pyridine (5 mL) and *p*-nitrobenzoyl chloride (3 g) then heated for 45 min at 70-75^{0}C. Reaction mixture was poured into ice cold solution of sodium bicarbonate to obtain a precipitate which was filtered off. On cooling the filtrate gave crystals of glycerol tri-O-*p*-nitrobenzoate derivative which were separated by filtration. It on recrystallization with acetone had m.p and mixed m.p. 187.2^{0}C, Lit. m.p. 191^{0}C[13] and 186-188^{0}C[14].

II. Erythritol: Syrup (900 mg) was treated with aqueous solution of animal charcoal, filtered and filtrate concentrated to a syrup. It moved a single spot on paper chromatogram corresponding to erythritol. It was again dissolved in ethanol (5 mL), on cooling the crystals of erythritol was obtained after recrystallization with ethanol had m.p. and mixed m.p. 119-120^{0}C, Lit. m.p. 191^{0}C[15], 117-118^{0}C[14] and 120-121^{0}C[16].

Erythritol syrup (250 mg) was dissolved in anhydrous pyridine (4 mL) and added *p*-toluene sulphonyl chloride (1.5 g). Reaction mixture was allowed to stand for 24 hrs at room temperature and it poured into ice cold water (50 mL). On cooling the derivative crystallized out and crystals were washed with water followed by ethanol gave tetra-o-tosyl-erythritol had m.p. and mixed m.p. 163-164^{0}C, Lit. m.p. 165-168^{0}C[16.]

III. D-mannose: Sugar syrup (50 mg) moved as a single spot of D-mannose in traces on paper chromatographic examination and had R_f 0.32 in solvent mixture (A). The spot is visible only in ultra-violet light.

Quantitative estimation of polyalcohols

Polyalcohols of *Cassia hirsuta* Linn. seeds polysaccharide was quantitatively estimated by chromotropic acid method[17]. Respective polyalcohols were separated by descending technique of paper chromatographic technique on Whatman No. 3 MM filter paper sheets in upper phase of the solvent mixture (B) and used (R) as spray reagent.

Polyalcohols components were cut out with the help of guide spots and eluted with water according to the Dent's method[12], produced glycerol, erythritol in the molar ratio of 1.02 : 3.65 and D-mannose (in traces). The colour intensity and absorbance were recorded at 540 mμ in photoelectrocolori meter and results ate given in Table 1.

Table 1: Absorbance of polyalcohols from *Cassia hirsuta* Linn. seeds polysaccharide at different concentration

S. No.	Amount of micrograms		Klett reading (absorbance) at 540 mμ	
	Glycerol	Erythritol	Glycerol	Erythritol
1	2.0	2.0	24	20
2	4.0	4.0	45	38
3	6.0	6.0	71	59
4	8.0	8.0	92	81
5	10.0	10.0	116	110

RESULTS AND DISCUSSION

Water soluble polysaccharide was extracted from *Cassia hirsuta* Linn. seeds by usual manner as D-galactose and D-mannose in 1 : 4 molar ratio. The periodate oxidized polysaccharide was reduced with sulphuric acid and sodium borohydride by Smith degradation method. It yielded polyalcohols as glycerol, erythritol in 1.02 : 3.65 molar ratio with traces of D-mannose by paper chromatographic analysis. Large proportion of erythritol released with acid hydrolysis of polyalcohols produced by sodium borohydride serves as evidence that the main polymer linkages are of (1 → 4)-β type with D-mannopyranose. Ratio of erythritol to the amount of glycerol was obtained due to the presence of D-galactose at the non-reducing end with (1 → 6)-α type linkages in the main polymer chain of the polysaccharide structure. It indicated one branching point on the average of the 5 hexoses units are in the main polymer chain and side chains of polysaccharide structure. Derivative of glycerol and erythritol was obtained by usual manner as glycerol-tri-o-*p*-nitrobenzoate while erythritol as tetra-o-tosyl-erythritol. Absorbance of polyalcohols were recorded in photoelectrocolorimeter on 540 mμ for glycerol and erythritol. It indicated one branching point on the average of four sugar hexoses units are in the backbone and one sugar hexose unit in non-reducing end for the support of earlier proposed polysaccharide structure of *Cassia hirsuta* Linn. seeds polysaccharide as shown in Fig. 1.

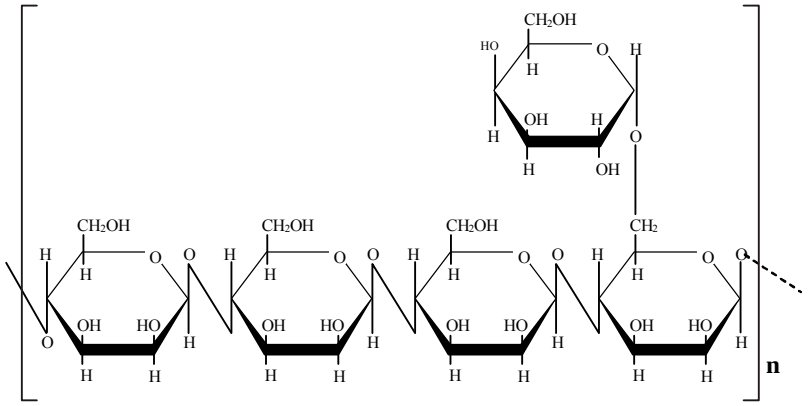

Fig. 1: Polysaccharide structure from *Cassia hirsuta* Linn. seeds galactomannan

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