



Polyalcohols from periodate oxidised seeds polysaccharide of *Cassia javanica* linn plant by smith degradation method

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Abstract

Water soluble seeds polysaccharide was extracted from *Cassia javanica* Linn. on acid hydrolysis with sulphuric acid and obtained hydrolysate on paper chromatographic analysis led to separation of D-galactose and D-mannose in 1:2 molar ratio. Purified seeds polysaccharide was reduced after periodate oxidation with sodium borohydride and sulphuric acid by Smith degradation method. The obtained hydrolysate produced polyalcohols as glycerol and erythritol in 1.00:2.05 molar ratio alongwith D-mannose by paper chromatography. The derivatives of polyalcohols were produced from seeds polysaccharide as glycerol-tri-O-*p*-nitrobenzoate and tetra-O-tosyl-erythritol. The absorbance of polyalcohols were recorded in photoelectrocolorimeter at 540m μ for glycerol and erythritol.

Keywords: polyalcohols, glycerol, erythritol, *cassia javanica* linn seeds polysaccharide

Introduction

Cassia javanica Linn. plant^[1] belong to Caesalpiniaceae family and commonly called as *Java-ki-rani*, is a medium to large size tree upto 25m in height and 3m girth. It occurs in Himalayan region of Northern India, Sumatra, Java, Borneo, Japan, Singapore and Malaysia. Plant is medically used in the Indigenous system of medicine for the treatment of diarrhoea, asthma, eye, skin disease and other human diseases. Leaves are also used as cattle fodder in hills. Seeds yielded a water soluble polysaccharide as D-galactose and D-mannose in 1:2 molar ratio by TLC, column and paper chromatographic analysis. In our earlier communications, the nature of seeds polysaccharide^[2], methylation studies^[3], periodate oxidation studies for the confirmation of seeds polysaccharide structure^[3] and structure elucidation of oligosaccharides^[4] have already been studied. Present manuscript mainly deals with the determination of polyalcohols from reduction of periodate oxidised seeds polysaccharide by Smith degradation method^[5] for the confirmation of proposed water soluble seeds polysaccharide structure of *Cassia javanica* Linn. plant. Recently the polyalcohols from seeds polysaccharide were determined from *Wrightia tinctoria* R.Br. (Roxb.)^[6], *Withania somnifera* Dunal^[7], *Cassia hirsuta* Linn.^[8], *Cassia auriculata* Linn.^[9] plant etc.

Materials and Methods

Separation of polyalcohol products

The polyalcohols sugar product were obtained from water soluble *Cassia javanica* Linn. seeds polysaccharide were separated from periodate oxidised hydrolysed compounds by descending technique of paper chromatographic analysis^[10] on Whatman No. 3 MM filter paper sheet. The following upper phase of the solvent mixture (v/v) were used as: (A) *n*-butanol-ethanol-water (4:1:5)^[11] and (B) ethyl acetate-pyridine-water (2:1:5)^[12] for the identification of polyalcohols. The spray reagent (R) acetonical silver nitrate, alcoholic sodium hydroxide^[13] was applied for the

detection of polyalcohols. All evaporation were carried out under reduced pressure at 45-50°C. The syrupy product yielded glycerol, erythritol and traces of D-mannose on paper chromatogram.

Identification of polyalcohols by Smith degradation method:

Purified water soluble seeds polysaccharide (1.0gm) was oxidized^[14] with sodium metaperiodate (0.125, 250ml) in dark for 48 hrs in refrigerator at 4-8°C. The obtained periodate oxidized compound was treated with ethylene glycol (5 ml) to decompose the excess of periodate ions and reaction mixture was dialysed against running water for 48 hrs then concentrated to a thin syrup (30 ml). The resulting solution was reduced^[15] by mechanical stirring with sodium borohydride (1.00 gm) at room temperature for 24 hrs. The excess sodium borohydride was acidified with glacial acetic acid (5 ml) and content was dialysed against running water then the solution was evaporated to dryness. The obtained residue was distilled with methyl alcohol to remove the borate ions as methyl borate. The borate free reduced product was again dialysed against running water for 48 hrs to remove the complete inorganic ions. It was concentrated to a thin syrup and further hydrolysed with sulphuric acid (1N, 10 ml) for 12 hrs on boiling water-bath. The hydrolysed product was neutralized with barium carbonate slurry with the help of mechanical stirrer then the reaction mixture left for 24 hrs. It was filtered off and obtained filtrate was deionised by Amberlite ion-exchange resins^[16], IR-120 (H⁺) and IR-45 (OH⁻) then concentrated to a thin syrup.

Characterization of Polyalcohols

The hydrolysed product of periodate oxidised water soluble *Cassia javanica* Linn. seeds polysaccharide was resolved into its components by descending technique of paper chromatographic separation method on Whatman No. 3MM filter paper sheets. The

solvent mixture (A) and used (R) as spray reagent to revealed the presence of three spots of polyalcohols corresponding to the glycerol, erythritol and D-mannose. The component sugar strips were cut out with the help of guide spots corresponding to the

authentic sample of polyalcohols. It was eluted with water according to the Dent's method [17], after evaporation of syrup which were characterized and identified as glycerol and erythritol as shown in Figure-1.

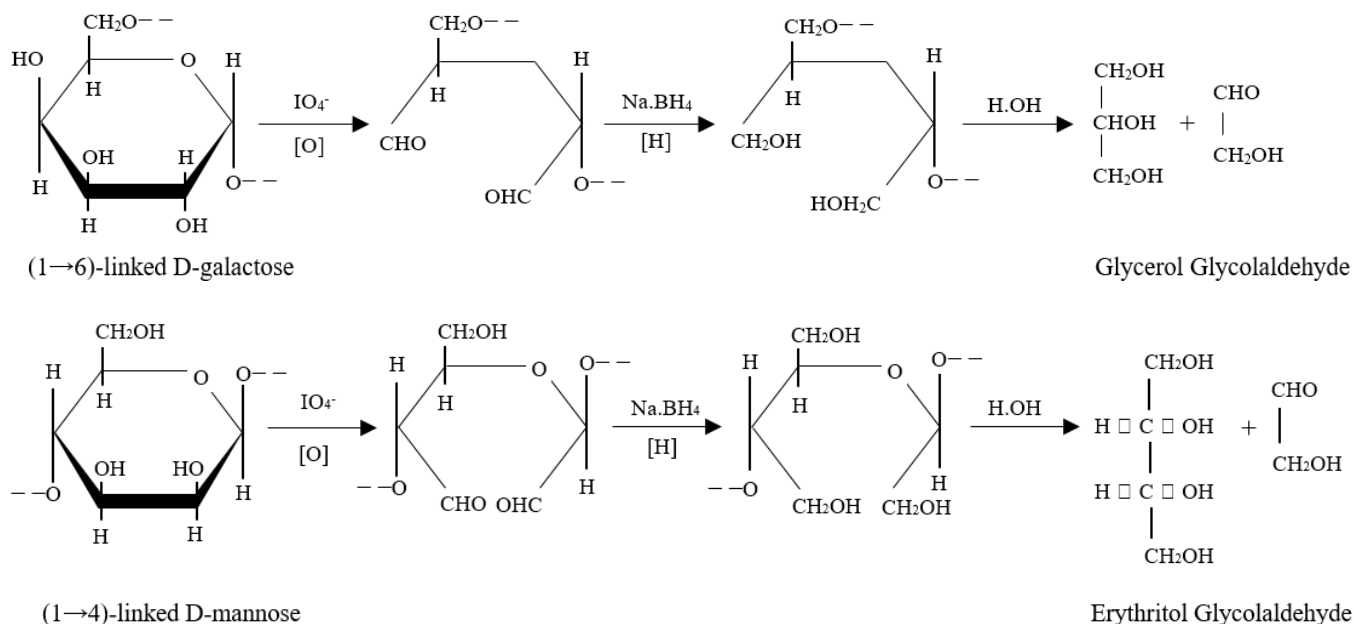


Fig 1: Smith degradation of polyalcohols from *Cassia javanica* Linn. Seeds polysaccharide.

Fraction-I: Glycerol

Sugar syrup (280 mg) was dissolved in ethanol (50 ml) and it decolourised with aqueous solution of animal charcoal (50 ml) for 24 hrs then filtered off. The filtrate was concentrated to syrup and it moved a single spot on paper chromatogram corresponding to the authentic sample of glycerol. The derivative was prepared by dissolving the residue (350 mg) in pyridine (5 ml) and *p*-nitrobenzoyl chloride (2.4 gm) then the content was heated for 1 hr at 70-75°C. The reaction mixture was poured into ice-cold solution of sodium bicarbonate to obtain a precipitate which was filtered off. The filtrate gave crystals of glycerol-tri-*O*-*p*-nitrobenzoate derivative were obtained on cooling the reaction mixture, which were separated by filtration. It on recrystallization with acetone, had m.p. & mixed m.p. 188-189°C, Lit. m.p. 186-188°C [18] and 190-191°C [19].

Fraction-II: Erythritol

Sugar syrup (850 mg) was treated with aqueous solution of animal charcoal (50 ml) for 20 hrs, then filtered and filtrate concentrated to a syrup. It moved a single spot on paper chromatogram corresponding to the authentic sample of erythritol. It was again dissolved in ethanol (5 ml), on cooling the crystals of erythritol was obtained after recrystallization with ethanol then filtrated off. It had m.p. and mixed m.p. 118-119°C, Lit. m.p. 117-118°C [18], 121-122°C [20] and 119-120°C [21]. Derivative of erythritol syrup (200 mg) was prepared by dissolving it in anhydrous pyridine (5 ml) and *p*-toluene sulphonyl chloride (1.5 gm) at room temperature for 20 hrs. The

content was poured into ice-cold water (50 ml) to Crystallised out the needle shaped derivative of erythritol. The crystals were washed with water followed by ethanol were dried in air. On recrystallization with acetone and ethanol mixture gave tetra-*O*-tosyl-erythritol, had m.p. & mixed m.p. 164-165°C, Lit. m.p. 166-168°C [20].

Fraction-III: D-mannose

Sugar syrup (50 mg) was moved as a single spot on paper chromatogram parallel to D-mannose. It was obtained in traces, which have R_f values 0.11 and 0.32 in solvent mixture (A) and (B) respectively were identified as D-mannose. The sugar spots of D-mannose is visible only in ultraviolet light.

Quantitative estimation of polyalcohols

Polyalcohols obtained from water soluble seeds polysaccharide of *Cassia javanica* Linn. were quantitatively estimated by chromotropic acid method [22].

The respective polyalcohols were separated by descending technique of paper chromatographic examination [10] on Whatman No. 3 MM filter paper sheet in upper phase of 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 [17], producing glycerol and erythritol in 1.00:2.05 molar ratio alongwith traces of D-mannose.

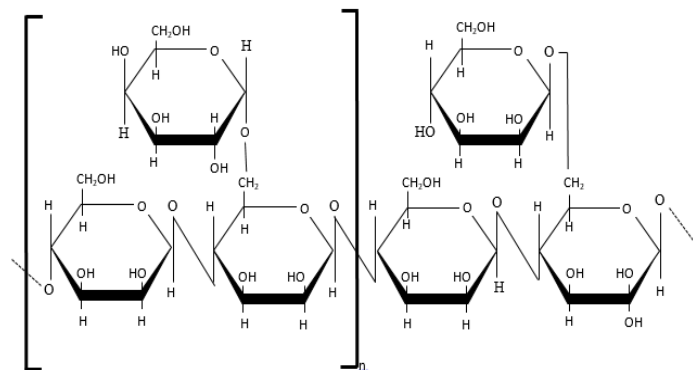
The colour intensity and absorbance were read at 540 mμ in photoelectrocolorimeter and results are given in Table-1.

Table 1: Absorbance of polyalcohols from *Cassia javanica* Linn. seeds polysaccharide.

S. No.	Amount in micrograms		Klett reading (Absorbance at 480 m μ)	
	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	101

Results and Discussion

Cassia javanica Linn. Seeds yielded a water soluble seeds polysaccharide by usual manner as D-galactose and D-mannose in 1:2 molar ratio on paper chromatogram. Periodate oxidised seeds polysaccharide was reduced with sodium borohydride and sulphuric acid by Smith degradation method. It yielded polyalcohols as glycerol and erythritol in 1.00:2.05 molar ratio alongwith traces of D-mannose by paper chromatographic analysis. The large proportion of erythritol was released by acid hydrolysis of polyalcohols, produced by sodium borohydride serves as evidence that the main polymer linkages are of (1 \rightarrow 4)- β -type with D-mannopyranose units. The ratio of erythritol to the amount of glycerol was obtained due to the presence of D-galactose at the non-reducing end with (1 \rightarrow 6)- α -type linkages in the main polymer chain of the polysaccharide structure. It indicated one branching point on the average of four hexose unit in the main polymer chain two hexose unit are in side chain in polysaccharide structure as shown un Figure-2. Derivative of glycerol was obtained by usual manner as glycerol tri-O-*p*-nitrobenzoate while erythritol as tetra-O-tosyl-erythritol. The absorbance of polyalcohols was recorded in photo electrocolori meter at 540 m μ for glycerol and erythritol. Polysaccharide structure showed one branch point on the average of two hexoses units are in the backbone and one hexoses unit in the non-reducing end for the support of the earlier proposed seeds polysaccharide structure of water soluble *Cassia javanica* Linn. Seeds polysaccharide as shown in Figure-2.

**Fig 2:** Seed polysaccharide structure of *Cassia javanica* Linn Plant

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