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Polyalcohols from periodate oxidised seeds polysaccharide of *Cassia glauca* Lam. plant by smith degradation method

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Abstract
Water soluble seeds polysaccharide was extracted from *Cassia glauca* Lam. seeds on acid hydrolysis with sulphuric acid and obtained hydrolysate on paper chromatographic analysis led to separation of D-galactose and D-mannose in 1:4 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, erythritol and traces of D-mannose in 1.00:3.60:0.50 molar ratio 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 540mμ for glycerol and erythritol.

Keywords: polyalcohols, glycerol, erythritol, *Cassia glauca* Lam. seeds polysaccharide

Introduction

Cassia glauca Lam. Plant [1] belong to Caesalpiniaceae family, is a medium to large size evergreen shrab up to 10m in height. It occurs in Himalayan region of Northern India, Japan, Nepal, Tropical Asia, Pakistan, Malaysia, Sri Lanka, Thailand and South Australia. Plant is medically used in the Indigenous system of medicine for the treatment of diarrhoea, asthma, and other human diseases. Ripe seeds are greedily eaten by birds [2] and Bark is extensively employed for diabetes Folk medicine. Leaves are also used for the treatment of blennorrhogia [3]. Seeds oil are used in Indigenous system of medicine for the treatment of skin and leucoderma diseases. Plant is also a good pollution tolerance and reduces chemical pollution from atmosphere [4]. Seeds yielded a water-soluble polysaccharide as D-galactose and D-mannose in 1:4 molar ratio on paper chromatogram. In our earlier communications, the nature of seeds polysaccharide [5], methylation studies [6], to obtained methyl sugars for the determination on proposed seeds polysaccharide structure and structural elucidation of oligosaccharides [7] have already been studied. Present manuscript mainly deals with the determination of polyalcohols from reduction of periodate oxidised seeds polysaccharide by Smith degradation method for the confirmation of proposed water-soluble seeds polysaccharide structure of Cassia glauca Lam. plant. Recently the polyalcohols from seeds polysaccharide were determined from Wrightia tinctoria R.Br. (Roxb) [9], Withania somnifera Dunal [10], Cassia auriculata Linn [12], Quercus incana Roxb. [13] Cassia javanica Linn. [14] plant etc.

Materials and methods

Separation of polyalcohol products:
The polyalcohols sugars were obtained from water soluble Cassia glauca Lam. seeds polysaccharide and separated from periodate oxidised hydrolysed compounds by descending technique of paper chromatographic analysis [15] 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) [16] and (B) ethyl acetate-pyridine-water (2:1:2) [17] for the identification of polyalcohols. The spray reagent (R) acetonical silver nitrate, alcoholic sodium hydroxide [18] was applied for the detection of polyalcohols. All evaporation were carried out under reduced pressure at (40-450C). The syrupy product yielded glycerol, erythritol and traces of D-mannose on paper chromatogram.

Identification of polyalcohols by Smith degradation method of periodate oxidised seeds polysaccharide

Purified water-soluble seeds polysaccharide (1.0gm) was oxidized [19] with sodium metaperiodate (0.125, 250ml) in dark for 50 hrs in refrigerator. The obtained periodate oxidized compound was treated with ethylene glycol (5 ml) to decompose the excess of periodate and reaction mixture was dialysed against running water for 48 hrs. It was-concentrated to a thin syrup (30 ml). The resulting solution was reduced [20] by mechanical stirring with sodium borohydride (1.00 gm) at room temperature for 12 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 [21], 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 glauca Lam. 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 [22], after evaporation of syrup which were characterized and identified as glycerol, erythritol and D- mannose and reaction as shown in Figure-1.

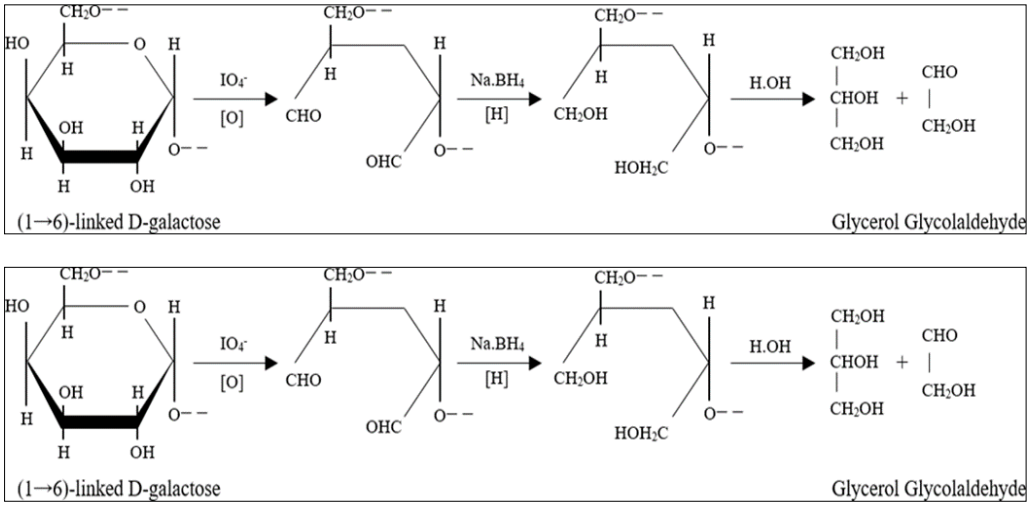

Fig 1: Smith degradation of polyalcohols from Cassia glauca Lam. seeds polysaccharide

Fraction-I: Glycerol

Sugar syrup (350 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 of glycerol (320 mg) was prepared by dissolving the residue (300 mg) in pyridine (5 ml) and p- nitrobenzoyl chloride (2.5 gm) then the content was heated for 1 hr at 70-750C. 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. and mixed m.p. 188-1900C, lit. m.p. 186- 1880C [23].

Fraction-II: Erythritol

Sugar syrup (860 mg) was treated with aqueous solution of animal charcoal (50 ml) for 24 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. 118-1190C, Lit. m.p. 117-1180C [23] and 120-1220C [24].

Derivative of erythritol syrup (200 mg) was prepared by dissolving it in anhydrous pyridine (5 ml) and p-toluene sulphonyl chloride (1.5 gm) Then the reaction mixture was left 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. and mixed m.p. 164-1650C, Lit. m.p. 166- 1680C [24].

Fraction-III: D-mannose

Sugar syrup (50 mg) was moved as a single spot-on paper chromatogram parallel to D-mannose. The spot of D-mannose is visible only in ultraviolet light. It had Rf. values 0.11 and 0.32 in solvent (A) and (R) used as spray reagents for the detection of sugars and it is identified as D-mannose which was obtains in traces as D-mannose sugar.

Quantitative estimation of polyalcohols

Polyalcohols obtained from water soluble seeds polysaccharide of Cassia glauca Lam. were quantitatively estimated by chromotropic acid method [25]. The respective polyalcohols were separated by descending technique of paper chromatographic examination [15] 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 [22], producing glycerol, erythritol and D-mannose is 1.00: 3.60: 0.50 molar ratio. The colour intensity and absorbance were read at 540 mμ in photoelectrocolorimeter and results are given in Table-1.

Table1: Absorbance of polyalcohols from Cassia glauca Lam. seeds polysaccharide

S. No.	Amount in micrograms		Klett reading (Absorbance at 480 mμ)	
	Glycerol	Erythritol	Glycerol	Erythritol
1.	2.0	2.0	25	20
2.	4.0	4.0	46	39
3.	6.0	6.0	72	60
4.	8.0	8.0	96	82
5.	10.0	10.0	118	103

Results and discussion

Cassia glauca Lam. seeds yielded a water-soluble seeds polysaccharide by usual manner as D-galactose and D-mannose in 1:4 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, erythritol and D-mannose in 1.00: 3.60: 0.50 molar ratio 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→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→6)-α-type linkages in the main polymer chain of the polysaccharide structure. It indicated one branching point on the average of four hexoses sugar unit are in the main polymer chain and one hexose sugar unit in non-reducing end for the support at the earlier proposed seed polysaccharide structure of Cassia glauca Lam. 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 electrocolorimeter at 540 mμ for glycerol and erythritol.

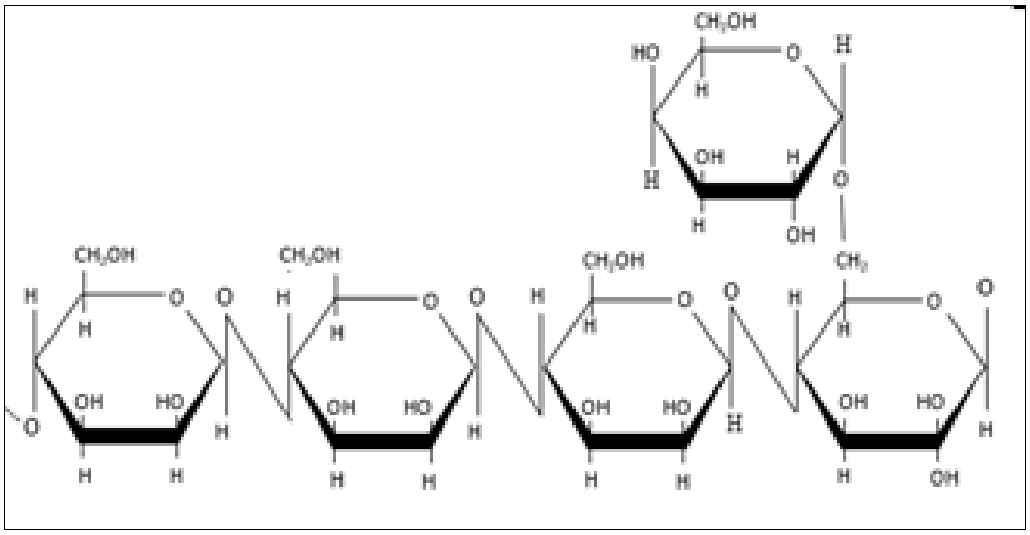

Fig 2: Seed polysaccharide structure of Cassia glauca Lam. plant

References

1. Chopra RN, Nagar SL, Chopra IC. Glossary of Indian Medicinal Plants, CSIR Pub., New Delhi (India),1956:1:54-55.
2. Kirtikar KR, Basu BD. Indian Medicinal Plants, Periodical Experts, New Delhi (India),1999:2:2333-2334.
3. Kirtikar KR, Basu BD. Indian Medicinal Plants, Allahabad (India),1944:4:1139.
4. Warrier PK, Nambiar VPK, Ramankulty CJ. International Medicinal Plants, Orient Langman Pvt. Ltd, Hyderabad (India), 1996, 423-424.
5. Singh RB. International Journal of Multidisciplinary Sciences SE RBD,2018:(1):01-03.
6. Singh RB, Jindal VK. Acta Ciencia Indica Chemistry,1989:15-C(I):37-4.
7. Singh RB. Acta Ciencia Indica Chemistry,1992:18(3):205-208.
8. Smith F, Chem J. Soc.,1940:47:1035-1038.
9. Singh RB. International Journal of Ayurveda and Pharmaceutical Chemistry,2014:1(2):124-129.
10. Singh RB. Advances in Applied Science Research, Pelagia Research Library,2013:4(1):71- 73.
11. Singh RB. International Journal of Chemical Sciences,2012:10(2):793-798.
12. Singh RB. International Journal of Academic Research and Development,2020:5(1):09-11.
13. Singh RB. International Journal of Chemistry Studies,2020:4(2):25-27.
14. Singh RB. International Journal of Chemical Research and Development,2020:2(1):20-23.
15. Partridge SM. Nature (London), 158: 270-274 (1946).
16. Partridge SM, Westall RG, Biochem J.,1948:72:238-241.
17. Jermyn MA, Isherwood FA, Biochem J.,1949:44:402-405.
18. Trevelyan WE, Procter DP, Harrison JS. Nature (London),1950:166:444-446.
19. Abdel AM, Hamilton JK, Montogomerg R, Smith FJ. Amer. Chem. Soc.,1952:74:4970-4973.
20. Morimoto LJY, Unrau CJ, Unrau AMJ. Agri. Food Chem,1960:10:134-136.
21. Adams BA, Halmes ELJ, Soc Chem. Ind.,1955:54:17-20.
22. Dent CE, Biochem J.,1947:41:240-243.
23. Unrau AM. J. Org. Chem., 1961:26:3097-3101.
24. Hamilton JK, Smith FJ, Amer Chem. Soc.,1956:75:5907-5910.
25. Lambert M, Neish A, Can J. Res.,1950:28-B:83-86.