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E: ISSN NO.: 2455-0817 *Remarking An Analisation*

Studies on the Structure of Polysaccharides occurring in The Seeds of Plants Cassia Emerginata Part-I (Hydrolytic Studies)

Abstract

Cassia Emarginata belong to the family leguminance and sub family caesalpinaceae. It was originated from tropical America and was introduced later into many tropical countries like India, Java, Sumatra etc, for its beauty. It seeds are rich in water soluble mucilage (absent 46%) the pure Polysaccharides was white amorphous powder with negligable ash content (0.52%) $[\alpha]^{31}_{D}$ 29.05 (NaOH). Pure polysaccharides content 1.816% furfural 3.569% Pentosans 3.145%, Pentose, Nitrogen, Sulphur, Halogen Methoxy and anhydrourenic acid were found to be absent. The I.R. spectrum showed a broad bond for (-OH, 3400 cm^{-1}) alongwith other bonds at 2850, 2300, 880, 800 cm^{-1} molecular weight of Polysaccharides 11983. On complete acid hydrolysis polysaccharides given D-galactose and D-Mannose in the 2:5 ratio. On gladed hydrolysis with dilute acid give two disaccharides two trisaccharides and one tetra saccharides.

Keywords: Polysaccharides, Structure, Solubility, Confirmation, Solution behaviour.

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Introduction

Cassia Emerginata belongs to the family Leguminance and subfamily ceasalpinaceae. Cassia is an old greak name of disscorides in allusion to the beauty of the tree when it is in full bloom. It is deciduous tree reaching about 35 to 40 feet in height. It was originated from tropical America and was introduced later into many tropical countries like India, Java, and Sumatra etc. for its beauty. It is ornamental, tree. The pulp of fruit is used as a purgative; seeds are almond shaped and their length ranging from 1.25 to 1.75 cms. The Preliminary analysis of seeds of the cassia-emarginata showed following characteristics.

Total weight of 100 seeds is 35.49 gm.
Moisture content 9.60%
Ash Contest 3.90%
Endo Sperm content 46%
Proteinous Matter content 7.6%

Isolation and Purification of Cassia Emarginata Seed Polysaccharides

Matured seeds of Cassia Emarginata were cleaned in a laboratory grinder and endospermic materials was separated by winnowing-

The poured in water for 24 hours, water soluble extract was acidified with 4N acetic acid and then 4 times ethanal was added. When a white flocutant precipitate was obtained. This process was repeated to four-five times to get finally to white grannular sample of the Polysaccharides. It was further purified by deionisation of its aqueous solution by simultaneous treatment with freshly regenerated duolite C-25 and Duolite A-7 ion exchange resins. The amorphous powder of pure polysaccharide thus obtained has negligible ash contednt-0.52% specific rotation of polysaccharides was found $[\alpha]^{31}_{D}$ + 29.05 (NaOH). It did not reduce Fehling solution on further analysis it was found to contain furfural 1.816% (estimated as phloroglucide by distilling the Polysaccharide with 13% hydrochloric acid, Pentosans 3.569% and Pentoses 3.145% (Calculated from the furfural value). Nitrogen, sulphur, halogen, methoxyl and anhydrouronic acid were found to be absent in Polysaccharides. The I.R spectrum was taken in KBr (Potassium Bromide) Pallets showed a

broad band for (-OH 3400 cm^{-1}) alongwith other bands at 2850, 2300, 880, 800 cm^{-1}. Paper Electrophoresis techniques was used 1, 2 for judging the homogeneity of a Polysaccharides. The electrophoretis experiments were performed with 0.5% solution of pure Polysaccharides in 0.05M sodium tetra borate buffer (P^H 9.2) using laboatorium Felzenclsk Gyara apparatus type DE 201. Electrophoretic pattern showed that Polysaccharide migrated as a single spot. The calculation of ionic mobility (μ) of the Borate complex indicates a value of $0.24x10^{-5}$ $cm^2volt^{-1}sec^{-1}$. The molecular weight of homogenous sample of Polysaccharides had been found by adopting sedimentation velocity method by using following formula.

$$M.W = \frac{RTS}{D}(1 - \frac{\delta}{d})$$

Where S is sedimentation coefficient (1.346×10^{-13}) (Svedberg unit) D is diffusion coefficient (7.14×10^{-7})) δ is partial specific volume (0.5929) and d is density molecular weight of the pure polysaccharides was calculated to be in order of 11983.

Hydrolytic studies of Polysaccharides

Complete hydrolysis of Polysaccharide was done by heating Polysaccharides with sulphuric acid (2N) for 24 hrs on boiling, water bath, resulted in a mixture of neutral Sugars. Preliminary chromatographic examination of mixture revealed the presence of two sports corresponding to D-glactose and D-Mannose. Fractionation of mixture cellulose Column 3-4 using n-butanol half saturated with water as elvant^{+5}. The first-sugar was identified as D-Mannose from its mp 132^0C optical rotation $(x)^{32}_D$ + 16.3% and by preparing P-Nitro–N, Phenyl D-Mannosty amine m.p-210-11^0C and second sugar identify as D-galactose from its m.p-167^0C and optical rotation $[\alpha]^{32}_D$ + 79.6 and by preparing p-Nitro, N-Phenyl D-Mannose amine m.p.-205^0C. The Polysaccharide had been subjected to quantities hydrolysis with 2N sulphuric acid for 24 hrs. in a sealed tube. D-Galactose, D-monmosyl after being separated by paper chromatography was estimated by periodate oxidation according to the procedure of Hirst. It was absorbed that D-galactose, D-Mannose were present-in the ratio of 2:5.

Aim of the Study

Studies on the structure of the Polysaccharides occurring in the seeds of plants Cassia Emerginata

Isolation of Oligosaccharides

The Polysaccharides upon hydrolysed with dilute Sulphuric acid (0.1N) for 8 hrs. Furnished a mixture of Neutral (five) oligosaccharides along with D-mannose and D-galactose. Solution of mixture into its different component was first attempted on charcoal celite column, Appropriating tractions were them combined and concentrated to a syrup which was finally resolved into its compounds by paper chromatographically on whatmann no3 mm filter paper sheets, thus five oligosaccharide could be obtain in an homogenous state. Resolution of the syrup into its component-sugar was carried out by adsorbing the syrup on a column (50x2.0 cms) of charcoal celite (1:1) w/w. The column was first eluted with water to remove monosaccharides, mannose and galactose. Subsequently the column was washed with water proportions of Ethanol and differed–fraction (100 ml) each fraction were examined paper chromatographically and result were tabulated below.

Fractions No.	Eluant	Volume of Eluant (ml)	Oligosacchaside (Raellobiose)
1-34	0.5% Ethanol	3.40	1.31, 1.18, 0.06
35-64	5% Ethanol	3.00	0.60, 0.82, 0.16,
65-85	15% Ethanol	2.00	0.52, 0.01

The mixture of Oligosaccharides was finally resolved into its compounds by chromatography on whatmann no.3 mm filter paper sheets. Degree of Polymerization of oligosaccharides were determined according to the method of peat wheldan and Robert's. Degree of polymerisation of the oligosaccharides were found 2.167 (s_1) 2.154 (S_2) 3.22 (S_3) 3.101 (S_4) and 4.259 (S_5)

Characterisation of Oligo Saccharides

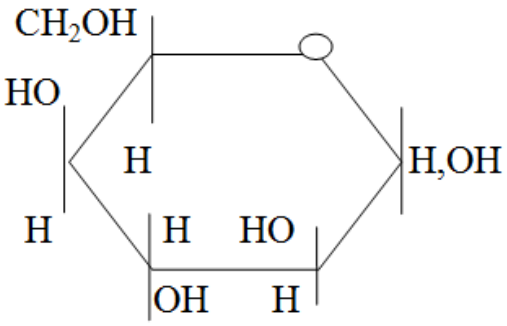
S_1 was characterised as man P(1-4) Man P by complete hydrolysis, periodate oxidation method (methyl glycoside of disaccharides (S_1) Consumed 3 Moles of periodate and liberate/mole of formic acid.

S_2 was characterised Gal-P(1-6) Man-P by complete hydrolysis, periodate oxidation methods (methyl glycoside of disaccharides (S_2) Consumed 4 Mole of periodate and liberate/mole of formic acid.

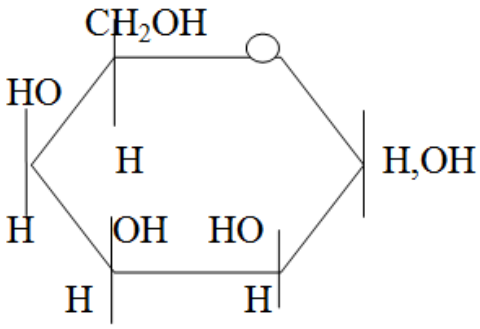
S_3 was characterised Gal-P(1-6) Man-P(1-4) Man-P by complete hydrolysis, periodate method (methyl glycoside of trisaccharides (S_3) Consumed 5 Moles of periodate and 2 moles of formic acid.

S_4 was characterised as man P(1-4) Man-P (1-4) Man-P by complete hydrolysis, periodate oxidation method (methyl glycoside of saccharides (S_4) Consumed 4 Moles of periodate and liberate/mole of formic acid.

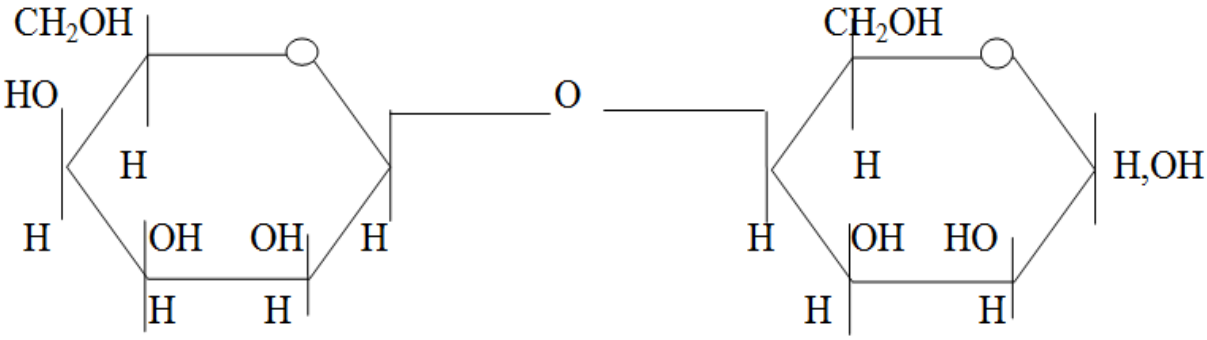
S_5 was characterised as man P(1-4) Man P (1-4) man-p (1-4) map p by complete hydrolysis, periodate oxidation method (methyl glycoside of tetrasaccharides (S_5) Consumed 5 Moles of periodate and 1 moles of formic acid.



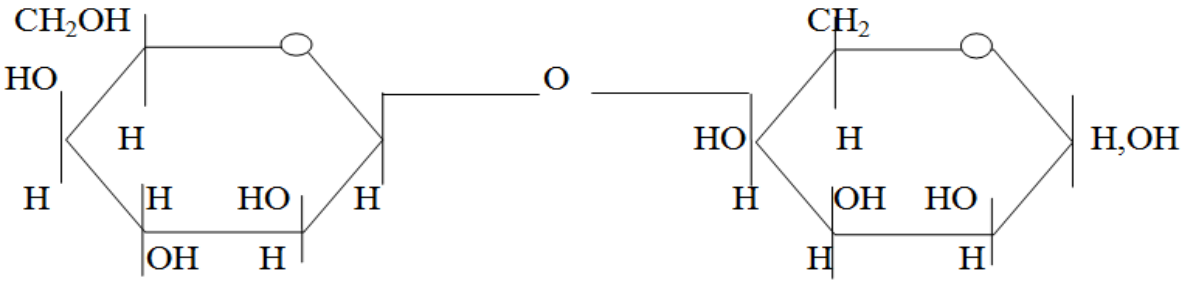
D-Galacotose



D-Mannose



(S_1)



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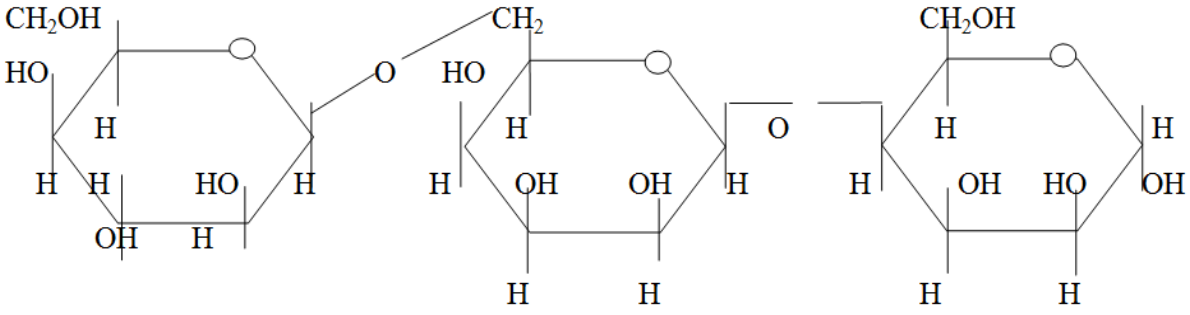

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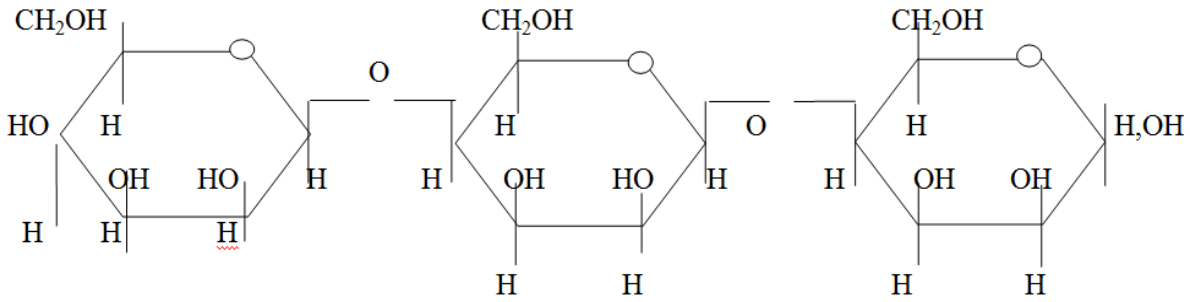

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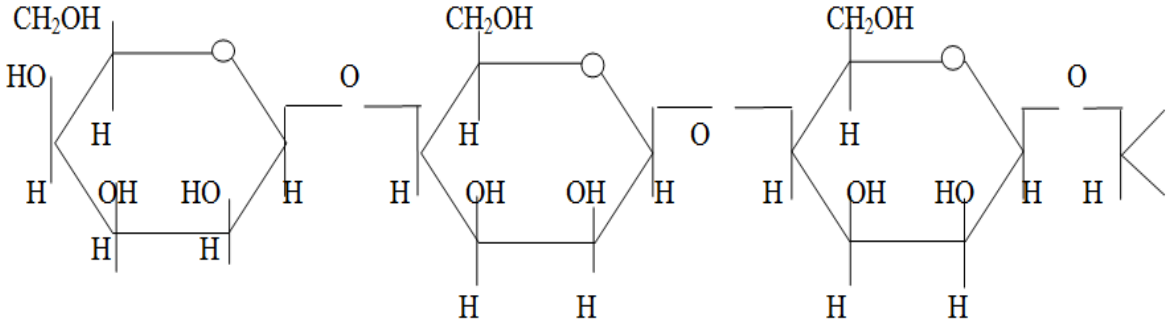

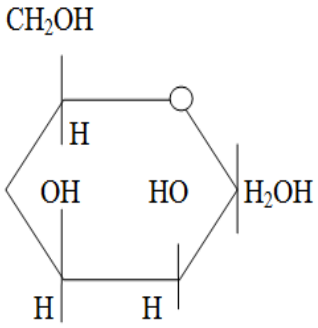

(S_5)

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