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Extraction of Natural Product from Plant Source from Cassia Glauca

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ABSTRACT

Having D-galacto-D-mannan constitute in structure and in molar ratio of 1:2, a new polysaccharide has been isolated from ripe seeds of Cassia glauca[1] Hydorlysis of methylated polysaccharide gave 2,3,4,6 tetra O methyl-D-galactose, 2,3 di- O methyl-D mannose and 2,3,6, tri-O- methyl D- mannose in the molar ratio of 1:1:1 respectively. Both periodate oxidation and methylation studies indicated 32.84% of end group. This shows similarity with the structure of polysaccharide obtained from Guar gum. The above findings together with date of oligosaccharide formed on partial acid catalysed hydrolysis showed the galacto-mannan to consist of a linear chain (14) linked-ß- mannopyranosyl residues some of which are substituted at 6 positions by galactopyranosyl unit, glycosidically. This polysaccharide in non-ionic in nature & having presence of D-glactose in the peripheral position.

Keywords- Extract of seed gum, acidic hydrolysis, methylation periodate oxidation oligosaccharide.

I. INTRODUCTION

Majority of plants of Cassia Glauca[2] genus are reputed for their therapeutic value. A Galactomannan was isolated from crushed; defated and decolurised seed with 1.2% aqueous acetic acid and precipitated with 4 Vol of ethanol. It is purified by repeated precipitation deproteinisation with chloroform complexation with Fehling's solution. Homogeneity of the galactomannan was verified by fractional precipitation, zone electrophoresis and via acetylation and deacetylation, the dry polysaccharide $(\alpha)_d^{25}+40^0$ (water) water soluble at room temperature and had negligible methoxyl Acetyle and Uronic acid contents. One complete hydrolysis os galactomannan yilded D-galactose and D-mannose in the molar ratio 1:2 when graded hydrolysis of galactomannan was monitored by paperchromatography. D- galactose was found to appear first followed by D-mannose suggesting presence of D- mannose in the main chain and linked D- galactose units at periphery as end groups.

Experiment of chemical analysis of non-ionic gum.

To determine the position of linkages between the building units of the galactoman, it was exhaustively methylation by conventional Haworth-Purdie method, to afford a brown mass $(\alpha)_d^{25}+40.5^0$ (chloroform) Hydrolysis of the methylation product and quantitative analysis of the methylated sugars with alikaline hypoiodite gave 2,3,4,6 tetra- O-methyl D-galactose (1 mole) 2,3, 6-tri-o-methyl-D-mannose (1 mole). The identity of these methylated monosaccharide was established on the basis of their R_{TMG} values and optical rotation. The percentage of end groups calculated from methylation studies was 32.84% Oxidation of polysaccharides with metaperiodate indicate that end groups is 32.85% (cf.methylation).;

Acid catalysed partial hydrolysis of the seed gum gave disaccharide and trisaccharides Along with the component sugars. All the small oligosaccharide are characterized. These results corroborated in the earlier findings. The forgoing data accord with the following structure.

4- β-D Manp (1 → 4-β-D Manp (1→4)—β-D Mannp (1 → 4)- β-D Manp (1)n

II. EXPERIMENTAL

Paper chromatography was conducted on whatman no. 1 and 3 papers by descending technique using the following systems U/V Solvent A>1-Butanol-Ethanol – Water (5:1:4)[3] Solvent B) 1- Butanol, -isopropanol-Water (2:1:2)[4] Solvent C 1-Butanol-isopropanol-Water (11:6:3)[4], Solutions were concentrated at diminished pressure and at low temperature. All residues were dried in vacuum over anhydrous calcium chloride, melting points are uncorrected and values are for equillbria.

Dried and crushed seeds (1 kg) were successively extracted with light petroleum ether and ethanol to remove fatty and colouring substances respectively. Polysaccharide was extracted with 1% aqueous acetic acid and precipitated with 90% ethanol. It was purified by repeated deproteinisation[6] with chloroform and complication with Fehling's solution[7]. Homogeneity was tested by fractional precipitation[8], zone electrophoresis[9] and via acetylation[10] and deacetylation[11]. The pure polysaccharide was hydrolysed with 2N sulphuric acid, and the hydrolysate was fractionated by paper chromatography. (solvent-C) on preparative scale. Quantification[11] by periodate oxidation showed the molar ratio of 1:2 between D- galactose and D-Mannose.

Graded hydrolysis 12 hours with 25 mm sulphuric acid (monitored by Paper chromatography solvent-C) resulted in the favoured removal of D-galactose (20 min.) followed by D-mannose 30 min.

III. RESULT AND DISCUSSION

Polysaccharide was echausive methylated first by Haworth's method[13] and then by purdies method[14] completely methylated polysaccharide $[\alpha]_d^{28}$ + 41^0 (chloroform) was hydrolysed[15] and the hydrolysate was fractionated (paper chromatography_solvent-A) on preparative scale using whatman No.3 paper. The resulting methylated sugars were quantified by alkaline hypoiodite method[16] gives following.

1. 2,3,4,6-tetra-o-ine thy 1-D-galactose (3mol.) R_{TMG} (Solvent-A), 0.87, m.p. 72.730 $[\alpha]_D^{32}$ +120^0 (water) The anilide had m.p. 192-193^0 $[\alpha]_d^{32}$ + 43^0) acetone).
2. 2,3,-di-o-nmethy 1-D-mannose (3 mol): R_{TMG} (solvent-A)-0.52, syrup
3. $[\alpha]_D^{28}$ +14^0 (water) the anilide had m.p. 136^0.
4. 2,3,6-tri-o-methyl 1-D-mannose (4 mol): RTMG (solvent-A) 0.80m syrup
5. $[\alpha]_D^{28}$-11^0.

The derived phenylhydrazide had m.p. 130^0. Polysaccharide (380) was oxidized with 25 ml of 0.25 M sodium metaperiodate by the method of Andrews et al 1.02 mole of periodate were consumed with the simultaneous liberation of 0.202 mole of formic acid per 100 g of the polysacharids.

The polysaccharide (6.0g) was partially hydrolyzed with 50 mm sulphuric acid for 12h at 100^0 and the hydrolysate was examined by paper chromatography (solvent B) Following oligosaccharides were detected. These oligosaccharides were separated and identified.

1. Epimelibiose α-D Galp (1 ⟶6) β-D $Manp_D^{22}$ m.p. 200^0 + $[\alpha]_D^{25}$+120^0 water, phenyl osazsone m.p. 173^0, acid hydrolysis gave paper chromatography (solvent-B) galactose and mannose, and methylation followed by hydrolysis gave (solvent-A) 2,3,4, 6-tetyra-O- Methyl galactose and 2,3,4- tri-O methyl-D-mannose.
2. Mannobiose : β-D-Manp (1 ⟶4) β D-$Manp^{23}$, : M.P. 203-205^0 (from enthanol) $[\alpha]_D^{25}$-9^0 (water) The derived phenylosazone had m.p. 204^0. Acid hydrolysis gave mannose only paper chromatography (solvent-c) and emulsion hydrolysed the disaccharide, indicating β-Linkage, Methylation followed by hydrolysis gave paper chromatography (solvent-A) 2,3,4,6- tatra-O- methyl, and 2,3,6. tri-o-methyl-D- Mannose.
3. Mannotrioses β-D- Manp (1 ⟶ 4) - β-D Manp (1 ⟶ 4) D-Manp $[\alpha]_D^{25}$ m.p. 161- 163^0 (from ethanol) $[\alpha]_D^{25}$ -23^0 (water) acid hydrolysis gave paper chromatography (solvent-c) mannose only and partial hydrolysis with acid gave paper chromatography (solvent-c) mannobiose and mannose, methylation followed by hydrolysis gave paper chromatography (solvent-A) 2,3,4, 6 terta- O- methyl 2,3, 6-tri-omethyl-D-mannose. The trisaccharide was cleaved by emulsion, enzyme showing inter sugar linkage as β.
4. Galactosyl mannobios : α-D-Galp (1 ⟶ 6) – β-D- Manp (1 ⟶ 4)-D- manp α_D^{22} m.p. + 227^0 $[\alpha]_D^{25}$ +92-93^0 (water) Partial hydrolysis gave paper chromatography. (solvent-c) mannobiose, epimeliiose, galactose and mannose. During 48 h the trisaccharide consumed 6.02 mol of formic acid.

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