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GALACTOMANNAN FROM MEDICINAL PLANT CASSIA TORA

Manoj Singh
Department of chemistry S.G.R.P.G. College Dobhi Jaunpur .
(V.B.S. Purvanchal University Jaunpur)

Co - Author Details :

J. P. Singh , Rajeev Dubey and D. S. Singh
Department of chemistry S.G.R.P.G. College Dobhi Jaunpur .
(V.B.S. Purvanchal University Jaunpur)

ABSTRACT

A water soluble galactomannan having D-Mannose (4-part) and D-Galactose (2-parts) was isolated from Cassia Tora. In cassia Tora Hydrolysis of methylated seed gum furnished three methylated sugars, 2, 4-di-O-methyl-D-mannose, 2, 4,6-tri-O-methyl-D-mannose and 2, 3,4,6-tetra-O-methy-D-galactose was present in molar ratio 2:3:2. Partial acid catalysed hydrolysis of the seed gum gave four oligosaccharides epimelibiose, mannobiose, galactosyl mannobiose, mannotriose along with the component sugars. Periodate oxidation and methylation studies in cassia Tora showed 44% of end groups from the recent studies it is the concluded that galactose unit in galactomannan only terminal positions.

KEYWORDS :*medicinal plant Cassia Tora , water soluble galactomannan , Medicinal plants .*

INTRODUCTION :

An annual foeited herb, with a height of 30 cm to 90 cm, cassia Tora is mainly found in the state of Uttar Pradesh and Madhya Pradesh, in India. It has pinnate leaves, which are about 10 cm long. Each leaf has three pairs of leaflets that are opposite ovate, oblong and oblique at the base. The yellow colored flowers are bearded in the axel of the leaves. The flowers comprises of five petals, each about half inch in diameter. Seed: 30-50 rhombohedral and gathered in autumn and dried in sun (1, 2, 3). Seeds contain Anthraquinone namely, aurantio-obtusin, chryso-obtusin, obtusin, chrysoobtusin-2-O-beta-D-glucoside, physcion, emodin, chrysophanol, obtusifolin, obtusifolin-2-Obeta-D-glucoside (4) and Anthraquinone glucoside, namely, alaternin 2-O-,-Dglucopyranoside.

Medicinal plants still play an important role in emerging and developing countries of Asia, both in preventive and curative treatments as these are relatively safe, cost effective and efficacious solutions to primary health care (5, 6). The leaves, roots, and even the whole plant are employed in the

treatment of impetigo, ulcers, and helmenthiasis and as a purgative (7). Recently in vitro study find out that leaves of Cassia Tora is used as anticancer drug (8). Plant pacifies vitiated tridosha, dandruff, constipation, cough, hepatitis, fever and haemorrhoids (9). Hydroalcoholic extracts of Cassia Tora whole plant showed significant decrease in the levels of serum markers, indicating the protection of hepatic cells and significant dosedependent protection against paracetamol induced hepatocellular injury (10)

MATERIALS AND METHODS:

Polysaccharide was isolated from crushed seeds of cassia fistula which were decolourized and defeated by ethyl alcohol and petroleum ether and against treatment with 1% of acetic acid solution. For complete precipitation of the polysaccharide was extracted with 90% solution of ethyl alcohol. To purification of polysaccharide using three methods, repeated precipitation, Deproteinization, Complexation with Fehling's solution. After purification of polysaccharide was found a white amorphous material which was easily dispersed in water forming viscous solution at the room temperature. It was showed sulphated ash o.188% .The methoxy and acetyl was neglible and found to be free from halogen, nitrogen and sulphur. Extraction, purification and characterization of galactomannans from non-traditional SOURCES (11)

The homogeneity of the polysaccharide was checked by following methods:

(A) FRACTIONAL PRECIPITATION: (12, 13)

As per experimental knowledge show totally different fraction and their chromatography analysis (fraction I and fraction II), gave D-galactose and D-mannose. each the fraction indicating consistent nature of saccharide (B) ZONE- ionophoresis

(B) ZONE- ELECTROPHORESIS

Complete chemical reaction of the saccharide with one N acid and so the saccharide was subjected to traditional zone ionophoresis on whattman no.-1 natural process paper in salt buffer that pH - nine.2. . The intensity of characteristic yellow orange colour developed in aqueous eluates of each segment was measured in Klett Summerson photo-electric colorimeter using filter no-44. The plot of absorbance against segment number showed only single and sharp spot in chromatogram which identified the polysaccharide to be homogeneous. For calculating the quantity of polysaccharide used the following formula.

$$M = \frac{31.74 \times \text{absorbance}}{0.211}$$

Where M is in microgram per ml of polysaccharide solution .absorbance was measured as following formula.

$$\text{Absorbance} = \frac{2 \times \text{klett–reading}}{1000}$$

(c) Acetylating and Deacetylation: The acetylation of the polysaccharide was done by acetic anhydride and sodium acetate. The deacetylated polysaccharide had the same optical rotation as original polysaccharide, which further confirmed that the polysaccharide is homogeneous.

(D) Paper chromatographic examination in different mobile phase: The hydrolyzed polysaccharide was neutralized, filtered and concentrated the filtrate under reduced pressure up till a syrupy form. It was studied that the selected mobile phase. The result in all the mobile phase was identical which showed the polysaccharide to be homogeneous.

Sugar identification was identified by chromatographic analysis. Paper Chromatographic analysis show RF and RG value of two spots corresponded to D-galactose and D-mannose. The identity of the sugars D-galactose and D-mannose was confirmed by the co-chromatography developed in different mobile phase. In column chromatographic analysis the hydrolysate polysaccharide was dissolved in the solution of water and methanol in 1: 1 and the solution absorbed over a cellulose column. Each fraction by paper chromatography compare with standard sample of D-galactose and D-mannose. Two fractions were obtained in crystalline form. Quantitative estimation of sugars after the total recovery of D-ribose result shows that the ratio of D -mannose and D-galactose in the seeds are 4:2. Graded hydrolysis result was showed that D-galactose was found to be liberated first and then D-mannose. D-galactose units are present at the periphery as end groups and D-mannose forms the basic chain of the polysaccharide. Methylation of the pure polysaccharide done by Haworth's method by using dimethyl sulphate and sodium hydroxide then by Purdie's method using silver oxide and methyl iodide. Methylated product was as brownish masses. methylated sugar 2, 4-di-O-methyl-D-mannose, 2, 4, 6–tri–O–methyl–D-mannose, 2, 3, 4, 6 – tetra –O –methyl–D-galactose as 2:3:2. . 2, 4 -di-O-methyl-D-mannose was obtained as syrup R_{TMG}, in the mobile phase no. 5, 0.56, O-me: 29.8% calculated for dimethyl hexose, O-me: 29.9% $[\cdot]_D^{27}$-16.8° (water), melting point 192-193°C. For 2, 4, 6-tri-O-methyl D-mannose was obtained as syrup R_{TMG} in the mobile phase no. 5 0.84, $[a]_D^{27}$ -13° (water), melting point-167-170°C, $[a]_D^{27}$-33° (chloroform). 2, 3, 4, 6-tetra-O-methyl − D − galactose was obtained as a solid material with melting point 69°-70°C and, RTMG in mobile phase.no-5, 0.89, $[a]_D^{30}$ -121° (water) lit. These oligosaccharides were obtained and identified.

Epimelibiose:(a-D-galactosyl (1-6) −D-mannopyranose) m.p. 200-203 ºC, [a]D30+ 120.5 º water.(reported m.p. 201-203 ºC, [a]D30+ 120.5 º). Acid hydrolysis gave galactose and mannose; it reducing R glu in solvent (A) was found 0.58, and R_{man}. In mobile phaseno.-2, 5, and 7 was 0.18, 0.36 and 0.24 respectively. It reduced Fehling solution and tollen's reagent. (1→? linkage between galactose and mannose unit .the disaccharide was not hydrolysed by emulsion indicating the presence of αglycoside linkage show high positive optical rotation.

Mannobiose (βD-Mannopyranosyl (1→? ?D-Mannopyranose m.p.2023-204^{0}C , { α D2}- 8.9 ^{0} C (reported m.p.202-205^{0}C , { α D2}- 8 to -9 ^{0}C). Acid hydrolysis showed the presence of mannose units only. Emulsin hydrolysis showed the presence of βlinkage between the mannose units which were also confirmed by the optical rotation. Rglu in mobile phase no-2, 0.68, and Rman , in mobile phase 5, 6 & 7 0.30, 0.48 and 0.34.

Mannotriose (β D- Mannopyranosyl) (1→ ? ?β D- Mannopyranosyl (1→ ? D-mannopyranose, m.p.165- 167 ^{0}C , { α D2}- 18 ^{0}C. (Reported m.p.165- 166 ^{0}C , { α $_D^{25}$} -16 to - 18 ^{0}) Rman in mobile phase no-2 was found 0.23 and Rglu in mobile phase no-3 was found 0.35.it reduced Fehling's solution tollen's reagent. Anhydrous hydrolysis indicated the presence of mannose units only

and partial hydrolysis resulted in the formation of mannose, mannobiose. Further emulsion hydrolysis suggested mannose units are linked through β linkage.

Galactosyl mannobiose: (α D-galactopyranosyl (1→? – Dβ–mannopyranosyl (1→? D-mannopyranose) experimental m.p.226-229^{0}C {α 表25 - 92.8^{0}C (water) (Reported m.p. 227-230^{0}C {α D25 -93 C^0).R $_{glu}$ in mobile phase no-2, 0.32,and R $_{man}$, in mobile phase 6 & 7 0.19 and 0.08 respectively, and was found to be pure in solvent mixture.

Hydrolysis yielded galactose and mannose 1:4, its equivalent weight 265 .0 corresponded to monohydrated tri-saccharide. 3.0 moles of HCCOH methylation and subsequent hydrolysis gave 2, 3, 4, 6 –tetra-o-methyl-D-galactose, and 2, 3, 6- tri-o methyl-D- mannose. Hydrolysis with emulsin gave mannose and epimelibiose indicating one α and β linkage.

RESULTS AND DISCUSSION:

The polysaccharide was extracted with 1% aqueous Acetic acid and precipitated with 4 volume of ethanol. This process was repeated until minimum ash content, 0.188 % was obtained. Homogeneity of the polysaccharide was tested by fractional precipitation and zone – electrophoresis methods. The dry polysaccharide was soluble at room temperature and had negligible methoxy, acetyl and uronic acid contents. Complete hydrolysis yielded D- galactose and D- mannose in molar ratio 2:4 respectively. On graded hydrolysis with 0.05 M H_2SO_4 galactose was liberated first, followed by mannose.

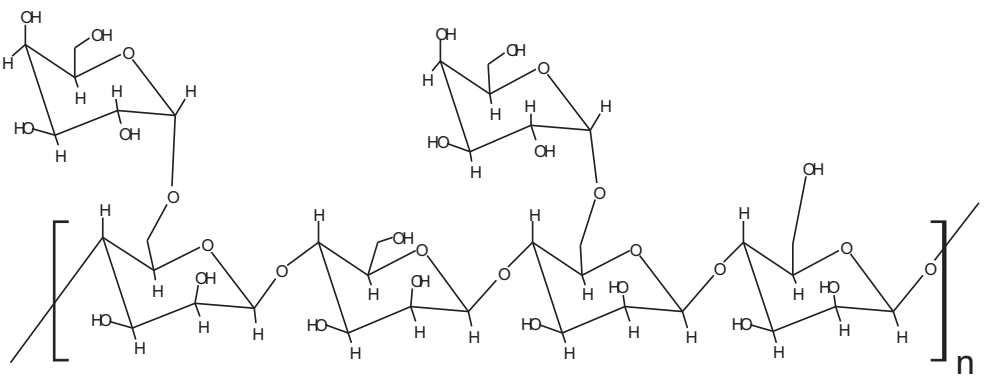
Completely methylated polysaccharide on hydrolysis gave 2,4,-di-O-methyl D- mannose, 2,4,6-tri-O-methyl-D- mannose and 2,3,4,6-tetra-O-methyl-D- galactose in the molar ratio 2:3:2.

Analysis of the percentage of end groups by the periodate method was close agreement with the methylation studies. Partial acid hydrolysis of the polysaccharide with 0.5 M H_2SO_4 for 14 hours liberated the following oligosaccharides (i) Mannotriose (ii) galactosyl mannobiose, (iii) Epimelibiose and (iv) Mannobiose,

They were identified by PC and by the preparation of their derivatives as well as by periodate oxidation. It was found to give four oligosaccharides where two were homogeneous and two were heterogeneous, in addition to the two component mannoschharides D- galactose, and D-mannose.

The homogeneous oligosaccharides were found to have (1→? linkage between mannose units but the heterogeneous members had (1→? linkage between galactose and mannose units.

These structural studies of the polysaccharides and of different oligosaccharides obtained by partial acid hydrolysis suggest the following structure of the repeating units of polysaccharide.



Previosly reasercher well known studied seed galactomannan (14). The above structure contains six units of monosaccharide, two galactose units and five mannose units. It explains the formation of oligosaccharides as well as, the percentage of end groups which are incomplete agreement with analytical data of the polysaccharide.

CONCLUSION

This research work based on improves the yield and quality of polysaccharide. As per experimental data it was found improve yield of polysaccharide up to 5%. Description, melting point, optical rotation, paper chromatographic analysis, NMR & Mass analysis of our experimental sample is nearly same as our reported sample.

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