Structure of the Polysaccharide
Occurring in the Seeds of Cassia Mimosa
: Hydrolytic Studies

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ABSTRACT

The seeds of Cassia Mimosa have been reported to be highly rich in mucilaginous matter. The preliminary screening of the seeds of the plants belonging to above mentioned genus has been conducted to locate a good source of polysaccharides. Cassia Mimosa (Linn), is commonly known as a “Horse Cassia” belongs to the family Leguminasceae (Sub-family : Cesalpinaceae). It is an ornamental and deciduous tree, reaching about 35 to 40 feet in height. Its seeds are almond-shaped and creamy in colour, their length ranging from 1.25 to 1.75 cms. During this investigation the seed of Cassia Mimosa plant has been found to contain a polysaccharide in high yield.

The several kinds of application of polysaccharides made great attention towards the study of molecular structure of polysaccharides, for the production of several derivatives, which are applicable in various forms in industries. The investigation of the structure of polysaccharides gives good idea about their biogenesis by which simple sugar transformed into their hexose, pentoses, uronic acid and methyl pentoses. A systematic chemical investigation of the polysaccharides from its seeds has now been undertaken which constitutes the subject matter of the present paper.

KEY WORDS: Polysaccharides, Leguminous plant Cassia Mimosa, Industrial applications, Quantitative acid hydrolysis, Paper chromatography analysis, Endosperm, Monosaccharides and Heteropolysaccharides.

INTRODUCTION

Polysaccharides are high molecular weight carbohydrates. As the name expresses a polysaccharide includes many sugar units, which is formed by condensation of many monosaccharides unit or uronic acid, amino sugar etc. They are essential constituents of almost all living organisms. The classification of plant polysaccharide mainly depends upon their composition and structure. After hydrolysis, the polysaccharide give rise to only one type of monosaccharides are called homoglycan while containing two or more kinds of monosaccharides unit are known as heteroglycans or heteropolysaccharides e.g. plant gum, mucilage and neutral polysaccharide like galactomannan (Anderson, 1949). Polysaccharide an essential part of all living organisms, are found in higher order of land plants mainly as structural unit of the cell wall and also found in algae, fungi, sea weeds, mucous and skin etc. Mostly plant bearing polysaccharides are in the form of gums, mucilage and hemicellulose. The mucilage is either in the stem of the plant or the endosperm of seeds (Anderson et al., 1969). Extraction of polysaccharide from the seeds of Cassia Mimosa
with water furnished crude polysaccharide, which was subsequently purified by repeated fractionation with ethanol followed by de-ionization with ion exchange resins. The polysaccharide isolated was neutral and free from nitrogen, sulphur, halogen, methoxyl and uronic acid groups (Andrews et al., 1953).

The further study of polysaccharides of various plants gives good output in many fields of industrial applications (Leo et al., 1968 and Weinstein 1958). The study of leguminous plants especially Cassia Mimosa, which are rich sources of mucilages, give a great attention for their immense importance in industries such as food, textile, cosmetic, paper (Yuch et al., 1971; and Schuppver 1969), painting product (Littl, 1968 and Fath et al., 1972), bakery, tooth paste (Gilcksman and Foarkar, 1972), soap and pharmaceuticals (Pettelt, 1972), wall drilling and mining explosive and fire-fighting etc. The polysaccharides found in the endospermic part of the seed caesalpinia are a water-soluble neutral galactomannan, which is composed of galactose and mannose unit linked glycosidically. The ratio of galactose and mannose differ in various plant, the biological function of galactomannan is double physiological, i.e serve as food for germinating seed and prevent from complete drying of seeds, hence causes protein denaturation.

Paper electrophoresis showed that the polysaccharide migrated as a single spot having ionic mobility \( \mu = 19 \times 10^{-7} \) cm\(^2\) volt\(^{-1}\) sec\(^{-1}\). The sedimentation pattern of the compound as revealed from ultra-centrifugal analysis showed a single symmetrical peak, thus establishing its homogeneity (Boggs et al., 1950; Biswas et al., 1970; Baker et al., 1920 and Bailey et al., 1960). Complete acid hydrolysis of the polysaccharide gave a mixture of neutral sugars, which was resolved into D-galactose and D-mannose by paper chromatography as well as on a cellulose column. The individual sugars were further characterized on the basis of their melting points, specific rotations and melting points of their crystalline derivatives. Quantitative acid hydrolysis of the polysaccharide showed that galactose and mannose were present in a molar ratio of 1:3 (Bose et al., 1971; Hirst et al., 1949; Hough et al., 1950 and Patridge et al., 1946).

**MATERIAL AND METHOD**

Unless otherwise stated all evaporations were carried out at 40-50\(^\circ\) C under reduced pressure. Specific rotations are equilibrium values and melting points are uncorrected. Paper chromatographic analyses were carried out by using the following solvent system by volume (V/V).

\[
\begin{align*}
S_1 &= \text{n-Butanol} : \text{ethanol} : \text{water} (4:1:5 \text{ upper layer}) \\
S_2 &= \text{n-Butanol} : \text{acetic acid} : \text{water} (4:1:5 \text{ upper layer}) \\
S_3 &= \text{Ethyl acetate} : \text{acetic acid} : \text{water} : \text{butanal} (4:3:3:3) \\
S_4 &= \text{2-Butanol} : \text{water} : \text{azotrope} \\
S_5 &= \text{Benzene} : \text{ethanol} : \text{water} (167:47:15) \\
S_6 &= \text{2-Butanol} : \text{water} : \text{ammonia} (200:17:2) \\
S_7 &= \text{Ethyl acetate} : \text{acetic acid} : \text{water} (9:2:2)
\end{align*}
\]

The spray reagents used for detecting the sugars were:

\[
\begin{align*}
R_1 &= \text{Acetonical silver nitrate-alcoholic sodium hydroxide} \\
R_2 &= \text{p-Anisidine phosphate} \\
R_3 &= \text{p-Anisidine hydrochloride} \\
R_4 &= \text{Diphenylamine-p-Anisidine} \\
R_5 &= \text{Sodium metaperiodate-Benzidine}
\end{align*}
\]
**Isolation and purification**: The seeds of *Cassia mimosa* (200 gm) were pulverized in a laboratory grinder and the endosperm was separated from kernel and husk by winnowing. The endosperm (93 gm) was finally powdered and extracted with water (2 litres) at 50-60°C for 10 hrs under constant stirring. The resulting highly viscous suspension was filtered through a muslin cloth and the undissolved material again extracted with water as above. The combine filtrate was centrifuged (3000 r.p.m.) for 50 minutes and yellow supernatant solution decanted off. It was then acidified (pH: 2.0-3.0) with glacial acetic acid and aqueous extract was poured slowly into four times its volume of rectified spirit with continuous stirring. The polysaccharide was obtained as a white flocculent precipitate. After the removal of aqueous ethanol, the precipitate was found to be sticky in nature. At this stage, acetone (350 ml) was added and the solution kept overnight. Subsequently, acetone was replaced by absolute alcohol (150 ml) and this operation was repeated thrice when the precipitate became granular. Finally, the compound was purified by passing its aqueous solution successively through columns of freshly regenerated cation (Duolite C-25) and anion (Duolite A-7) exchange resins. The Combined effluent was treated with a large granular powder (40 gm). [α]°D = 20.07° (C, 0.53 gm in 1 N NaOH). Its aqueous solution was almost neutral (pH - 6.5). The polysaccharide was non-reducing and did not contain nitrogen, sulphur, halogens, and methoxyl and uronic acids groups. It formed a O-acetyl derivative [α]°D = 38.62° (C, 0.5 gm. in Acetone) (found: Acetyl, 43.92 % calculated for acetylated polysaccharide: Acetyl, 44.20 %). IR spectrum of the polysaccharide showed a prominent band for OH (3400 cm⁻¹) besides other bands at 2850, 2300, 880 and 800 cm⁻¹.

Polysaccharide did not move at all under various condition of paper chromatography. However when subjected to paper electrophoresis on strips (45.4 cms) of what-man No.1 filter paper in borate buffer (0.005 M Sodium Tetraborate deca-hydrate pH = 9.2). Under field strength of 700 volt for 7 hours the compound moved as a single spot. The presence of polysaccharide spot in the paper was detected by spray R₃ and washing afterwards with acetone.

The ionic mobility of the polysaccharide was calculated from the equation.

\[ \mu = \frac{dqaK}{tl} \]

Where
d = Distance moved in cms = 20 cm
qa = Cross section area of the paper = 0.5 cm² strip
K = Conductivity of buffer = 1/18, 180
\( t \) = Time in second = 420 × 60 sec.
\( l \) = Current in ampere = 0.020
\( \alpha \) = Ionic mobility = 19 × 10⁻⁷ cm² sec⁻¹ volt⁻¹

Ultra-centrifugal analysis of the compound in 0.05 M borate buffer at 5000 r.p.m. for appropriate time intervals showed only one peak confirming the homogeneity of the sample. The molecular weight of polysaccharide was calculated from the values of:

Sedimentation constant (S\(^{20} \)) = 1.496 × 10⁻¹³

Diffusion coefficient (D\(^{20} \)) = 8.04 × 10⁻⁷

The partial specific volume of the compound was determined at 20°C by the procedure described earlier at three different concentrations of the polysaccharide in buffer pH-8.4.

The values are given in the Table 1.

Weight of pyknometer = 26.1074 gms
Weight of pyknometer + water = 75.8328 gms
Weight of pyknometer + buffer = 75.9216 gms
Weight of water = 49.725 gms
Weight of buffer = 49.7842 gms

Substituting all values in the following formula:

\[ \text{M.W.} = \frac{R\times T}{S \times D \times (1-\delta/d)} \]

Where
M.W. = Molecular weight of the polysaccharide
R = 8.31×10^7
T = 298°C K
S = Sedimentation constant = 1.496 × 10^3
D = Diffusion coefficient = 8.04×10^{-7}
\( \delta \) = Partial specific volume = 0.5439
d = Density of water at 20°C = 0.9982

The molecular weight of a pure sample of Cassia Mimosa polysaccharide was found to be in the order of 10684.

**RESULT AND DISCUSSION**

Complete acid hydrolysis of the polysaccharide: Purified polysaccharide (5.0 gm) was subjected to hydrolysis with sulphuric acid (2N, 250 ml) for 20 hours on a boiling water-bath. The course of hydrolysis was followed by iodometric titration (Smith et al., 1964).

After definite intervals of time on aliquot (2 ml) of the hydrolyte was withdrawn in an earlyMeyer flask and mixed with iodine solution (0.1N, 20 ml) and sodium hydroxide solution (0.1N 30 ml). The mixture was kept for 20 minutes in dark. The solution was acidified with sulphuric acid (10 ml) and excess of iodine was treated with standard thiosulphate (0.05 N). The time taken for completion of hydrogen along with the variation of iodine adsorption during the progress of the reaction is recorded in Tables 2 & 3 with the graphical representation as process of acid hydrolysis of polysaccharide and its industrial applications are shown in (Figures 1 to 4).

The acid hydrolyte was neutralized (barium carbonate) and evaporated to thin syrup. Its paper chromatography in solvent S1 revealed spots corresponding to galactose, mannose and xylose. Resolution of the sugars on a cellulose column using N-butanol half saturated with water as the eluant (Smith et al., 1964). Resulted in the isolation of two individual sugar and faint spot. The faint spot sugar melting point 143-44°C was characterized as a D-xylose from the measurements of optical rotation the second sugar was identified as D-galactose from its melting point 131°C. Optical rotation [\( \alpha \)]^30 D + 16.2 and by preparing p-nitro-N-phenyl-D-mannosylamine derivative melting point 210-21°C. Identify of the slowest moving sugar melting point 167°C was established as D-galactose from measurement of its optical rotation [\( \alpha \)]^30 D + 79.6 and by preparing N-phenyl galactosylamine derivative melting point 205°C.

**CONCLUSION**

The proposed scheme of the research work will be another new attempt towards the determination of the structure of the polysaccharide occurring in the seeds of Cassia Mimosa. Periodate oxidation and Smith’s degradation studies on oxidation gives rise to polysaccharide. The result of these experiments will be helpful for confirming the structure of the polysaccharide deduced earlier for methylation experiments. Determination of molecular weight of polysaccharide occurring in seeds of Cassia Mimosa has been studied through mathematical calculation. The interpretation
of the results of these experiments will be helpful in elucidation of the complete structure of the polysaccharide occurring in the seeds of Cassia Mimosa. Plants belonging to Cassia genus and family Leguminasceae (Sub-family: Cesalpinaceae), which has not been investigated so far and is further considered to elaborate the study in laboratory by experimental processes of polysaccharides.

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REFERENCE


Table 1. Partial specific volume of polysaccharide

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Weight of</th>
<th>$\rho_o$ e/c</th>
<th>$\rho_w$ e/c</th>
<th>Average</th>
</tr>
</thead>
</table>


AUTHOR

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<table>
<thead>
<tr>
<th>Time (Hrs)</th>
<th>Volume (ml) of thiosulphate solution (0.5) required for excess iodine</th>
<th>Volume (ml) of iodine solution consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>14.8</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>13.7</td>
<td>1.1</td>
</tr>
<tr>
<td>6</td>
<td>13.1</td>
<td>1.7</td>
</tr>
<tr>
<td>9</td>
<td>12.8</td>
<td>2.2</td>
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<tr>
<td>12</td>
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<td>2.5</td>
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<td>15</td>
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</tr>
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<td>27</td>
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<td>3.2</td>
</tr>
<tr>
<td>30</td>
<td>11.6</td>
<td>3.2</td>
</tr>
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</table>

Table 2. Process of acid hydrolysis of polysaccharide

<table>
<thead>
<tr>
<th>Polysaccharide w/w Solution</th>
<th>pyknometer + polysaccharide</th>
<th>specific volume $\delta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.05</td>
<td>76.2017</td>
<td>1.00181</td>
</tr>
<tr>
<td>1.75</td>
<td>76.3201</td>
<td>1.00181</td>
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<td>2.25</td>
<td>76.4394</td>
<td>1.00181</td>
</tr>
<tr>
<td>Tones (‘000)</td>
<td>USD million</td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>CMC</td>
<td>42</td>
<td>125</td>
</tr>
<tr>
<td>Hydroxyethylmethyl</td>
<td>22</td>
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<tr>
<td>Methyl</td>
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<tr>
<td>Microcrystalline</td>
<td>6</td>
<td>27</td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>84</td>
<td>313</td>
</tr>
</tbody>
</table>

Figure 1. Process of acid hydrolysis of polysaccharide
Figure 2. Natural view of carrageenan obtained from red seaweeds

(a) Electron microphotograph of microorganism producing both polysaccharide and PHB (*Deleya marina*)

(b) PHB (Left) and polysaccharide (right) obtained from *Deleya marina*

Figure 3. Production of polysaccharides and poly-*f*-hydroxybutyrate through microorganisms

(a) GEMPOLYM GUAR 200 FOODGRADE

(b) Gempolym Explosives Applications

(c) Gempolym Drilling Applications

Figure 4. Industrial applications of Guar gum