Chemical analysis of water soluble polysaccharide isolated form medicinal plant *Leucaena Leucocephala*

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**ABSTRACT**

In the present paper we experimentally show that polysaccharides extracted form defatted seeds of *Leucaena leucocephala* is water soluble with ash content 0.41% and have negligible content of methoxyl, acetyl and uronic acid. Acidic hydrolysis of polysaccharide gave D-galactose and D-mannose in the molar ratio 1: 4. Graded hydrolysis, liberated galactose first, indicates that these residues were present at end groups. Methylated polysaccharides having $\alpha_D + 11.20$ (chloroform) and hydrolysis gave 2,3,6 tri-o-Methyl D-mannose, 2,3-di-o-methyl, D-mannose and 2,3,4,6-terta-o-methyl D-galactose in the molar ratios 2:3:2. Periodate oxidation shows 25% end group and this result accord with methylation studies. Partial hydrolysis of polysaccharide with 0.05 in H$_2$SO$_4$ at 100°C for 12hr gave mannobiose, mannotriose, galactosyl mannobiose together with galactose and mannose. The experimental observation indicates that polysaccharide is a (1→4)-β-D mannose substituted at position 6 by galactosyl group.

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**KEYWORDS**

Acidic hydrolysis; Methylation; Periodate oxidation; Enzymatic hydrolysis; Mucilage; Endgroup analysis.

**INTRODUCTION**

*Leucaena leucocephala* (leguminoseae) majority of plant genus leucaena described to be highly medicinal¹ great economic value, high source of polysaccharides. In Mexico it is shown by its famous name oavena, which is derived form pre Columbian word “Uravin” place where the leucaena grows. This genus is being used in research and also as research tool in bio-chemistry due to the presence of rhizobium² seed that are shiny and its emerald leaves are long used in dwelling as ornamental, various part of the plant are reputed to have medicinal property such as disease of stomach and used as contraception and abortion³. Here we obtain watersoluble polysaccharides by applying different chemical progresses i.e. acidic hydrolysis, periodate oxidation etc.

**EXPERIMENTAL**

The solutions were concentrated under diminished pressure at (60°C-62°C). All residues were dried in vacuo over anhydrous CaCl$_2$. Melting points are uncorrected and $\alpha_D$ values for equilibria. P.c. was carried out at the room temperature with A, 1-butanol-ethanol- water (5: 1: 4); B, 1-butanol-ethanol-water (4: 1: 5); C, 1-butanol-2-propanol water (11: 6: 3); D,
Short Communication

1-ethylacetate-pyridine-water (2: 1: 2) and spots were detected using aniline hydrogen phthalate.

Isolation of polysaccharide

Dried crushed seeds were extracted successively with petroleum ether and ethanol to defat and decolourise. The defatted and decolourised seeds were extracted with 1% aqueous acetic acid solution and extract was added slowly with stirring to a large excess of ethanol. The crude polysaccharide was collected washed, dried and precipitated in aqueous 1% acetic acid with ethanol, the product yield 3.2g/100g, had $\alpha_25^0 + 68^\circ$ (c 1, water), and gave 0.41% of ash. The homogeneity of polysaccharide was tested by fractional precipitation form its aqueous solution with ethanol. Each fraction had $\alpha_25^0 + 27.5^\circ$ (c 1.2 chloroform). Deacetylation generated material has $\alpha_25^0 + 57.5^\circ$ (c 1.3 water).

Investigation of structure of polysaccharide

The purified polysaccharide was completely hydrolysed with 2 M $\text{H}_2\text{SO}_4$ at 100°C for 20hrs of the hydrolysate revealed galactose (Rf =0.21). The absolute configurations were confirmed by the preparation of D-galactose by Hawroth then Purdie[9] method. The product has $\alpha_25^0 + 11^\circ$ (c 1.2 chloroform) was hydrolyzed with 0.25M formic acid at 100°C for 6hrs, then $\text{M} \text{H}_2\text{SO}_4$ for 14 hrs. at 100°C, and product were fractionated on Whatmann No. 3 paper (solvent A) to give the following compounds. 2, 3, 4, 6 Tetra-o-methyl-D-galactose m.p., 72-73°C, $\alpha_25^0 + 120^\circ$ (c1, water); lit.[8] m.p. 74°C, $\alpha_25^0 + 121^\circ$ (water). 2,3-Di-o-methyl-D-mannose, m.p. 107-108°C, $\alpha_25^0 + 16^\circ$ (c 1.5, water); lit.[8] m.p. 106°C, $\alpha_25^0 + 15.8^\circ$; the anilide[7] had m.p. 136°C. 2,3,6-Tri-o-methyl D-mannose, $\alpha_25^0 + 11^\circ$ (water); lit.[8] $\alpha_25^0 + 10^\circ$ (water); the hydrazide has m.p. 121°C-131°C. The methylated polysaccharide together with D-glucose as reference was treated with the M $\text{H}_2\text{SO}_4$ at 100°C for 18hr. The resulting methylated sugars were subjected to p.c. (Solvent A) and their molar ratios were determined by alkaline hypoiodite.[8] The molar ratios of three methylated sugars were 2: 3: 2.

CONCLUSION

The polysaccharide was hydrolyzed with 0.25M $\text{H}_2\text{SO}_4$ at 100°C for 12hrs. Preparative p.c. (solvent D and E) of the hydrolysate gave D-galactose, D-mannose and the following oligosaccharide. Mannobiose [β-D-Manp-(1→4)-D-Manp], m.p. 203-205°C (form ethanol), $\alpha_25^0 - 9^\circ$ (c 1.2, water); lit.[8] m.p. 193-210°C. Epimellabiose [α-D-Galp-(1→6)-D-Manp], m.p. 199°C, $\alpha_25^0 + 120.5^\circ$ (c 1.3, water); lit.[9] m.p. 200°C, $\alpha_25^0 + 121^\circ$ (water). Mannotriose [β-D-Manp-(1→4)-β-D-Manp-(1→4)-D-Manp], m.p. 211-213°C (form ethanol), $\alpha_25^0 - 13^\circ$ (c 1.2, water); lit.[9] m.p. 214-215°C.

Galactosylamannobiose [α-D-Galp-(1→6)β-D-Manp-(1→4)-D-Manp], m.p. 225-227°C, $\alpha_25^0 + 93^\circ$.
(c 1.5, water); m.p. 228-229° lit.\textsuperscript{[10]}, \(a_0^{\circ} + 93.3\) (water). The result indicates that the main chain of the polysaccharide consists of \((1 \rightarrow 4)\)-\(\beta\)-D-Manp substituted position 6 by D-galactosyl group. A possible repeating unit of the polysaccharide has been assigned as below:

\[ a - D - Galp \]

\[
\begin{array}{c}
1 \\
6 \\
\end{array}
\]

\[ [4 - \beta - D - Manp(1 \rightarrow 4) - \beta - D - Manp(1 \rightarrow 4) - \beta - D - Manp(1 \rightarrow 4)]_n \]

Designation: Galp = Galactose, Manp = Mannose

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**REFERENCES**