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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 defated 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, librated galactose first, indicates that these residues were present at end groups. Methylated polysaccharides having α_D^{25} **+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_2SO_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.

KEYWORDS

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

INTRODUCTION

Leucaena leucocephala (leguminoseae) majority of plant genus leucaena described to be highly medicinal[1] 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 rhizobium[2] 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 abortion[3]. Here we obtain water soluble polysaccharides by applying different chemical progresses i.e. acidic hydrolysis, periodateoxidation 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 α_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,

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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 $\alpha_D^{25} + 68^\circ$ (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 $\alpha_D^{25} + 68^\circ$ (water) and on hydrolysis with M sulphuric acid at 100°C 20hr gave D-galactose and D-mannose in molar ratio 1:4. The polysaccharide was subjected to zone electrophoresis on Whatmann No. 1 paper in borate buffer (pH- 9.2) at 320 volt and 3.7mA for 6 hrs. The paper was cut into 31 equal segments and each was eluted with distilled water. The intensity of characteristics yellow orange color developed in each elute by adding aqueous 8% phenol (1mL) and concentrate H_2SO_4 (6ml) was measured in Klett-Summerson photoelectric colorimeter (filter No. 50). A plot of absorbance against segment number showed only a single sharp peak. The polysaccharide was treated with sodium acetate-acetic anhydride and resulting acetate had $\alpha_D^{28} + 27.5^\circ$ (c 1.2 chloroform). Deacetylation generated material has $\alpha_D^{28} + 57.5^\circ$ (c 1.3 water).

Investigation of structure of polysaccharide

The purified polysaccharide was completely hydrolysed with 2 M H_2SO_4 (solvent D) at 100°C for 20hrs of the hydrolysate revealed galactose (R_F=0.21). The absolute configurations were confirmed by the preparation of D-galactose phenyl osazone, m.p. 164°C has $\alpha_D^{30} + 80^\circ$ (water) and and D-mannose m.p. 131°C has $\alpha_D^{30} + 14^\circ$ (water). The polysaccharide (300mg), together with D-Ribose (30mg) as a reference was treated with M H_2SO_4 at 100°C for 20hrs P.c. and quantification of the components in the hydorlysate revealed the molar ratio of the galactose and mannose to be 1: 4.

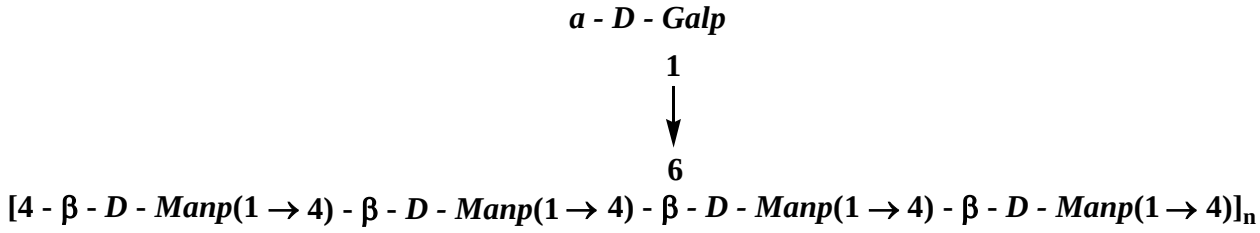
The polysaccharide was hydrolyzed[4] with 0.25M H_2SO_4 at 100°C for 6hrs. P.c. (solvent C) of the hydorlysate showed that the galactose was librated first. To a solution of polysaccharide were added KCl and 0.25M sodium metaperiodate. The amount of formic acid librated was 0.230 mol/100g (72hr) corresponding to 25.7% if the end groups. The polysaccharide was methylated by Hawroth then Purdie[5] method. The product has $\alpha_D^{25} + 11^\circ$ (c 1.2 chloroform) was hydrolyzed with aq. 90% formic acid at 100° for 6hrs, then M H_2SO_4 for 14 hrs. at 100°C, and product were fractioned on Whatmann No. 3 paper (solvent A) to give the following compounds. 2, 3, 4, 6 Tetra-o-methtyl-D-galactose m.p., 72-73°, $\alpha_D^{32} + 120^\circ$ (c1, water); lit.[6] m.p. 74°C, $\alpha_D^{32} + 121^\circ$ (water). 2,3-Di-o-methyl-D-mannose, m.p. 107-108°C, $\alpha_D^{25} - 16^\circ$ (c 1.5, water); lit.[6] m.p. 106°C, $\alpha_D^{25} - 15.8^\circ$; the anilide[7] had m.p. 136°C. 2,3,6-Tri-o-methyl D-mannose, $\alpha_D^{25} - 11^\circ$ (water); lit.[8] $\alpha_D^{25} - 10^\circ$ (water); the hydrazide has m.p. 121°C-131°C. The methylated polysaccharide together with D-glucose as reference was treated with the M H_2SO_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 H_2SO_4 at 100°C for 12hrs. Preparative p.c. (solvent D and E) of the hydorlysate gave D-galactose, D-mannose and the following oligosaccharide. Mannobiose [β-D-Manp-(1→4)-D-Manp], m.p. 203-205°C (form ethanol), $\alpha_D^{25} - 9^\circ$ (c 1.2, water); lit.[8,9] m.p. 193-210°C. Epimellabiose [α-D-Galp-(1→6)-D-Manp], m.p. 199°C, $\alpha_D^{32} + 120.5^\circ$ (c 1.3, water); lit.[10] m.p. 200°C, $\alpha_D^{32} + 121^\circ$ (water). Mannotriose [β-D-Manp-(1→4)-β-D-Manp-(1→4)-D-Manp], m.p. 211-213°C (form ethanol), $\alpha_D^{25} - 13^\circ$ (c 1.2, water); lit.[9] m.p. 214-215°.

Galactosylamannobiose [α-D-Galp-(1→6)β-D-Manp-(1→4)-D-Manp], m.p. 225-227°C, $\alpha_D^{25} + 93^\circ$

(c 1.5, water); m.p. 228-229° lit.[10], α_D^{25} +93.3° (water). The result indicates that the main chain of the polysaccharide consists of (1→4)-β-D-Manp substituted position 6 by D-galactosyl group. A possible repeating unit of the polysaccharide has been assigned as below:



Designation: Galp = Galactose, Manp = Mannose

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