

## Characterization of polysaccharides isolated from gums of two venezuelan specimens of *Albizia niopoides* var. *colombiana*

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### Abstract

The genus *Albizia durazzini* comprises 150 species; some of these species are disseminated in Venezuela. *Albizia niopoides* var. *colombiana* exudes a clear gum, very soluble in cold water at room temperature. Analytical data of the gum exudates from two specimens of that species showed little variation. The polysaccharide isolated from the gums contains galactose, arabinose, rhamnose, glucuronic acid and its 4-O-methyl derivative. The high content of rhamnose is comparable to that reported for *A. forbessii*. It was not detected mannose. Although, the presence of this sugar in most of *Albizia* gums has been taken as a feature of great diagnostic significance. The analytical data reported in this work is a contribution to augment the data existing for the gums from *Albizia* spp. The NMR spectra of the polysaccharides studied showed many interesting structural features which are according to the analytical data.

**Key words:** *Albizia niopoides* var. *colombiana*; gum exudates; Mimosaceae; polysaccharide; <sup>13</sup>C-NMR spectroscopy.

## Caracterización de los polisacáridos aislados de gomas de dos especímenes venezolanos de *Albizia niopoides* var. *colombiana*

### Resumen

El género *Albizia durazzini* comprende 150 especies; algunas de estas especies están diseminadas en Venezuela. *Albizia niopoides* var. *colombiana* exuda goma clara muy soluble en agua fría a temperatura ambiente. Los datos analíticos de los exudados gomosos de dos especímenes de esta especie mostraron poca variación. El polisacárido, aislado de la goma, contiene galactosa, arabinosa, ramnosa, ácido glucúronico y su 4-O-metil derivado. El alto contenido de ramnosa es comparable al reportado para *A. forbessii*. No fue detectada manosa; sin embargo, la presencia de esta azúcar, en la mayoría de las gomas de *Albizia* ha sido tomado como rasgo

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diagnóstico de gran significado. Los datos analíticos reportados en este trabajo es una contribución para aumentar los datos existentes para las gomas de especies de *Albizia*. Los espectros de RMN de los polisacáridos estudiados mostraron muchos rasgos estructurales interesantes, los cuales están de acuerdo con los datos analíticos.

**Palabras clave:** *Albizia niopoides* var; colombiana; espectroscopía de RMN de Carbono-13; exudados gomosos; Mimosaceae; polisacárido.

## Introduction

The genus *Albizia durazzini* (family Mimosoidae, tribe Ingae) comprises 150 species (1). Most of these species are present in the world tropics and some of them are disseminated in Venezuela. The composition and properties of the gum from *Albizia lebbbeck* (L.) Benth. have been reported (2).

The use of *Albizia* spp. in agroforestry, the great potential as soil improvers through fixation of nitrogen and other uses (3) are important features of the genus. This paper presents data on gum exudates of two Venezuelan specimens of *Albizia niopoides* var. colombiana in comparison with others *Albizia* gums.

## Materials and Methods

### Origin and purification of the gum samples

The gum samples from two specimens of *Albizia niopoides* var. colombiana (Britton & Killip) Barneby & Grimes (hueso de pescado) were collected during January-March, 1996 by Lic. Antonio Vera, after two weeks of an incision was made at the trunk level. The two samples were collected from trees growing at 48 Km. West (specimen 1) and 40 Km East (specimen 2) of Maracaibo City, Zulia State, Venezuela, South America. The identification of voucher specimens was confirmed by Dra. Lourdes Cárdenas de Guevara, a botanical taxonomist of Universidad Central de Venezuela, as *Albizia niopoides* var. colombiana, Section XV, Anthrosamanea (Britton & Rose) Barneby & Grimes. Serie I (Paniculatae).

The gum samples, were very soluble in cold water. They were purified as described previously (4, 5).

### Analytical methods

The standard methods of analysis used have been described (4). Descending paper chromatography was performed on Whatman No. 1 and 3 MM sheets. The organic phase of the following chromatographic solvent systems were used: (a) C<sub>6</sub>H<sub>6</sub> BuOH-C<sub>5</sub>H<sub>5</sub>N-H<sub>2</sub>O (1:5:3:3), (b) HOAc-EtOAc-HCOOH-H<sub>2</sub>O (3:18:1:14); (c) EtOH-0.1 M HCl-nBuOH, (10:5:1). Chromatograms were developed with aniline oxalate.

The nitrogen content was determined by the Kjeldahl method. Specific rotations were measured with a Perkin-Elmer 343 polarimeter at 589 nm at 30°C. The sugar composition was determined by the phenol-H<sub>2</sub>SO<sub>4</sub> method (6), and by high performance liquid chromatography (HPLC), which was carried out with a Waters system provided with a refraction index detector. A nova pack amino column (250 mm x 4.6 mm) was used with acetonitrile: water (75:25, v/v) as a solvent at room temperature and a flow rate of 1.4 ml min<sup>-1</sup>. The acidity was determined by the m-hydroxybiphenyl method (7).

NMR-spectra (<sup>1</sup>H, <sup>13</sup>C) of the two gum samples were recorded with a Brüker AM-300 spectrometer. <sup>13</sup>C-NMR data were accumulated overnight at 37°C with complete proton decoupling and the spectra were calibrated by the addition of d-methanol. The polysaccharide (100-200 mg) was dissolved in D<sub>2</sub>O (1 mL).

## Results and Discussion

*Albizia niopoides* var. *colombiana* exudes a clear gum very soluble in cold water at room temperature. The solubility of the gum increases when samples are collected as soon as the gum is exuded. This behavior has been observed in many gum bearing species (8).

The analytical data published so far for *Albizia* gums (2, 3) and those corresponding to *A. niopoides* var. *colombiana*, Table 1, support the botanical observation that *Albizia* is a complicated genus (1).

The gums from the two specimens of *A. niopoides* var. *colombiana*, Table 1, showed quite similar analytical parameters. This small variation interspecimen has been reported for *Acacia* gum (5, 9). The gums studied have relative high negative specific rotations as have been observed for gums from species included in the Section 2 (*Eualbizia*) according to Bentham, i.e., *A. sericocephala*, *A. glaberrima* and *A. procera*. The acidity is comparable to that reported for *A. anthelmintica* gum (3). The nitrogen content is in the range (0.24-2.8%) reported for *Albizia* gums (2, 3). The limit viscosity number is relatively low in comparison with others *Albizia* gums (3).

The polysaccharide, isolated from the gums studied, contains galactose, arabinose, rhamnose, glucuronic acid and its 4-O-methyl derivative, Table 1. The high content of rhamnose (21-24%) is comparable to that of *A. forbesii* but higher than that reported for gums from *A. harveyii*, *A. amara*, *A. adiantifolia*. (3). Mannose was not detected. The presence of this sugar in most of *Albizia* gums (2, 3) has been taken as a feature of great diagnostic significance. In this respect the gum from *Albizia niopoides* var. *colombiana*, deviates from the typical *Albizia* pattern.

*A. niopoides* var. *colombiana* gum, in deuterium oxide, shows very well resolved <sup>13</sup>C-NMR spectra, Figures 1 and 2. Chemical composition of this gum, Table 1, and previ-

Table 1  
Analytical data for gums from two specimens from *Albizia niopoides*, var. *colombiana*

	Specimen	
	1	2
Moisture, (%)	6	19
Ash, (%) <sup>a</sup>	7	3
Nitrogen, (%) <sup>a</sup>	0.68	0.64
Hence protein, (%) <sup>a</sup>	4.25	4.00
[α] <sub>D</sub> in H <sub>2</sub> O (°) <sup>a</sup>	-59	-58
Intrinsic viscosity, (ml g <sup>-1</sup> ) <sup>a</sup>	7	8
Acidity, (%) <sup>b</sup>	19	19
Sugars composition after hydrolysis, (%)		
Galactose	49	51
Arabinose	8	9
Rhamnose	24	21

<sup>a</sup>Corrected for moisture. <sup>b</sup>Uronic acids are represented by glucuronic acid and its 4-O-methyl derivative.

ous results of *A. lebbeck* gum (5) and of analogous polymers (10, 11) led to do unequivocal signals assignment, Table 2.

The spectrum of the gum studied, Figure 1, contains the resonances due to 3-O- and 6-O-β-D-galactose residues (10, 12) terminal and 3-O-α-L-arabinofuranose (10) and uronic acid residues (10, 12), Table 2. The uronic acids are represented by glucuronic acid and its 4-O-methyl derivative. The former acid is partly neutralized, which is according to the high intensity signal that appears at low field (181.0 ppm), Figure 1, that is assignable to -D-glucuronic acid substituted by metals (12). This signal is also related to the high ash content exhibited by the gum, Table 1; there are not unequivocal resonances due to 4-O-methyl -D- glucuronic acid, except that of the methoxyl group (60.8 ppm) (11, 12). The anomeric region of the spectrum, expanded in Figure 2, contains the carbons due to β-L-arabinopyranose and α-L-rhamnose residues (101.56 ppm) (11, 12), 6-O-β-D-galactose residues (102.44 ppm) and 3-O-β-D-galactopyranose; (103.50; 103.71 ppm) (10), β-D-glucuronic acid (104.67 ppm) (11) and α-L-arabinofuranose residues

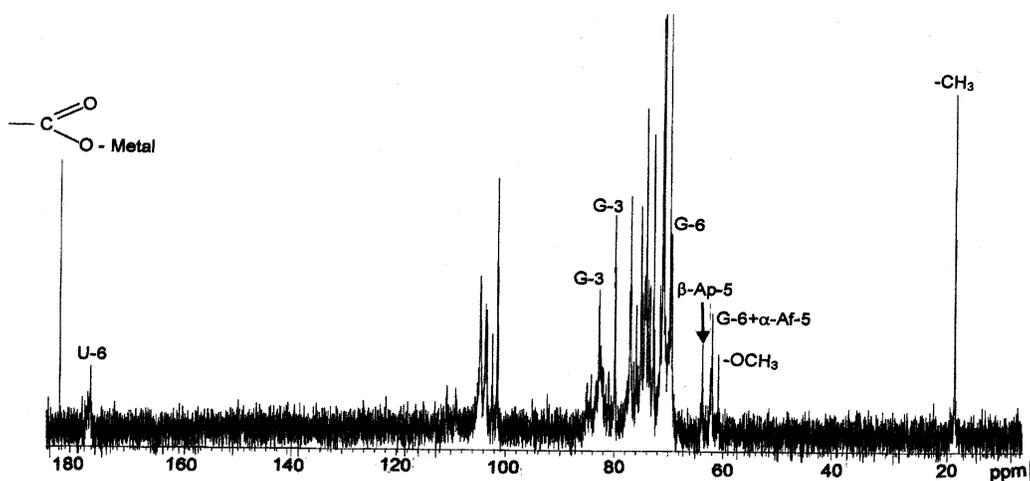


Figure 1.  $^{13}\text{C}$ -NMR spectrum of *A. niopoides* var. colombiana gum exudate (0 – 200 ppm) G=  $\beta$ -D-galactose U= Uronic acids.  $\dot{\text{C}}$ = Carbon involved in glycosidic linkage.  $-\text{OCH}_3$  = methoxyl group of 4-O-Me- $\alpha$ -D-glucuronic acid.  $-\text{CH}_3$  = methyl group of rhamnose.

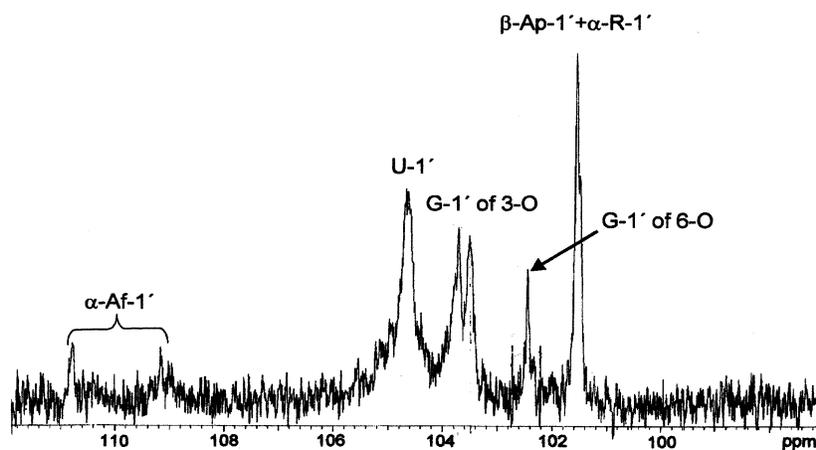


Figure 2.  $^{13}\text{C}$ -NMR spectrum of *A. niopoides* var. colombiana (98 – 110 ppm). G=  $\beta$ -D-galactose. U= Uronic acids. Ap=  $\beta$ -L-arabinopyranose. Af=  $\alpha$ -L-arabinofuranose. R=  $\alpha$ -L-rhamnose.  $\dot{\text{C}}$  = carbon involved in glycosidic linkages.

(109.20; 110.8; ppm) (10). The overlapping of the resonances that appear at 101.56 ppm is according to the unequivocal signals due to the methyl group of rhamnose (17.42 ppm) (12) and to C-5 of  $\beta$ -L-arabinopyranose residues. (65.00 ppm) (10). The resonance of low intensity (20.73 ppm) may be assignable to acetyl groups (12, 13).

There is a well resolved signal at 79.93 ppm, assignable to terminal  $\alpha$ -L-arabinofuranose linked to C-3 of  $\beta$ -D-galactose or to C-4 linked of  $\beta$ -D-galactose residues as has been observed in the polysaccharides from others gums (10).

The presence of rhamnose and acetyl groups was confirmed by proton NMR spectroscopy, Figure 3. The existence of -

Table 2  
 $^{13}\text{C}$ -NMR spectral data<sup>a</sup> of neutral and uronic acid residues of the gum polysaccharide from *Albizia niopoides* var. *colombiana*

Type of linkage	C-1	C-2	C-3	C-4	C-5	C-6
$\rightarrow 3\text{-D-Galp}(1\rightarrow^b)$	103.50 103.71	71.19	82.77	69.81 -	74.17 75.15	61.99
$\rightarrow 6\text{-D-Galp}(1\rightarrow^b)$	102.44	70.94	72.83		73.49	69.49
$\rightarrow 3\alpha\text{-L-Araf}(1\rightarrow^b)$	109.20	80.74	85.18	84.44	61.99	
$\alpha\text{-L-Araft}(1\rightarrow^b)$	110.80	81.48	77.03	84.44	61.99	
$\beta\text{-D-Glucp A}(1\rightarrow^c)$	104.67	75.99	77.03	73.85	77.03	176.30

<sup>a</sup>Values relative to the signal of methanol (49.00 ppm) <sup>b</sup>León de Pinto et al, 1994a <sup>c</sup>León de Pinto et al., 1998

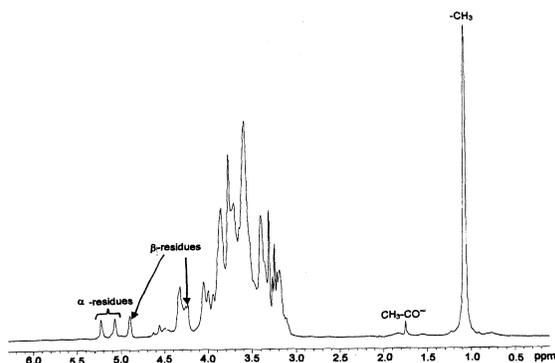


Figure 3.  $^1\text{H}$ -NMR spectrum of *A. niopoides* var. *colombiana* gum.  $-\text{CH}_3$  = methyl group of  $\alpha\text{-L-rhamnose}$ .  $\text{CH}_3\text{CO}^-$  = acetyl group.

(5.1-5.8 ppm) and  $\beta$ - residues (4.3-4.8 ppm) in the structure of the polysaccharide from this gum was also confirmed (14).

The presence of acetyl groups, reported for *Combretum* gum and absent in *Acacia* gums (3) is an atypical feature for *Albizia* gums which may distinguish gums from *Acacia* and *Albizia* species.

The analytical data reported in this work may be useful to support taxonomic classification of *A. niopoides* var. *colombiana* and is a contribution to augment the data existing for the gums from *Albizia* spp. The NMR spectra of the polysaccharides studied showed many

interesting structural features which are according to the analytical data.

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