



## New structural features of *Acacia tortuosa* gum exudate



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### ABSTRACT

*Acacia tortuosa* produces a clear gum, very soluble in water. Previous reports showed that it was constituted by four fractions, one of them an arabinogalactan–protein complex. The elucidation of the *A. tortuosa* gum structure by the combination of classical chemical methods, size exclusion chromatography and NMR spectroscopy, was the objective of this investigation. The data obtained show that the heteropolysaccharide is an arabinogalactan type II, highly ramified, with lateral chains at C-2 as well as at C-6 of the galactose 3-O residues; mono-O-substituted galactoses were not detected. There are residues of mannose, the arabinose, pyranose predominantly, is terminal and 2-O-linked. The abundance of the 4-O-methyl- $\alpha$ -D-glucuronic acid was not previously reported. The proteic fraction is probably represented by an arabinogalactan–protein complex that binds poorly with  $\beta$ -glucosyl Yariv reagent, and two glycoproteins. The NMR spectra suggest that the carbohydrate links to hydroxyproline through the galactose (galactosylation).

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### 1. Introduction

Polysaccharides possess interesting applications as hydrocolloids, mainly due to their ability to (a) retain water, (b) increase the viscosity of aqueous dispersions, (c) form viscoelastic materials and (d) provide a suitable texture for specific commercial products. For this reason, they are widely used as moisture-control products, thickeners, gelling agents, stabilizers of different kinds of dispersion, crystallization inhibitors and even as emulsifiers (Braun & Rosen, 2010; Imeson, 2010; Phillips & Williams, 2009).

Only a few plant species are cultivated, at present, to obtain gums used in the food industry as additives: *Acacia senegal*, source of arabic gum; *Astragalus* spp., source of tragacanth; *Cyamopsis tetragonolobus*, source guar gum; *Ceratonia siliqua*, source of locust bean gum.

*Acacia tortuosa* (Leguminosae), a tropical American specie, located in Venezuela, produces a clear gum, very soluble in water (León de Pinto, Martínez, De Bolaño, Rivas, & Ocando, 1998; León de Pinto, Martínez, Ortega, Villavicencio, & Rojas, 1993). Previous

studies demonstrated that the gum contains mainly a polysaccharide and a proteinaceous material (Beltrán, León de Pinto, Martínez, & Rincón, 2005). The polysaccharide contains galactose (69%), arabinose (13%), xylose (<1%) and uronic acid (18%). The nitrogen content is relatively high (6%), in comparison with those reported for *A. senegal* (0.29%) gum and for other *Acacia gummiferae* gums, such as, *Acacia seyal* (0.14%), *Acacia gerrardii* (1.8%), *Acacia robusta* (2.8%) and *Acacia tortilis* (1.9%) (Beltrán, León de Pinto, Martínez, & Rincón, 2005; Islam, Phillips, Sliivo, Snowden, & Williams, 1997; Sidigg, Osman, Al-Assaf, Phillips, & Williams, 2005). The amino acid composition showed high proportions of hydroxyproline, serine, lysine and glycine; threonine glutamine, proline, alanine, valine, isoleucine, tyrosine, phenylalanine and histidine are also found as minor constituents, similar to those reported for *A. seyal*, *A. gerrardii*, *A. robusta* and *A. tortilis* (Beltrán, León de Pinto, Martínez, & Rincón, 2005). Size exclusion chromatography, with multiangle laser light scattering data demonstrated that *A. tortuosa* gum is a highly polydisperse system (Beltrán, León de Pinto, Martínez, Picton, et al., 2005). The weight average molar mass ( $M_w = 410,000 \text{ g mol}^{-1}$ ) and the number average molar mass ( $M_n = 170,000 \text{ g mol}^{-1}$ ) are within the typical ranges reported for many *Acacias* (Al-Assaf, Phillips, & Williams, 2005).

*A. tortuosa* gum aqueous dispersions (15–40% w/v) displayed shear-thinning non-Newtonian flow properties at 20 °C, fitting the Sisko model, since a tendency towards a high-shear limiting Newtonian viscosity was observed. The aqueous dispersion gum of *A. tortuosa* (40% w/v) exhibited clear viscoelastic properties from

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5 to 25 °C (Muñoz et al., 2007). The surface tension of *A. tortuosa* gum (42.6 mN/m), at a given concentration (0.5% w/v) (Muñoz et al., 2007), is below the value reported for gum arabic (46.9 mN/m) at the same concentration (Huang, Kakuda, & Cui, 2001); the emulsifying properties of this last gum (Ibanoglu, 2002; Tan, 2004) have been related to an arabinogalactan–protein complex (Picton, Bataille, & Muller, 2000). The fractionation and characterization of *A. tortuosa* gum suggested the presence of an arabinogalactan (AG) and an arabinogalactan–protein complex (AGP) (Beltrán, León de Pinto, Martínez, Picton, et al., 2005).

Structural studies, by sugar composition, methylation analysis and 1D-NMR spectroscopy, suggested that the backbone of the polysaccharide structure is mainly a  $\beta$ -D(1  $\rightarrow$  3) galactan (León de Pinto et al., 1998). This nucleus also contains arabinose and uronic acid residues; the presence of these residues, vulnerable to periodate oxidation, requires a further study (León de Pinto et al., 1998).

This work reports new structural features of *A. tortuosa* gum exudate by the combination of classical chemical methods, size exclusion chromatography and 1D- and 2D-NMR spectroscopy.

## 2. Materials and methods

### 2.1. Origin and purification of gum samples

Gum from *A. tortuosa* (L) Wild (uveda), was collected in Zulia State (East of Maracaibo lake), Venezuela, South America, by the authors in January–March, 2012. The identification of voucher specimens was confirmed by Dr. Lourdes Cárdenas de Guevara, a botanical taxonomist of the Universidad Central de Venezuela. The gum exudate was purified as described previously (León de Pinto et al., 1998).

### 2.2. General methods

Neutral sugar compositions were determined by HPLC, with an IR detector (Waters 410, 35 °C) and a carbohydrate column (Waters, WAT 044355, oven 35 °C), an Acetonitrile:water (80:20) mixture was used as eluent (flow 1 ml/min). The amino acid content was determined with a post-column derivatizer (Pickering laboratories) adapted to a Perkin Elmer 785 UV–visible detector (570 nm), a sodium cation exchange column (Pickering Laboratories) and buffer solutions (pH: 3.8 and 7.14) as eluent (0.5 ml/min) were used. The uronic acids values were determined by the Blumenkrantz–Asboe-Hansen method (Blumenkrantz & Asboe-Hansen, 1973). The total protein and carbohydrate contents were evaluated, using the Lowry and phenol–sulfuric methods respectively (Dubois, Geilles, Hamilton, Reberts, & Smith, 1956; Lowry, Rosenbough, Farr, & Randall, 1951).

### 2.3. Classical methods

#### 2.3.1. Preparation of the degraded gums A and B

Purified gum (7.2 g) was hydrolyzed with sulfuric acid (5 mM, 290 ml, 96 h, 100 °C). After cooling, neutralization and filtration, the solution was dialyzed against running tap water (48 h), using a Spectra/Por molecular membrane tubing (MWCO 12–14,000). The degraded gum A was obtained by freeze-drying. This hydrolysis gives information about the structure of the lateral chains.

Degraded gum B was prepared by a drastic Smith-degradation of degraded gum A. Degraded gum A (1.5 g) was subjected to oxidation (50 ml, 0.25 M, NaIO<sub>4</sub>), reduction (0.5 g, NaBH<sub>4</sub>) and then acid hydrolysis (0.5 M, H<sub>2</sub>SO<sub>4</sub>) at room temperature for 2 days. The product was obtained by freeze-drying, after dialysis against tap water (48 h). This drastic degradation is used to obtain information about the nucleus of the studied polymer.

#### 2.3.2. Methylation analysis of the original gum

The original gum was permethylated by two successive methylation processes (Haworth, 1915; Purdie & Irvine, 1903). The corresponding alditol acetates were prepared from the permethylated polysaccharide, as described previously (León de Pinto et al., 2000). The alditol acetates were analyzed by GC–MS in a Hewlett Packard HP6890 GC coupled with a Hewlett Packard 5973A Mass-selective detector. The gas chromatograph was equipped with a capillary HP-5MS column (30 m  $\times$  0.25 mm i.d. 0.25 mm). The carrier gas was Helium (1 ml/min). The oven temperature was raised from 70 to 260 °C at a rate of 6 °C/min and then to 280 °C at a rate of 25 °C and held for 2 min.

### 2.4. NMR spectral studies of the degraded gums A and B

Experiments were recorded in a Bruker Avance DRX-400 NMR spectrometer operated at 500.13 and 125.77 MHz, for <sup>1</sup>H and <sup>13</sup>C NMR, respectively. Studies included correlation spectroscopy (COSY), heteronuclear multiple quantum correlation (HMQC) and heteronuclear multiple bond correlation (HMBC). The degraded products (50–100 mg) were dissolved in deuterium oxide (0.5–1.0 ml); the analyses were performed at probe temperature (27 °C). NMR data were reported in ppm ( $\delta$ ) down field from Me<sub>4</sub>Si; 1,4-dioxane was used as internal standard.

### 2.5. Size exclusion chromatography of the original gum

Size exclusion chromatography (SEC) was used in order to obtain the different molecular weight fractions of the original gum, this technique was performed in an open column, packed with Sephacryl S-500 HR (Sigma), and sodium chloride solution (0.5 M) was used as a carrier (0.5 ml/min). The protein, at 280 nm, and carbohydrate (by sulfuric acid method, at 490 nm) contents were monitored with an UV–visible spectrometer. The elution profile was obtained plotting absorbance vs elution volume (León de Pinto, Sanabria, Martínez, Beltrán, & Igartuburu, 2002).

### 2.6. Yariv reaction of the original gum

Aliquots (4 and 40  $\mu$ g) of the original gum, were subjected to rocket electrophoresis in agarose gels (1% w/w) containing  $\beta$ -glucosyl Yariv reagent, which selectively precipitates and stains AGPs as they move through the gel. Gum arabic was used as standard (Komalavilas, Zhu, & Nothnagel, 1991).

**Table 1**  
Sugar composition of *Acacia tortuosa* original gum and its fractions.

Sample Sugar	Proportion (% m/m)			
	OG <sup>a</sup>	F1 <sup>b</sup>	F2 <sup>c</sup>	F3 <sup>d</sup>
Galactose	53 $\pm$ 1.5	32 $\pm$ 0.6	48 $\pm$ 0.3	52 $\pm$ 0.7
Arabinose	28 $\pm$ 1.5	38 $\pm$ 0.2	39 $\pm$ 0.6	32 $\pm$ 0.8
Xylose	2 $\pm$ 0.26	6 $\pm$ 0.2	2 $\pm$ 0.2	3 $\pm$ 0.5
Mannose	7 $\pm$ 0.5	13 $\pm$ 0.2	5 $\pm$ 0.3	5 $\pm$ 0.3
Uronic acid	10 $\pm$ 0.26	11 $\pm$ 0.3	6 $\pm$ 0.2	8 $\pm$ 0.4
Total	100	100	100	100

<sup>a</sup> OG: Original gum.

<sup>b</sup> F1: Fraction one.

<sup>c</sup> F2: Fraction two.

<sup>d</sup> F3: Fraction three.

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