Comparative study of the gum exudates from two Citrus spp. (Rutaceae) located in Venezuela (Citrus spp. gum exudates)

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Estudio comparativo de los exudados gomosos de dos especies de Citrus spp. (Rutaceae) localizadas en Venezuela(Citrus spp. gum exudates)

Estudi comparatiu dels exsudats gomosos de dues espècies de Citrus spp. (Rutaceae) localitzades a Veneçuela (Citrus spp. Gum exudates)

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RESUMEN

La familia Rutaceae se encuentra representada en Venezuela por 26 géneros y 84 especies. Se han reportado pocos estudios sobre las gomas de esta familia. *Citrus aurantifolia* Swings y *Citrus paradisi* MacFayden (Rutaceae) y exudan gomas, claras y muy solubles en agua. La caracterización de las gomas se llevó a cabo mediante metodología clásica para carbohidratos y espectroscopía de Carbono-13. La goma de *C. aurantifolia* tiene una estructura más compleja, contiene galactosa, arabinosa, xilosa, ramnosa y ácidos urónicos, mientras que la goma de *C. paradisi* presenta únicamente galactosa, arabinosa y ácidos urónicos. La ausencia de xilosa y ramnosa y de residuos α-L arabinofuranosos se confirmó por espectroscopía de Carbono-13 de la goma de *C. paradisi* y la de residuos α-L-arabinofuranosos. Ambas gomas son polisacáridos, predominantemente, pero también contienen aminoácidos y ácidos grasos en su estructura. Los estudios químicos y espectroscópicos revelaron semejanzas y diferencias importantes relacionadas con los rasgos estructurales de las gomas de *C. aurantiifolia* y *C. paradisi.*

Palabras clave: Espectroscopía de RMN de Carbono-13, *Citrus aurantiifolia*, *Citrus paradisi*, exudados gomosos, Rutaceae

SUMMARY

The Rutaceae family is represented in Venezuela by 26 genera and 84 species. Few studies have been published about Rutaceae gums. *Citrus aurantiifolia* Swing. and *Citrus paradisi* MacFayden (Rutaceae) exudate, a clear, highly water-soluble gums. The characterization of their gum exudates was done by classical analytical methods and Carbon-13 NMR spectroscopy. *C. aurantiifolia* gum has the most complex structure, containing galactose, arabinose, xylose, rhamnose and uronic acids. This sugar composition differs from that of the *C. paradisi* gum, which contains only galactose, arabinose and uronic acids. *C. paradisi* Carbon-13 NMR spectrum confirmed the absence of xylose and rhamnose and in contrast to *C. aurantiifolia* gum, showed no evidence of α-L-arabinofuranose residues in its structure. Both gums are predominantly polysaccharides but they also contain amino acids and fatty acids. Chemical and spectroscopic studies showed important similarities and differences in the structural features of the *C. aurantiifolia* and *C. paradisi* gums.

Key words: Carbon-13 NMR spectroscopy, *Citrus aurantiifolia*, *Citrus paradisi*, gum exudates, Rutaceae

RESUM

La família Rutaceae es troba representada a Veneçuela per 26 gèneres i 84 espècies. S'han publicat pocs estudis sobre les gomes d'aquesta família. *Citrus aurantifolia* Swings i *Citrus paradisi* MacFayden (Rutaceae) exsuden gomes clares, molt solubles en aigua. La caracterització de les gomes es va dur a terme mitjançant la metodologia clàssica per carbohidrats i espectroscòpia de Carboni-13. La goma de C. *aurantifolia* té una estructura més complexa, i conté galactosa, arabinosa, xilosa, ramnosa i àcids urònics, mentre que la goma de C. *paradisi* presenta únicament galactosa, arabinosa i àcids urònics. L'absència de xilosa i ramnosa es va confirmar en l'espectre de Carboni-13 de la goma de C. *paradisi* així com en l'absència de residus α-L-arabinofuranosos. Les dues gomes són predominantment polisacàrids, però també tenen aminoàcids

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i àcids grassos en la seva estructura. Els estudis químics i espectroscòpics van revelar semblances i diferències importants relacionades amb els trets estructurals de les gomes de C. *aurantiifolia* i C. *paradisi*.

Paraules clau: Espectroscòpia de RMN de Carboni-13, *Citrus aurantiifolia*, *Citrus paradisi*, exsudats gomosos, Rutaceae

INTRODUCTION

Rutaceae, is a wide family which contains about 150 – 160 genera, and 1,600 species in tropical and subtropical countries (Badillo *et al*. 1985). About 84 species, in 26 genera, have been reported for Venezuela (Hokche *et al*. 2008).

Few studies have been published about Rutaceae gums. The structural features for *Feronia elephantum* Correa 1800 (Mathur and Mukherjee 1952) and *Citrus limonia* Osbeck 1765 gum exudates (Stoddart and Jones 1968) have been investigated. Also, the isolation and characterization of five oligosaccharides from *Aegles marrmelos* (L.) Correa 1800 (bael gum) (Roy *et al*. 1975) have been described. The analytical data for the gum from *Chloroxylon swietenia* DC. 1824 (Anderson *et al*. 1986) as well as the physicochemical, rheological characteristics and emulsification activity of *Zanthoxylum tessmanii* (Engl.) J. F. Ayafor (syn. *Fagara melanorhachis* Hoyle1933) have been reported (Orafidiya 1989, Orafidiya *et al*. 1992a, 1992b). The application of Carbon-13 NMR to *Amyris elemifera* L. 1759 exudate (Mexican elemi) confirmed its kinos-resinous character (Lambert *et al*. 2005).

Today, there is a growing interest in the study of polysaccharides from sources different to that of the arabic gum (*Acacia senegal* Willd. 1806) (Anderson *et al*. 1990). The analytical parameters of these polymers are regarded as a fingerprint of chemo-taxonomic importance (Anderson and Dea 1969).

Citrus aurantiifolia (Christm) Swingle 1913 and *Citrus paradisi* Macfad 1830, species cultivated in Venezuela, excrete a clear, highly water-soluble gums. To date, there are no documented published reports on the gum exudates from these two species. This analysis compares the physochemical properties and spectroscopic features of the gums from these two *Citrus* species.

MATERIALS AND METHODS

Origin and purification of gum samples

Gum samples were collected in Maracaibo, Venezuela, during January-March, 2010, from cultivated trees of *C. aurantiifolia* ("limón criollo") and *C. paradisi* ("grapefruit", "toronja roja").

The exudates were collected three weeks after incisions were made on the trunk, to obtain the appropriate yield. The identification of voucher specimens was confirmed by José Grande, plant taxonomist at the Fundación La Salle, Venezuela.

Gum samples were purified using methods described previously (León de Pinto 1991).

Analytical methods

The nitrogen content was determined by Kjeldahl method and the ash cationic composition by atomic absorption spectroscopy. Optical rotation, at equilibrium, was measured at room temperature in a Perkin-Elmer 241 polarimeter, using water diluted (1%) samples. Neutral sugar composition was estimated by the phenol - H_2SO_4 method (Dubois *et al*. 1956) and by HPLC, and the uronic acid content by the m-hydroxybiphenyl method (Blumenkrantz and Asboe-Hansen 1973). Absence of D-galacturonic acid was demonstrated by an appropriate solvent system (Kinsley 1967) and a quantitative colorimetric method was used to show presence of tannins (Abed *et al*. 2003). Intrinsic viscosities were determined by the isoionic method, using an Ubbelode viscosimeter, and amino acid composition by HPLC. The fatty acids contents were determined by GC, after derivatization to methyl esters.

NMR Spectroscopy:

Carbon-13 NMR and DEPT-135, for both gums, were recorded with a ^{13}C-NMR (Bruker AM 400) spectrometer. Data points (6,000-7,000) were accumulated overnight at 30ºC and with complete proton decoupling. Samples (50 mg) were dissolved completely in D_2O (1 mL). Inverted signals in DEPT-135 corresponded to the free or linked hydroxyl primary groups of the sugar residues present in the structure.

RESULTS AND DISCUSSION

The sugar composition of *C. aurantiifolia* gum, Table 1, differs from that of *C. paradisi* gum, which contains only galactose, arabinose and uronic acids. Absence of xylose and rhamnose has also been reported for *C. swietenia* gum (Rutaceae) (Stoddart and Jones 1968). The galactose : arabinose ratio (2 :1), in both cases, was higher than that reported for other Rutaceae (Stoddart and Jones 1968, Anderson *et al*. 1986) but lower than that described for Meliaceae gums, a related family (Rutales order) (León de Pinto *et al*. 1996).

The acidity content of the *C. paradisi* gum is higher (37%) than that of *C. aurantiifolia*., Table 1. The high acidity content has also been reported for gums from Caesalpinaceae (León de Pinto *et al*. 1993), Combretaceae (León de Pinto *et al*., 1998) and Sterculiaceae (Larrazábal *et al*. 2006). The ash contents, Table 2, suggest that these uronic acids are partly neutralized by metals, calcium and sodium predominantly.

The low positive specific rotation values shown by both *Citrus* gums, Table 1, suggest the presence of β- D sugar residues, predominantly in their structure (León de Pinto 1991). The specific rotation is an important commercial parameter used for gum identity and purity criteria (Williams and Phillips 2000).

The intrinsic viscosity value for *C. paradisi* gum was relatively low, Table 1, as it has been reported for Venezuelan *Acacia* gums, and it may be related to a possible compact structure (Dror *et al*. 2006). On the other hand, the intrinsic viscosity value for *C. aurantiifolia* gum was similar to that of *A. senegal* gum, a hydrocolloid of wide industrial application (Dror *et al*. 2006).

The gum nitrogen content, and therefore the protein content, was very similar for both *Citrus* species, Table 1, although, their amino acid composition was different, Table 3. Phenylalanine was the main amino acid in *C. aurantiifolia* gum, Table 3, while hydroxyproline is the major component in *C. paradisi* gum, as has been reported for many *Acacia* gums (Beltrán *et al*. 2005). The participation of the last amino acid in the carbohydrate-protein linkage of a

***Table 1**. Analytical dataa of C. aurantiifolia and C. paradisi gum exudates*

Parameter	C. aurantiifolia	C. paradisi
Moisture, %	6.86	3.10
Ash, %[a]	5.10	7.74
Nitrogen, %[a]	0.68	0.54
Hence Protein (%N x 6.25)[a]	4.25	3.40
Specific rotation, ° [b]	+9	+24
η (mL/ g)[b]	22	11
Acidity, %[b,c]	15	37
Neutral sugar composition, %[b]		
Galactose	54	46
Arabinose	25	17
Xylose	2	-
Rhamnose	3	-

[a] Average [b]Corrected for moisture [c] The acidity is represented by uronic acid residues.

***Table 2.** Cationic composition of C. aurantiifolia and C. paradisi gum exudates.*

Cations	Concentration (ppm)	
	C. aurantiifolia	C. paradisi
Calcium	23498	22154
Sodium	22334	12112
Magnesium	1727	4088
Potassium	3875	2809
Aluminium	831	902
Vanadium	406	116
Copper	78	45

Lead was detected as traces.

***Table 3.** Amino acid composition of C. aurantiifolia and C. paradisi gum exudatesa*

Amino acid	*C. aurantiifolia*	*C. paradisi*
Asp	*0.13*	*0.05*
Glu	*0.17*	*0.06*
Hyp	*0.62*	*1.36*
Ser	*0.18*	*0.11*
Gly	*0.10*	*0.07*
His	*n.d.*	*0.12*
Arg + thre	*0.13*	*0.12*
Ala	*0.14*	*0.15*
Pro	*0.15*	*0.28*
Tyr	*0.21*	*0.20*
Val	*0.12*	*0.20*
Met	*0.22*	*0.11*
Cys	*Nd*	*Nd*
Ileu	*0.07*	*0.11*
Leu	*0.13*	*0.17*
Phe	*1.74*	*0.18*
Lys	*0.13*	*0.11*
% protein	*4.26*	*3.40*

[a] mg of amino acid/ mg of total sample

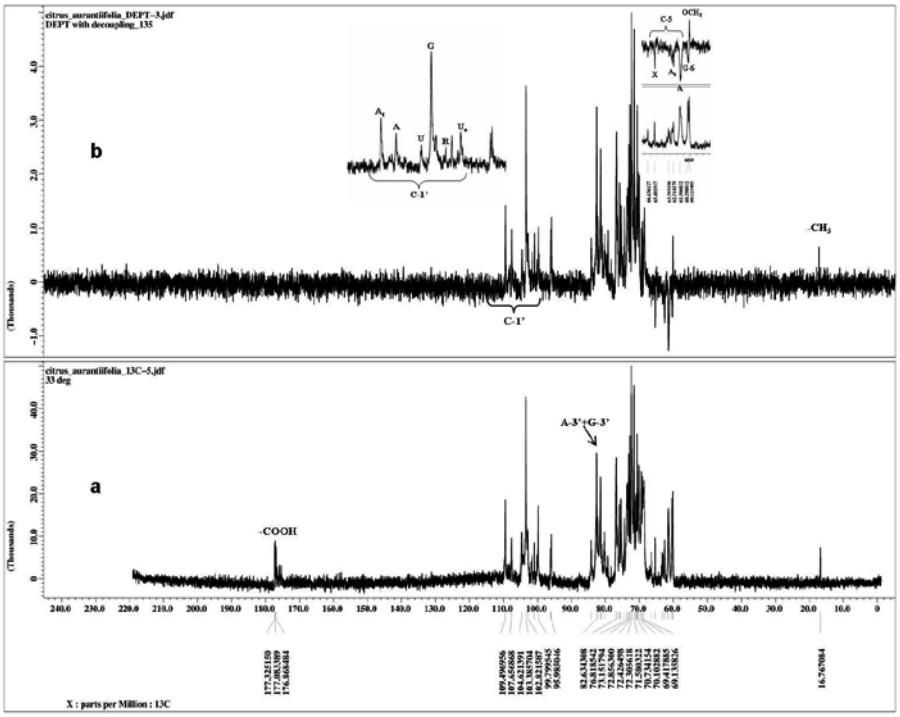

***Figure 1**. a) Carbon-13 NMR b) DEPT-135 of C. aurantiifolia gum. At = terminal α-L-arabinofuranose A = 3-O-α-L-arabinofuranose G = 3-O-β-D-galactopyranose R = rhamnose Ue = 4-OMe-α-D-glucuronic acid U = β-D-glucuronic acid X = β-D-xylopyranose . ' = carbon involucrated in a linkage*

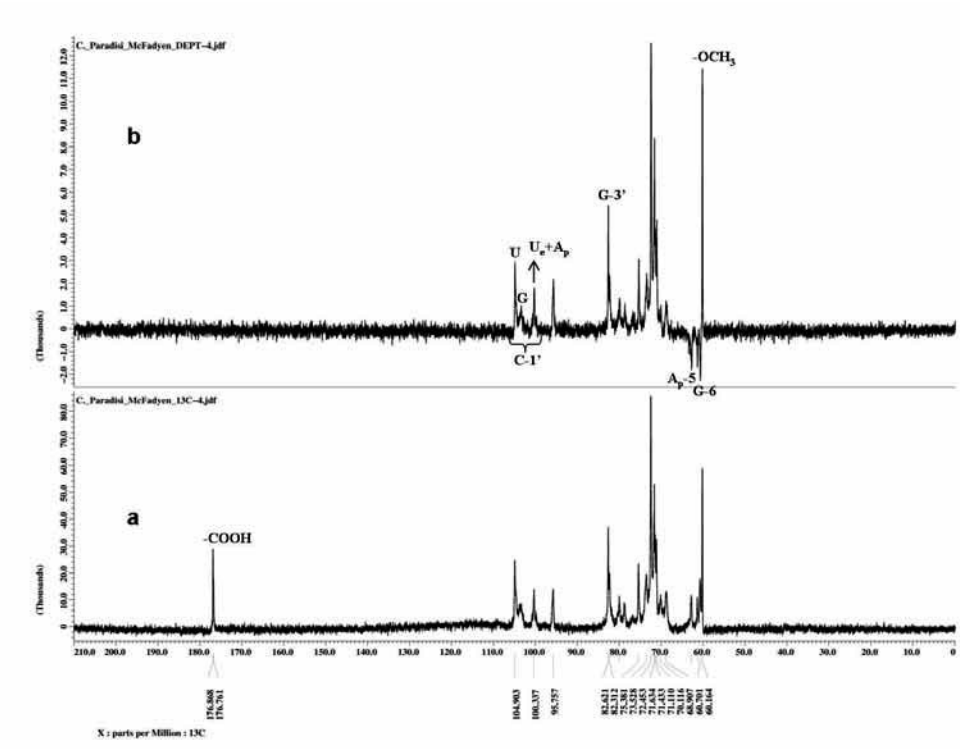

***Figure 2.** a) Carbon-13 NMR b) DEPT-135 of C. paradisi gum. Ap= β-L-arabinopyranose G=3-O-β-D-galacto-pyranose Ue= 4-OMe-α-D-glucuronic acid. U= β-D-glucuronic acid. ' = carbon involucrated in a linkage*

complex arabinogalactan-protein has been described for other gum exudates (Beltrán *et al*. 2005).

Fatty acids were similar for the two *Citrus* gums, Table 4: palmitic acid was the main component. Linoleic and linolenic acids were also present in *C. aurantiifolia* gum. However, the high proportion of saturated fatty acids may confer low nutritional value to these gums.

The NMR Carbon-13 and DEPT-135 spectra, for the gum samples, Figures 1 and 2, are according with the chemical data.

The DEPT-135 spectrum of *C. aurantiifolia*, Fig. 1b, showed, at high field, the non-inverted signal (16.76 ppm) due to a methyl group of rhamnose (Larrazábal *et al*. 2006) and two inverted signals (65.40 and 66.40 ppm) assignable probably to C-5 of β-D-xylopyranose residues (Añez *et al*. 2007). In the anomeric region (99.00 to 109.49 ppm) there are seven signals which correspond to seven types of linkages. The unequivocal signals, at low field (109.49; 107.66 ppm), are probably due to the C-1 of terminal and 3-O-α-L-arabinofuranose residues, respectively, and are related to C-2 (80.28; 81.12 ppm), free C-3 (76.81 ppm), C-3-linked (82.20 ppm), C-4 (84.28; 82.63 ppm) and C-5 (62.51; 61.51 ppm, inverted DEPT-135) resonances of those residues (León de Pinto *et al*. 1998). In addition, there were observed signals due to 3-O-β-D-galactose residues, i.e. C-1 (103.38 ppm), C-3-linked (82.63 ppm) and C-6 (60.30 ppm, inverted DEPT-135), sugar present in other Rutaceae gums (Roy *et al*. 1975). The non-inverted signal (60.12 ppm), due to the methoxyl group, and the corresponding anomeric carbon resonance (99.80 ppm) were assigned to 4-O-methyl-α-D-glucuronic acid (León de Pinto *et al*. 1998). The signals C-2 (75.68 ppm), C-3 and C-5 (76.81 ppm), due to terminal β-D-glucuronic acid (León de Pinto *et al*. 1998), were well resolved into the *C. aurantiifolia* gum spectrum, Figs 1a and 1b.

Table 4. Fatty acid compositiona of C. aurantiifolia and C. paradisi gum exudates

Fatty acid	C. auraniitifolia	C. paradisi
C12:0	31	5.99
C13:0	1.1	1.9
C14:0	4.21	5.6
C16:0	32	45
C16:1n9c	12.9	9.2
C17:0	1.6	4
C18:0	5.8	6
C18:1n9c	7.3	17.2
C18:2n6c	3.4	-
C18:3n3	0.39	-

[a]*Averages*

In other hand, the Carbon-13 NMR spectrum of *C. paradisi* gum, Figs 2a and 2b, contained the resonances due to 3-O-β-D-galactose and 4-O-methyl-α-D-glucuronic acid residues described above for *C. aurantiifolia* gum. The signals which correspond to the β-D-glucuronic acid are not well differentiated in this spectrum. However, the anomeric region showed an unequivocal resonance assignable to C-1 for these kind of residues (León de Pinto *et al*. 1998). No anomeric signals were detected, due to α-L-arabinofuranose. Nevertheless, there was observed a signal (100.34 ppm), which may be related to an inverted one, at high field (63.00 ppm), assignable to β-L-arabinopyranose residues (Bock *et al*. 1984).

The signals that appear at low field (176-177 ppm), in both spectra, are assigned to C-6 of the uronic acids, Figs 1 and 2. The absence of a lower field signal (181 ppm) - assignable to carboxylic groups, partly neutralized by metals - suggests that most of these functional groups in the structure of the studied gums are not substituted (Larrazábal *et al*. 2006).

CONCLUSIONS:

The chemical and Carbon-13 NMR spectra of the analyzed gums showed that the gum structure of C. *aurantiifolia* was more complex than that of C. paradisi. C. *aurantiifolia* gum spectra showed multiple signals attributed to rhamnose, β-D-xylopyranose, terminal and 3-O-α-L-arabinofuranose, 3-O-β-D-galactopyranose, β-D-glucuronic acid and its 4-O-methyl-α-ether while C. paradisi spectra confirmed the absence of xylose and rhamnose and suggested the presence of β-L-arabinopyranose residues, in liu of α-L-arabinofuranose, in its structure.

Citrus gums showed analytical data which could be applied as criteria for their use on diverse industries. In addition, this work represents a contribution to the study of gum exudates from other genera, different of Acacia, as substitute of gum arabic in its multiple industrial applications.

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