ELSEVIER

Food Hydrocolloids 19 (2005) 37–43

FOOD HYDROCOLLOIDS

www.elsevier.com/locate/foodhyd

1D- and 2D-NMR spectroscopy studies of the polysaccharide gum from *Spondias purpurea* var. *lutea*

Omaira Gutiérrez de G[a], Maritza Martínez[a], Lilian Sanabria[a], Gladys León de Pinto[a,*], J. Manuel Igartuburu[b]

[a]*Centro de Investigaciones en Química de los Productos Naturales, Facultad de Humanidades y Educación, Universidad del Zulia, Apartado 526, Maracaibo, Venezuela*

[b]*Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Cádiz, Apartado 40, 11510- Puerto Real, Cádiz, Spain*

Received 11 April 2003; revised 19 September 2003; accepted 19 September 2003

Abstract

Spondias purpurea var. *lutea* (Anacardiaceae) trees located in Venezuela, South America, produce a clear gum very soluble in water. The polysaccharide, from this gum, contains galactosyl, arabinosyl, xylosyl, rhamnosyl and uronic acid residues. Degraded gums A and B were prepared by mild acid hydrolysis and Smith degradation, respectively. Application of 1D- and 2D-NMR spectroscopy to the original gum and its degraded products, in combination with chemical data, led to confirm that the structure of the original polysaccharide contains 3-*O*- and 6-*O*-galactosyl residues, terminal and 3-*O*-α-L-arabinofuranosyl, terminal rhamnosyl residues and uronic acids, represented by β-D-glucuronic acid and its 4-*O*-methyl derivative. It was demonstrated that 2D-NMR spectroscopy is a good tool for structural elucidation of complex heteropolysaccharides.

Keywords: Anacardiaceae; *Spondias purpurea* var. *lutea*; Gum; Polysaccharide; 1D- and 2D-NMR spectroscopy

1. Introduction

Spondias spp. (Anacardiaceae) are very common in tropical regions over the world (Hoyos, 1994). Studies of exudate polysaccharides from *S. dulcis* (Basu, 1980), *S. pinnata* (Ghosal & Thakur, 1981) and *S. mangifera* (Haq & Chintalacharuvu, 1981) showed that galactose, arabinose and galacturonic acid are the main constituents. Gum from other Spondias located in Venezuela have been studied (León de Pinto et al., 2000a; León de Pinto, Martínez, Mendoza, Rivas, & Ocando, 1995). *Spondias purpurea* gum contained, in addition to galactose and arabinose, mannose, xylose and rahmnose (León de Pinto et al., 1996).

A preliminary study of the gum from *S. purpurea* var. *lutea* showed that it contained galactose, arabinose, mannose, xylose and rahmnose (León de Pinto et al., 2000b). The uronic acids present were represented by glucuronic acid and its 4-*O*-methyl derivative, in contrast to galacturonic acid reported for other Spondias gums (Ghosal & Thakur, 1981; Pérez, Sánchez, Pérez, & Vargas, 1995). On the other hand, the fruit from this species (caja fruit), used in the preparation of ice cream and liqueurs, has been tested for volatile components (Allegrone & Barbeni, 1993) and occurrence of *cis*-isomers of provitamin A (Godoy & Rodríguez, 1994) and its physicochemical properties have been reported (Silva, Maia, Oliveira, Figueiredo, & Brasil, 1998).

This work deals with the structural studies of the polysaccharide isolated from *Spondias purpurea* var. *lutea* gum by the application of 1D- and 2D-NMR spectroscopic studies.

2. Materials and methods

2.1. Origin and purification of gum samples

Gum from *Spondias purpurea* var. *lutea*, known in Venezuela as ciruelo amarillo, was collected by the authors

* Corresponding author. Tel.: +58-261-59-6269; fax: +58-261-59-6149.
E-mail address: gleon@luz.ve (G.L. de Pinto).

0268-005X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodhyd.2003.09.007

during the dry season (January–March, 1999) from trees growing in Maracaibo City, Zulia State, Venezuela, South America.

The gum exudate, dissolved in water, was filtered through muslin, Whatman N° 1 and N° 42 filter papers, dialysed against running tap water during two days and recovered by freeze-drying.

2.2. Analytical methods

The neutral sugar composition was determined by HPLC and by a combination of paper chromatography and the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Paper Chromatography was done on Whatman N° 1 and 3 MM papers in two solvent systems (v/v): (a) acetic acid, ethyl acetate, formic acid, water (8:18:3:9); (b) benzene, butan-1-ol, pyridine, water (1:5:3:3, upper layer).The uronic acid content was determined by direct titration with standard sodium hydroxide solution on exhaustively electrodialysed samples and by *m*-hydroxybiphenyl method (Blumenkrantz & Asboe-Hansen, 1973).

The specific rotation of water solutions of samples was determined at 25 °C using a Perkin–Elmer 343 Polarimeter.

2.3. 1D- and 2-Dimensional spectroscopy

^{13}C NMR spectra were recorded with a BRUKER AM-200 spectrometer. Data points (6000–7000) were accumulated overnight at 37 °C with complete proton decoupling. The spectrum width was 5000 Hz. The gum sample (150 mg) was dissolved in deuterium oxide (1 ml). Methanol-d was added as a reference. Two-dimensional spectroscopy was applied using Correlated Spectroscopy (COSY), Heteronuclear Multiple Quantun Coherence (HMQC) and Heteronuclear Multiple Bond Correlation (HMBC) with a BRUKER AM-400 spectrometer.

2.4. Preparation and examination of degraded gums A and B

Purified original gum (6.9 g) was hydrolysed with 5 mM H_2SO_4 (375 ml) for 96 h at 100 °C. The solution was neutralised, filtered, dialysed against distilled water for 24 h, then against running tap water for 48 h and finally freeze-dried. The dialysate, against distilled water, was concentrated and chromatographed in solvents (a) and (b). Degraded gum A (2.15 g) was hydrolyzed (0.25, 0.5, 1 M H_2 SO_4), and then analyzed by chromatography.

Degraded gum B (0.26 g) was obtained by a drastic Smith-degradation of degraded gum A (3.1 g). The polymer was subjected to oxidation (0.25 M $NaIO_4$), reduction (NaBH4) and then to acid hydrolysis (0.5 M H_2SO_4) at room temperature for two days. The product, obtained by freeze-drying, after dialysis, was examined by complete hydrolysis (1 M H_2 SO_4).

3. Results and discussion

The purified acidic polysaccharide, isolated from *Spondias purpurea* var. *lutea* gum, contains galactosyl, arabinosyl, xylosyl, rhamnosyl and uronic acid residues (Table 1). Most of the analytical parameters obtained from the gums from *S. purpurea* var. *lutea* and *S. purpurea* (León de Pinto et al., 1995) are comparable, but *S. purpurea* var. *lutea* gum is levorotatory and has a higher rhamnose content. It is interesting to note the absence of mannose in this gum specimen which was reported previously for *S. purpurea* var. *lutea* gum (León de Pinto et al., 2000b) and for other Spondias gums (León de Pinto et al., 1996; León de Pinto et al., 2000a).

Degraded gum A, prepared by mild acid hydrolysis, contains galactose and uronic acids as the major components (Table 2). During the preparation of this polymer, arabinose, xylose and rhamnose were removed as a possible indication of their position as terminal residues in the original polysaccharide structure.

Degraded gum B, obtained by drastic periodate oxidation (0.25 M) of the degraded gum A, the core of the structure, contains only galactosyl residues (Table 2). This homoglycan has been reported for the backbone of many polysaccharide gums (León de Pinto et al., 1996; León de Pinto, Martínez, & Sanabria, 2001a; León de Pinto, Martínez, Ocando, & Rivas, 2001b).

^{13}C NMR spectrum of the original polysaccharide (Fig. 1) shows resonances due to galactosyl, arabinosyl, rhamnosyl and uronic acid residues (Tables 3 and 4). There are unequivocal signals due to methyl group of rhamnose (17.51 ppm) (Bock, Pedersen, & Pedersen, 1984; León de Pinto, Martínez, Bolaño de, Rivas, & Ocando, 1998a) and to C-6 of uronic acids residues (175.81 ppm) (León de Pinto, Gutiérrez de G, Martínez, Ocando, & Rivas,1998b). The anomeric region contains at least six different glycosidic linkages in the structure of the complex polysaccharide, i.e. the anomeric carbon of 4-*O*-methylglucuronic acid (100.85 ppm) (León de Pinto, Martínez, &

Table 1
Analytical data[a] of the gum from *Spondias purpurea* var. *lutea*

Moisture (%)	12.0
Ash (%)	4.0
Nitrogen (%)	0.45
(N × 6.25)	2.81
Intrinsic viscosity (ml/g)	9
Specific rotation (°)	−35
Equivalent weight (g)	587
Hence uronic acid (%)	30
Sugar composition, after hydrolysis (%):	
Galactose	47
Arabinose	13
Xylose	3
Rhamnose	7

[a] Corrected by moisture.

Table 2
Sugar composition of original gum and degraded products of *Spondias purpurea* var. *lutea*

Sugar composition (%)	Original	Polysaccharide	
		A	B
Uronic acids[a]	30	46	–
Galactose	47	53	100
Arabinose	13	1	–
Xylose	3	Traces	–
Rhamnose	7	–	–

A, degraded gum A; B, degraded gum B.
[a] Uronic acids are represented by glucuronic acid and its 4-*O*-methyl derivative.

Rivas, 1994b), rhamnose (101.46 ppm) (Bock, Perdesen, & Pedersen, 1984; León de Pinto et al., 1998b), 6-*O*- and 3-*O*-β-D-galactosyl residues (103.69; 103.79 ppm) (León de Pinto, Martínez, Ludovic de Corredor, Ocando, & Rivas, 1994a), β-D-glucuronic acid (104.46 ppm) and α-L-arabinofuranosyl residues (109.03; 110.41 ppm) (León de Pinto et al., 1994b).

Degraded gum A, obtained by mild acid hydrolysis of the original polysaccharide, shows a simpler spectrum (Fig. 2) than that exhibited by the original polysaccharide. The signal of methyl group of rhamnose was not observed. The anomeric region contains predominantly two resonances due to C-1 of galactose (103.50 ppm) (León de Pinto et al., 1994a) and glucuronic acid residues (104.73 ppm) (León de Pinto et al., 1994b). The spectrum contains the low intensity signals assigned to arabinofuranose (107.38 ppm) and 4-*O*-methyl ether (100.29 ppm) (León de Pinto et al., 1994b); which confirms removal of the latter residues from the original polysaccharide structure during the preparation of degraded gum A and the presence of reducing sugar residues (92.57 ppm)(León de Pinto et al., 1998b) (Fig. 2).

Others resonances observed in the spectra of the original polysaccharide and its degraded gum A are shown in Tables 3 and 4. There were not observed any resonances attributed to arabinopyranosyl residues.

The DEPT 135 spectrum of degraded gum A shows two inverted signals (61.25; 69.38 ppm) assigned to C-6 of 3-*O*-β-D-galactosyl residues (León de Pinto et al., 2001a) and C-6 linked galactosyl residues (León de Pinto et al., 1994a), respectively. The absence of signal inversion at 60.00 ppm demonstrated the correct signal assignment of the methoxyl group of 4-*O*-methyl-α-D-glucuronic acid (León de Pinto et al., 1994b).

Bidimensional studies of the original polysaccharide from *S. purpurea* var. *lutea* and its degraded gum A showed the resonances of the carbon and proton of 3-*O*- and 6-*O*-galactose and those corresponding to uronic acid residues. HMQC of the original gum (Figs. 3 and 4) showed the unequivocal signal of methyl group (16.0 ppm) and its protons (1.1 ppm) (Agrawal, 1992), which confirmed

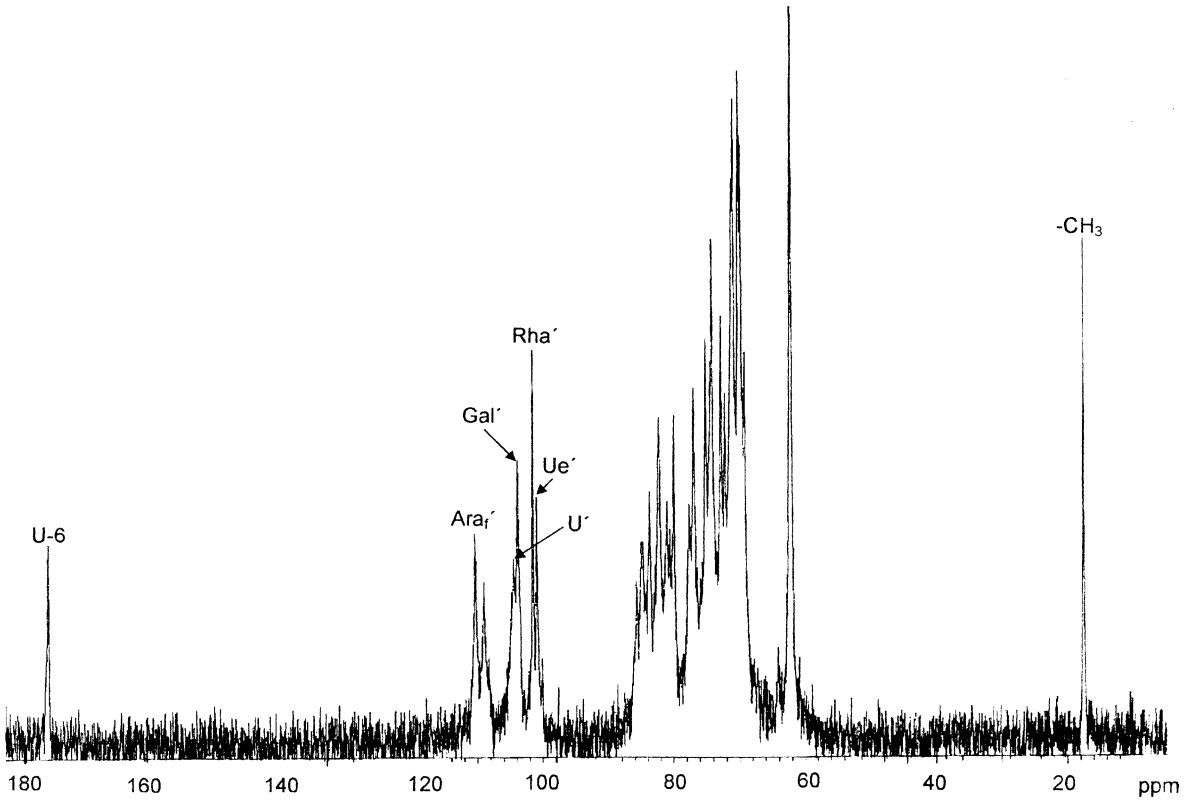

Fig. 1. ^{13}C-NMR spectrum of the original polysaccharide of *Spondias purpurea* var. *lutea*. U, uronic acid residues; U', linked β-D-glucuronic acid; Ue', linked 4-*O*-methyl-α-D-glucuronic acid.

Table 3
^{13}C NMR data[a] of neutral sugar residues of *Spondias purpurea* var. *lutea* gum

Type of linkage	Polymer	C-1	C-2	C-3	C-4	C-5	C-6
→ 3)β-Galp[b](1 →	Original gum	103.79	70.95	82.22	69.83	74.16	62.16
	A	103.80	71.15	83.04	69.50	74.55	62.02
→ 6)β-Galp[b](1 →	Original gum	103.69	70.12	72.21	–	72.89	69.18
	A	103.51	70.13	72.72	–	73.53	69.50
α-Araf[b](1 →	Original gum	110.41	82.22	76.97	84.74	62.16	
→ 3)α-Araf[b](1 →	Original gum	109.03	80.96	83.70	83.70	62.16	

[a] Values relative to methanol-d signal (49.00 ppm). A = degraded gum A. It was observed the unequivocal resonance of the methyl group of rhamnose (17.51 ppm) in the original gum. There are overlapping of some resonances (C-6 of Gal and C-5 of α-Araf).
[b] León de Pinto et al., 1994b.

Table 4
^{13}C NMR data[a] of uronic acid residues of *Spondias purpurea* var. *lutea* gum

Type of linkage	Polymer	C-1	C-2	C-3	C-4	C-5	C-6	4-OMe
β-D-GlcpA[b](1 →	Original gum	104.46	75.08	76.97	72.82	76.97	175.81	
	A	104.73	75.99	76.46	72.72	77.07	176.73	
4-*O*-Me-D-GlcpA[c](1 →	Original gum	100.85	72.21	72.82	82.22	70.95		62.16
	A	100.29	71.64	72.72	83.04	71.15		60.92

[a] Values relative to methanol-d signal (49.00 ppm) A = degraded gum A. The spectra of the original gum and degraded gum A showed high intensity signals (62.16; 60.92 ppm) due to signals overlapping.
[b] León de Pinto et al., 1994b.
[c] León de Pinto et al., 1994a.

the presence of rhamnose residues in the structure. The proton (3.70 ppm) is linked directly to C-6 of 3-*O*-galactose residues (60.8 ppm) (Machytka, Klein, & Egge, 1994) (Fig. 5). Also was observed the proton (3.88 ppm) linked to C-6 of 6-*O*-galactose (69.6 ppm) (León de Pinto et al., 2001a). On the other hand, the anomeric carbons (106.60, 110.01 ppm) due to terminal and 3-*O*-α-L-arabinofuranose residues are linked directly to its protons (4.61, 5.05 ppm)

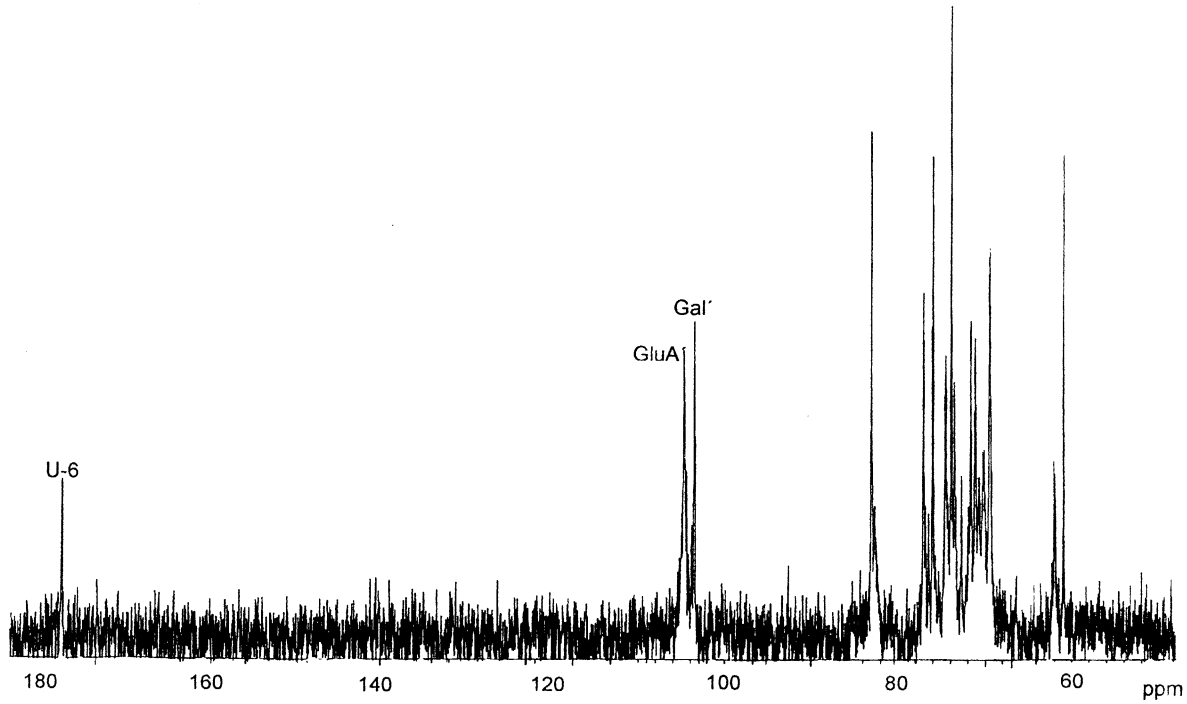

Fig. 2. ^{13}C-NMR spectrum of the degraded gum A of *Spondias purpurea* var. *lutea*. U, uronic acid residues; U', linked β-D-glucuronic acid.

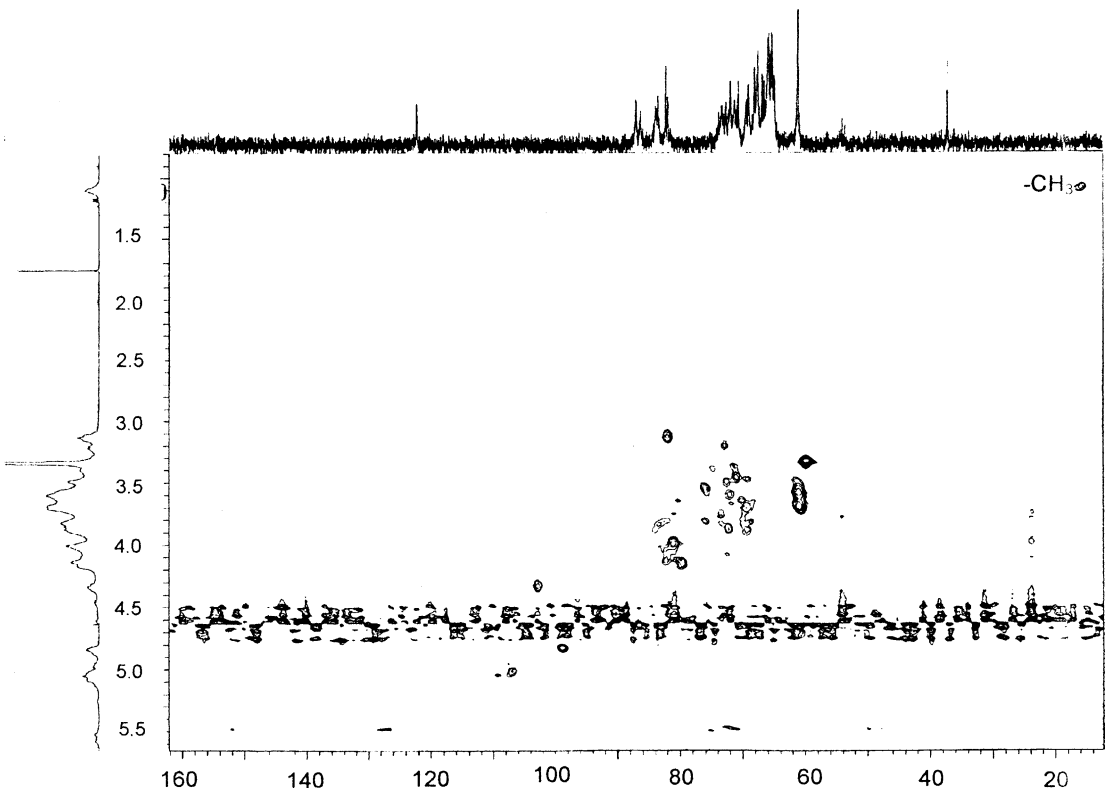

Fig. 3. HMQC spectrum of the original polysaccharide of *Spondias purpurea* var. *lutea* (20–160 ppm).

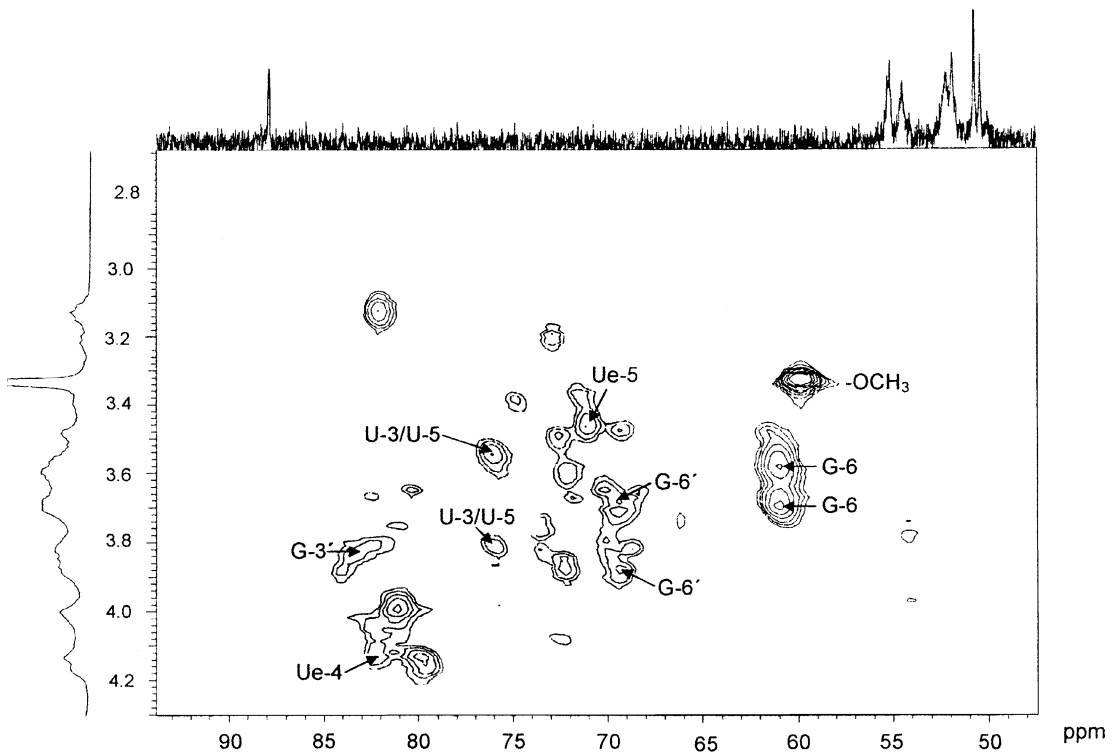

Fig. 4. HMQC spectrum of the original polysaccharide of *Spondias purpurea* var. *lutea*, (50–90 ppm). U, β-D-glucuronic acid; Ue, 4-*O*-methyl-α-D-glucuronic acid; G-3′ = 3-*O*-β-D-galactose; G-6, free C-6 of β-D-galactose; G-6′, 6-*O*-β-D-galactose.

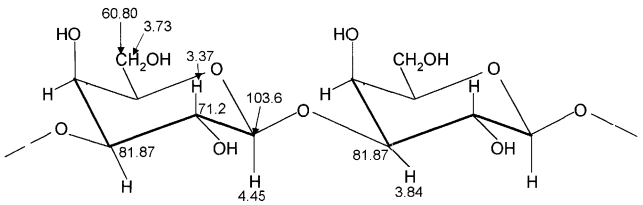

Fig. 5. Resonances (ppm) of 3-*O*-galactose residues present in the polysaccharide of *Spondias purpurea* var. *lutea*.

(Agrawal, 1992). It was also observed that the anomeric carbon of β-D-glucuronic acid (104.8 ppm) is linked to the proton that appears at 4.65 ppm. The chemical shifts for the carbon (60.0 ppm) and the protons (3.33 ppm) of methoxyl group, confirm the presence of 4-*O*-methyl-α-D-glucuronic acid residues (Agrawal, 1992). Carbon resonances of this group was overlapped in the ^{13}C NMR spectra (Figs. 1 and 2, Table 3).

HMQC and HMBC of degraded gum A, corroborated the presence of 3-*O*- and 6-*O*-galactosyl residues, as was discussed in the spectral information of the original polysaccharide; i.e. C-3 of linked galactose (81.87 ppm) (Fig. 5), as was shown in HMBC, is related, through four bonds, to the signal proton (3.73 ppm) which according to HMQC is linked directly to C-6 (60.80 ppm). HMQC also showed that the anomeric carbon (103.6 ppm) has a geminal proton (4.45 ppm) (León de Pinto et al., 2001a). This proton according to COSY is related to H2 (3.37 ppm) which may be correlated, through four bonds, to C-3 linked of another galactose residue. This intergalactosyl residue correlation confirmed the linkage 1,3 type between them (Fig. 5). There is a possible overlapping of the signals due to C-1 (103.6 ppm) of 3-*O*- and 6-*O*-galactosyl residues in the structure. Although, there were observed the resonances due to C-6 of linked galactose and to the protons (3.91 ppm) linked directly to that carbon, according to HMQC spectrum (León de Pinto et al., 1994a).

Heteronuclear bidimensional spectroscopy led also to corroborate the resonances due to uronic acid residues. There were observed, by HMQC, unequivocal signals due to anomeric carbon (104.8 ppm) of β-D-glucuronic acid linked to the proton (4.65 ppm) (Fig. 6A), which according to COSY is unidirectional related to H2 (3.72 ppm). The resonances due to C-3 and C-5 (76.4 ppm) of β-D-glucuronic acid are overlapping; but they are correlated to two protons (3.64, 3.70 ppm). It was also confirmed the presence of 4-*O*-methyl-α-D-glucuronic acid (Fig. 6). The resonances shown by HMQC and HMBC suggest that there are possibly two different kinds of 4-*O*-methyl derivative residues (Fig. 6B and C). The resonance of C-4 (81.67 ppm) of these residues is related through two bonds to the proton (3.73 ppm), which is linked directly to C-5 (70.0 ppm). There is also another resonance (80.83 ppm) due to C-4, related through two bonds, to the proton (3.83 ppm) linked directly to C-3 (73.6 ppm) of the ether. HMQC confirmed the resonances of carbon (60.00 ppm) and protons (3.33 ppm) of the methoxyl group of 4-*O*-methyl-α-D-glucuronic acid.

The NMR experiments carried out led to confirm many interesting structural features of the polysaccharide from *Spondias purpurea* var. *lutea* gum. The unequivocal signal of methoxyl group of 4-*O*-methylglucuronic acid was determined from DEPT 135 spectrum. Signal assignments of resonances of the sugars involved in the structure were confirmed by 2D-NMR techniques (COSY, HMQC and HMBC). The range of 2D-NMR experiments carried out

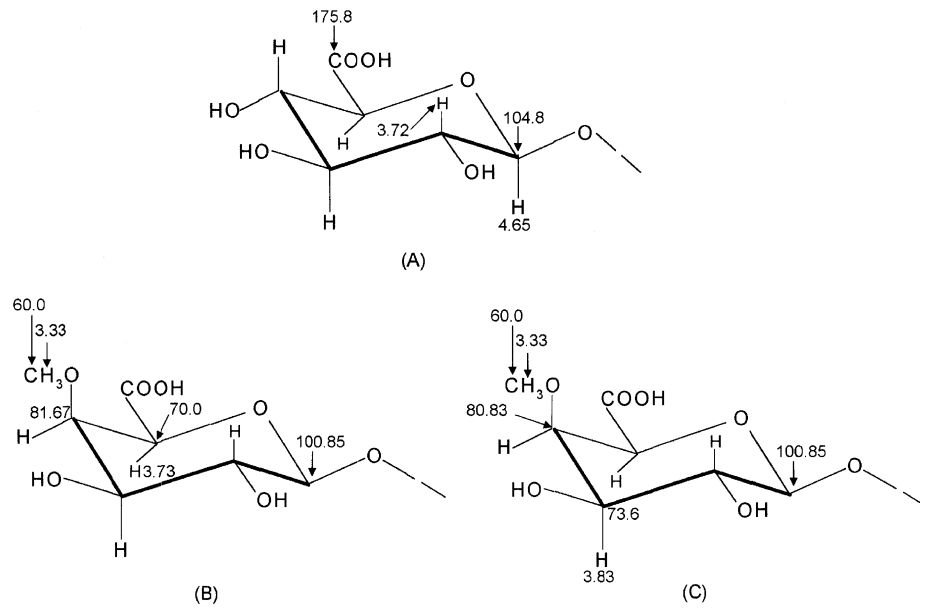

Fig. 6. Resonances (ppm) of uronic acid residues present in the polysaccharide of *Spondias purpurea* var. *lutea* β-D-glucuronic acid (A) and two types of 4-*O*-methyl-α-D-glucuronic acid residues (B,C).

have enabled to observe intracorrelations proton-proton and carbon-proton of galactose and uronic acid residues. It was also confirmed the interconnection between two 1,3-galactose residues.

Acknowledgements

The authors want to express their gratitude to the University of Zulia (CONDES), for the financial support to the research projects, about polysaccharides, from plants located in Venezuela.

References

Agrawal, P.K. (1992). NMR spectroscopy in the structural elucidation of oligosaccharides and glycosides. *Phytochemistry*, *31*, 3307–3330.

Allegrone, G., & Barbeni, M. (1993). Identification of volatile components of caja fruit (*Spondias lutea* L.) and chiral analysis of 3-hydroxy aliphatic esters. *Flavour and Fragance Journal*, *7*(6), 337–342.

Bock, K., Pedersen, C., & Pedersen, H. (1984). ^{13}C-Nuclear magnetic resonance data for oligosaccharides. *Advances in Carbohydrate Chemistry and Biochemistry*, *42*, 193–225.

Basu, S. (1980). Some structural studies on degraded *Spondias dulcis* gum. *Carbohydrate Research*, *81*, 200–201.

Blumenkrantz, N., & Asboe-Hansen, G. (1973). Method for quantitative determination of uronic acid. *Analytical Biochemistry*, *54*, 484–489.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.

Ghosal, P. K., & Thakur, S. (1981). Structural features of the acidic polysaccharide of *Spondias pinnata* gum exudates. *Carbohydrate Research*, *98*, 75–83.

Godoy, H., & Rodríguez, D. (1994). Ocurrence of *cis*-isomers of Provitamin A in Brazilian fruits. *Journal of Agriculture and Food Chemistry*, *42*(6), 1306–1313.

Haq, S., & Chintalacharuvu, V. (1981). Structural investigation of degraded *Spondias mangifera* gum. *Carbohydrate Research*, *94*, 215–224.

Hoyos, J. (1994). *Frutales en Venezuela*. Caracas, Venezuela: Sociedad de Ciencias Naturales, La Salle, p.33.

León de Pinto, G., Gutiérrez de Gotera, O., Martínez, M., Ocando, E., & Rivas, C. (1998b). The molecular characterization of the polysaccharide gum from *Laguncularia racemosa*. *Carbohydrate Polymers*, *35*, 205–213.

León de Pinto, G., Martínez, M., Beltrán, O., Rincón, F., Igartuburu, J. M., & F. Rodríguez, L. (2000a). Structural investigation of the polysaccharide of *Spondias mombin* gum. *Carbohydrate Polymers*, *43*, 105–112.

León de Pinto, G., Martínez, M., Bolaño de, L. M., Rivas, C., & Ocando, E. (1998a). The polysaccharide gum from *Acacia tortuosa*. *Phytochemistry*, *47*, 53–56.

León de Pinto, G., Martínez, M., Ludovic de Corredor, A., Rivas, C., & Ocando, E. (1994a). ^{13}C-NMR studies of *Enterolobium cyclocarpum* gum and its degradation products. *Phytochemistry*, *37*, 1311–1315.

León de Pinto, G., Martínez, M., Mendoza, J. A., Avila, D., Ocando, E., & Rivas, C. (1996). Structural study of the polysaccharide isolated from *Spondias purpurea* gum exudate. *Carbohydrate Research*, *290*, 97–103.

León de Pinto, G., Martínez, M., Mendoza, J. A., Ocando, E., & Rivas, C. (1995). Composition of three Anacardiacea gum exudates. *Biochemical Systematics and Ecology*, *23*, 151–156.

León de Pinto, G., Martínez, M., Ocando, E., & Rivas, C. (2001b). Relevant structural features of the polysaccharide from *Pithecellobium mangense* gum exudates. *Carbohydrate Polymers*, *46*, 261–266.

León de Pinto, G., Martínez, M., & Rivas, C. (1994b). Chemical and spectroscopic studies of *Cercidium praecox* gum exudate. *Carbohydrate Research*, *260*, 17–25.

León de Pinto, G., Martínez, M., & Sanabria, L. (2001a). Structural features of the polysaccharide gum from *Acacia glomerosa*. *Food Hydrocolloids*, *15*, 461–467.

León de Pinto, G., Martínez, M., Sanabria, L., Rincón, F., Vera, A., Beltrán, O., & Clamens, C. (2000b). The composition of two Spondias gum exudates. *Food Hydrocolloids*, *14*(3), 259–263.

Machytka, D., Klein, R. A., & Egge, H. (1994). Reporter resonances in the NMR spectra of oligosaccharides containing sialic acid linked to galactopyranose rings. *Carbohydrate Research*, *254*, 289–294.

Pérez, R. M., Sánchez, J., Pérez, S., & Vargas, R. (1995). An analytical study of gums from *Leucaena glabrata* and *Spondias purpurea*. *Journal of the Science of Food and Agriculture*, *68*, 39–41.

Silva, A., Maia, G., Oliveira, G., Figueiredo, R., & Brasil, I. (1998). Quality Characteristics of pulpy caja fruti juice (*Spondias lutea* L.) obtained by enzymatic-mechanical extraction. *Ciencia e Tecnologia de Alimentos*, *17*(3), 233–236.