ELSEVIER

Carbohydrate Research 290 (1996) 97–103

CARBOHYDRATE RESEARCH

Note

Structural study of the polysaccharide isolated from *Spondias purpurea* gum exudate

Gladys León de Pinto [a,*], Maritza Martínez [a], Julián Alberto Mendoza [b], Dinorah Avila [b], Edgar Ocando [c], Carlos Rivas [c]

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

[b] *Facultad Experimental de Ciencias, Universidad del Zulia, Maracaibo, Venezuela*

[c] *Centro de Química, Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela*

Received 12 December 1995; accepted in revised form 20 April 1996

Keywords: *Spondias purpurea*; Anacardiaceae; Structural study; Gum exudate; ^{13}C NMR spectroscopy

Spondias L. is a small genus (ca. 3 species). These species are widespread in tropical regions of the world [1]. Structural studies of gums from *S. pinnata* and *S. dulcis* have been published [2–4]. *S. cytherea*, *S. purpurea* L., and *S. mombin* L. are disseminated in Venezuela [1]. Analytical data of the two last *Spondias* gums have been published recently [5]. This paper deals with the structural study of the polysaccharide isolated from the *Spondias purpurea* gum.

1. Experimental

Origin and purification of the gum exudate.—The gum polysaccharide from *Spondias purpurea* L. (Anacardiaceae) was collected by Mendoza in northeast Venezuela, near lake Maracaibo, Zulia State, during February–March, 1991. The gum was purified as described previously [6].

General methods.—The specific rotation was measured with an Atago Polax-D polarimeter. The homogeneity of polysaccharides was tested on a column (30 cm) of Fractogel TSK H.W.-65 (S), using 0.1 M NaCl. Molecular weight determination was

* Corresponding author.

PII S0008-6215(96)00127-9

measured by gel filtration chromatography. Descending and ascending paper-chromatography was performed as reported previously [6–8]. ^{13}C NMR spectra of the original gum and its degradation products were recorded with an AM-300 spectrometer in D_2O and the experimental conditions have been reported [7,8]. The procedures for partial and total hydrolyses, isolation, and identification of neutral and acidic components by using HCO_2H (20%) and H_2SO_4 (0.25 M), quantitative analysis of sugars, methylation of the oligosaccharides and polysaccharides, and analysis of the resultant methyl glycosides by GLC have been reported [7–10].

Autohydrolysis experiments.—A solution of purified sample (5%; pH = 6) was heated for 120 h at 100 °C; portions (10 mL) were withdrawn at various intervals (4–120 h) dialyzed in distilled water (80 mL) and the dialyzate analyzed by PC. The polymer was isolated by freeze-drying (82.6%).

Preparation and studies of degraded gums A and B.—Unless otherwise stated, the experimental procedures used for the preparation and examination of degraded gums A and B were the same as those described previously [7–10]. Degraded gum A (1.82 g) was obtained from purified gum (13 g) by mild acid hydrolysis. Preliminary small-scale experiments showed that 96 h were required for the preparation of degraded gum B by periodate oxidation (0.25 M) of degraded gum A. This polymer (5.04 g) yielded degraded gum B (504 mg).

Preparation and studies of polysaccharides I–II.—A series of two sequential Smith degradations was performed with the pure gum as the starting material (60 g) to afford polysaccharide I (15 g), and the latter (12.87 g) yielded polysaccharide II (4.86 g). The experimental conditions for the preparation and examination of these polymers were, in general, as described previously [7–10].

2. Results and discussion

The homogeneity of the gum polysaccharide from *S. purpurea*, $[\alpha]_D$ +50°, molecular weight 1.6×10^6, was checked by gel-filtration, on Fractogel TSK HW-65(S) in 0.1 M sodium chloride. This polymer contains galactose, arabinose, mannose, xylose, rhamnose, and uronic acid residues (Table 1). These sugar acids are represented by

Table 1
Sugar composition [a] of *Spondias purpurea* gum and its degradation products

Polymer	Yield [b] (%)	Sugar composition (%)					
		Gal	Ara	Man	Xyl	Rha	U.A. [c]
Original gum	61	59	9	2	2	2	26
Autohydrolysis polymer	83	60	11	4	—	—	25
Degraded gum A	14	73	—	—	—	—	27
Degraded gum B	10	100	—	—	—	—	—
Polysaccharide I	25	75	12	2	—	—	1
Polysaccharide II	37	100	—	—	—	—	—

[a] Corrected for moisture.
[b] This is the yield of the pure gum obtained from the crude gum.
[c] U.A. = uronic acids.

Table 2
Methylation analysis of *S. purpurea* gum

Methyl ethers	T (min) [a]	Linkage
2,3,4-Me_3-L-Ara	0.46	L-Rhap(1 →
2,3,5-Me_3-L-Ara	0.67	L-Araf(1 →
2,5-Me_2-L-Ara	1.26; (2.20)	→ 3)-L-Araf(1 →
2,3,4-Me_3-D-Gal	1.65	D-Galp(1 →
2,3,6-Me_3-D-Gal	2.50; (3.60) (4.00)	→ 4)-D-Galp(1 →
2,4,6-Me_3-D-Gal	(3.60) (4.00)	→ 3)-D-Galp(1 →
2,3,4-Me_3-D-Gal	5.67; 6.00	→ 6)-D-Galp)1 →
2,4-Me_2-D-Gal	12.22; 13.80	→ 3,6)-D-Galp(1 →
2,3,4-Me_3-D-GlcA [b]	(2.20); 2.75	GlcA(1 →

[a] Relative to methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside. Figures in parenthesis indicate T values of components that were not completely resolved.
[b] As methyl ether methyl glycoside.

galacturonic acid, glucuronic acid, and its 4-methyl ether. The last two uronic acids are very common in the structure of the majority of the gums studied so far [7–10] while galacturonic acid, an unusual feature, has been reported in other *Spondias* gums [2,4] and in *Combretaceae* gums [11,12]. The presence of mannose and xylose has been reported in *Anacardium occidental* gum [13] and *Grevillea* gums [14], although those sugars were not evidenced in gums from *S. pinnata* [2] and *S. dulcis* [3,4]. Partial acid hydrolysis of the original gum led the isolation and further characterization by chromatography, hydrolysis, and methylation analyses of: 6-*O*-(β-D-glucopyranosyluronic acid)-β-D-galactose, R_{Gal} 0.27 (*a*) and 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose, R_{Gal} 0.59 (*a*). Neutral and acidic components, separated by column chromatography, corroborated the presence of the neutral sugars, the aldobiuronic acids mentioned above, and an oligosaccharide characterized by chromatography and methylation analyses as 6-*O*-(β-D-glucopyranosyluronic acid)-6-*O*-(β-D-galactopyranosyl)-D-galactose [15]. Methylation analysis of the original gum (Table 2) reveals that the structure of the gum polysaccharide contains 3-*O*-, 6-*O*-mono, and 3,6-di-*O*-substituted galactose residues, 3-*O*- and terminal L-arabinofuranose, and terminal D-glucuronic acid, and L-rhamnopyranose. Autohydrolysis of the original gum released rhamnose and xylose residues and the preparation of degraded gum A led the removal of the neutral sugars mentioned above, arabinose and mannose. The relatively easy release of these sugars residues of the polysaccharide gum, observed in others gums [7–9], suggests its vulnerability to the reaction conditions. Degraded gum B, obtained by periodate oxidation (0.25 M) of degraded gum A, consists of a β-(1 → 3) galactan (Table 1). This is a common structural feature of the *Spondias* gums [2–4] and the majority of the gums investigated so far [7,8,10]. Partial acid hydrolysis of the degraded gum B led the isolation of an oligosaccharide and its further characterization by chromatography, R_{Gal} 0.45 (*b*), hydrolysis studies, and methylation analysis as a (1 → 3)-linked galactobiose, which has been isolated previously [7,16].

Spondias purpurea gum was subjected to two successive Smith degradations, giving polysaccharides I and II. The composition of polysaccharide II, a galactan, is similar to that of degraded gum B (Table 1).

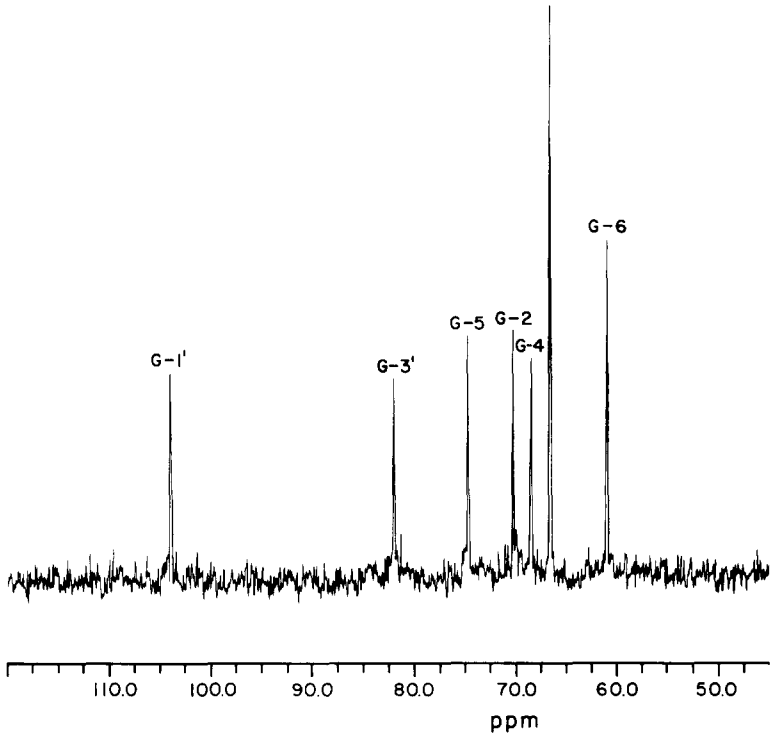

Fig. 1. ^{13}C NMR spectrum of polysaccharide II from *S. purpurea* gum. G = β-D-galactose.

Table 3
^{13}C NMR spectral data [a] of β-D-galactopyranose residues for *S. purpurea* gum and its degradation products

Type of linkage	Polymer	C-1	C-2	C-3	C-4	C-5	C-6
→3)β-D-Galp(1→ [b]		104.20	71.00	82.00	68.60	75.00	60.90
	II	103.90	70.17	81.89	68.34	74.63	60.84
	I	103.88	70.19	81.89	68.33	74.64	60.90
							61.12
	o.g.	103.51 [c]	71.09	81.97	68.32	74.95	61.31
	d.g.A	103.69	70.64	81.97	68.49	74.98	60.95
→6)β-Galp(1→ [b]	I	103.42	70.19	72.48	—	72.73	68.39
	o.g.	103.51 [c]	70.30	72.08	67.61	72.94	69.56
				72.34			
	d.g.A	102.50	70.14	72.52	–	72.71	69.22
						72.89	

[a] Values relative to the signal of 1,4-dioxane (δ 66.67 ppm).
[b] Ref. [9]. II = polysaccharide II; I = polysaccharide I; o.g. = original gum; d.g.A = degraded gum A.
[c] The wide signal at 103.51 ppm is assignable to C-1 of 3-*O*- and 6-*O*-galactose residues.

Table 4
^{13}C NMR spectra data [a] of uronic acid residues for *S. purpurea* gum and its degradation products

Type of linkage	Polymer	C-1	C-2	C-3	C-4	C-5	C-6	4-OMe
4-OMe-α-d-GlcA(1 → [b]		99.7	72.2	73.3	82.7	70.8	—	61.1
	I	99.80	72.18	73.26	82.77	70.71		59.25
						70.96		59.85
	o.g.	99.72	71.70	72.94	81.97	—		59.80
β-D-GlcA(1 → [b]		104.7	75.5	77.1	73.3	77.5	177.5	
	I	103.88 [c]	75.05	76.13	73.26	76.43	176.24	
	o.g.	103.51 [c]	75.70	76.02	72.94	76.02	175.67	
							176.31	
	g.A	103.69 [c]	75.01	76.04	73.50	76.04	175.65	
					73.60			

[a] Values relative to the signal of 1,4-dioxane (δ 66.67 ppm).
[b] Ref. [8]. I = polysaccharide I; o.g. = original gum; d.g.A = degraded gum A.
[c] C-1 of β-D-GlcA may be overlapped with C-1 of β-D-galactose residues.

Rhamnose and xylose, removed in the preparation of the polysaccharide I, may be present as terminal residues in the polysaccharide structure studied. The removal of arabinose, mannose, and uronic acids, during the preparation of the polysaccharides I

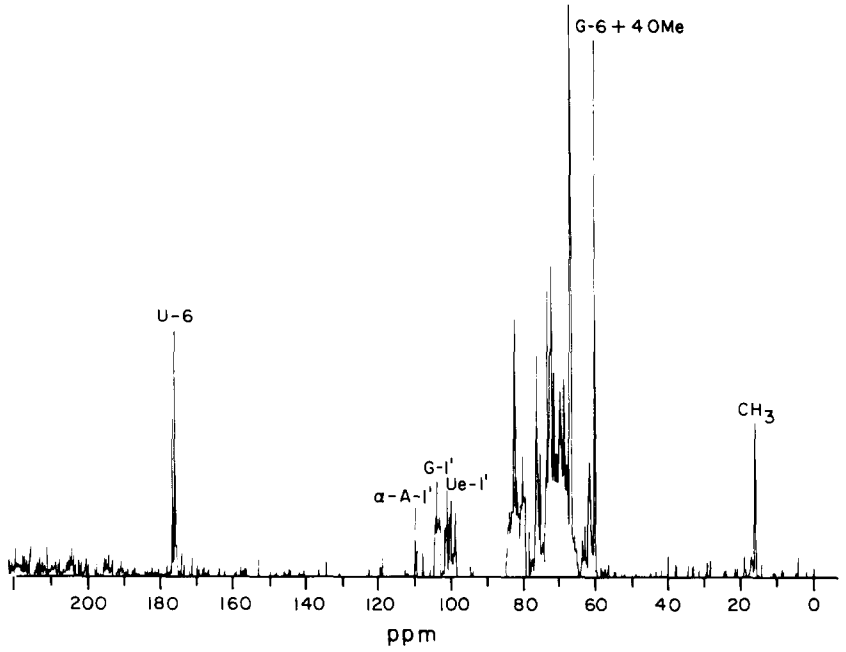

Fig. 2. ^{13}C NMR spectrum of *S. purpurea* gum. G = β-D-galactose; A = α-L-arabinofuranose; U_e = 4-*O*-methyl-α-D-glucuronic acid; U = uronic acids.

and II, suggests the presence of those sugars in the periphery as terminal and non-terminal residues. Partial hydrolysis studies of these polysaccharides led the separation of the galactooligosaccharide, R_{Gal} 0.45 (*b*), observed in degraded gum B, which was characterized as described previously.

The gum polysaccharide from *S. purpurea* and its degradation products, in deuterium oxide, gave well-resolved spectra. The ^{13}C NMR spectrum of polysaccharide II (Fig. 1 and Table 3), shows resonances due to 3-*O*-galactose residues [7,10], as supported by chemical studies (Table 1). The unequivocal signal assignments of this spectrum led the interpretation of the more-complex spectra. The spectrum of polysaccharide I shows, in addition to the signals observed in the previous spectrum, the resonances of residues of α-L-arabinofuranose [9], β-D-glucuronic acid, and its α-4-methyl ether [12] (Table 4). The spectrum of the original gum (Fig. 2) shows the resonances observed in the previous spectra and the unequivocal methyl signal of rhamnose (15.62 ppm) [7,9,10]. The resonances of 6-*O*-galactose residues are well resolved [9], although the C-1 signal of 3-*O*-galactose and 6-*O*-galactose are overlapped. The signals due to 4-*O*-methyl ether groups are unequivocally assigned, while the terminal α-L-arabinofuranose residues were detected by their anomeric carbon signal (109.30 ppm) [9]. It is noteworthy that there is evidence of galactose residues linked through C-3 to terminal α-L-arabinofuranose residues (79.81 ppm) [9]. The lowest-field peaks were due to C-6 atoms of uronic acid residues (175.67; 176.31 ppm).

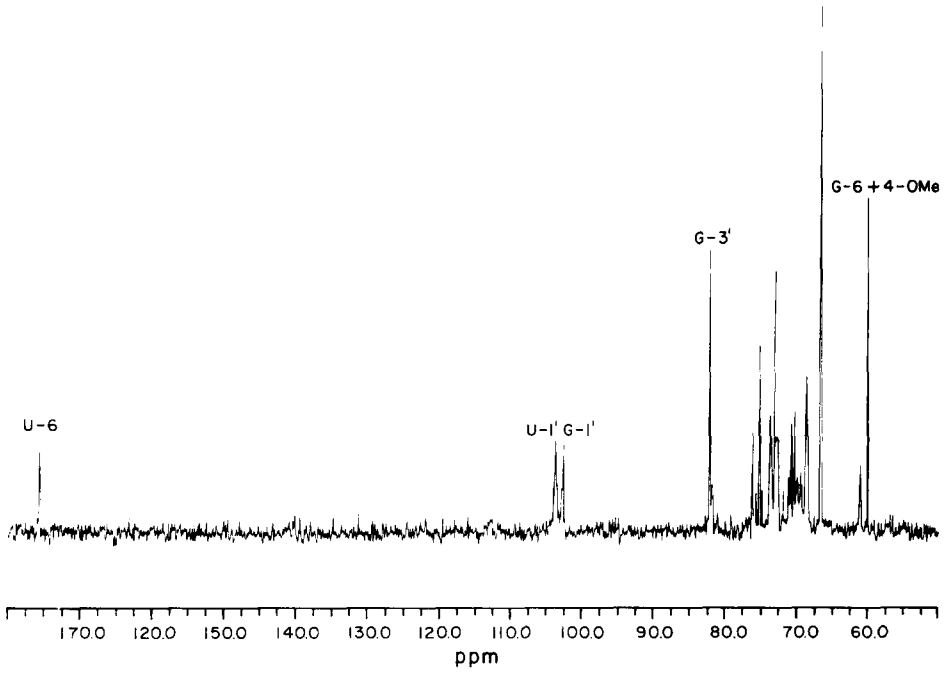

Fig. 3. ^{13}C NMR spectrum of degraded gum A obtained by mild acid hydrolysis of original gum. G = β-D-galactose; U = uronic acids.

The spectrum of degraded gum A (Fig. 3) shows the peaks for 3-*O*- and 6-*O*-galactose residues, and β-D-glucuronic acid residues (Tables 3 and 4). The resonances of α-L-arabinofuranose residues were not observed, as expected because of dearabinosylation during the preparation of this polymer. This fact must be related with the absence of the signal (79.81 ppm) due to C-3 of galactose linked to α-L-arabinofuranose residues.

Spectral evidence supports the existence of 3-*O*- and 6-*O*-galactose residues, β-D-glucuronic acid, and its α-4-methyl ether, linked to C-6 of galactose, and terminal α-L-arabinofuranose residues linked to C-3 of galactose.

Chemical and spectroscopic results thus reveal that the polysaccharide from *S. purpurea* gum is constituted of a β-(1 → 3) galactan, the backbone of the structure. The chains of arabinose are up to two units, while mannose, xylose, rhamnose, and uronic acid exist predominantly as terminal residues.

References

[1] J. Hoyos, *Frutales de Venezuela*, Sociedad de Ciencias Naturales La Salle, Caracas, 1989, p 33.
[2] P.K. Ghosal and S. Thakur, *Carbohydr. Res.*, 98 (1981) 75–83.
[3] S. Basu, *Carbohydr. Res.*, 81 (1980) 200–201
[4] S. Basu and C.V.N. Rao, *Carbohydr. Res.*, 94 (1981) 214–224.
[5] G. León de Pinto, M. Martínez, J.A. Mendoza, C. Rivas, and E. Ocando, *Biochem. Syst. Ecology.*, 23 (1995) 151–156.
[6] M. Martínez, G. León de Pinto, and C. Rivas, *Phytochem.*, 31 (1992) 535–536.
[7] G. León de Pinto, S. Alvárez, M. Martínez, A. Rojas, and E. Leal, *Carbohydr. Res.*, 239 (1993) 257–265.
[8] G. León de Pinto, M. Martínez, and C. Rivas, *Carbohydr. Res.*, 260 (1993) 17–25.
[9] G. León de Pinto, M. Martínez, A. Ludovic de Corredor, C. Rivas, and E. Ocando, *Phytochem.*, 37 (1994) 1311–1315.
[10] G. León de Pinto, *Carbohydr. Res.*, 220 (1991) 229–242.
[11] D.M.W. Anderson and P.C. Bell, *Carbohydr. Res.*, 57 (1977) 215–221.
[12] D.M.W. Anderson, J.R.A. Millar, and W. Weiping, *Food Addit. Contam.*, 8 (1991) 423–436.
[13] D.M.W. Anderson, P.C. Bell, and J.P.A. Millar, *Phytochem.*, 13 (1974) 2189–2193.
[14] D.M.W. Anderson and G. Pinto, *Carbohydr. Res.*, 2 (1985) 19–24.
[15] J.L. Di Fabio, G.G.S. Dutton, and P. Moyna, *Carbohydr. Res.*, 99 (1982) 41–50.
[16] G. León de Pinto and M. Martínez, *Acta Científica Venezolana.*, 46 (1995) 1–4.