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Structural features of an arabinogalactan gum exudates from *Spondias dulsis* (Anacardiaceae)

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Abstract

The tree *Spondias dulcis*, located in Venezuela, exudes a light-brown gum. The polysaccharide, isolated from the original gum, contains galactose, arabinose, mannose, rhamnose, glucuronic acid, and its 4-*O*-methyl derivative. Application of chemical methods, in combination with 1D and 2D NMR spectroscopy afforded interesting structural features of the gum polysaccharide. The unequivocal presence of rhamnose in the polymer structure was confirmed by chemical and spectral data [^{1}H (1.03 ppm); ^{13}C (16.92 ppm)]. Also confirmed was the existence of 3-*O*- and 6-*O*-substitutes galactose residues by the spectral data correlations observed in Heteronuclear Multiple Quantum Coherence (HMQC) and Heteronuclear Multiple Bond Correlation (HMBC). Also observed were unequivocal resonances for β-D-glucuronic acid and its 4-*O*-methyl derivative, and the presence of 3-*O*-α-L-arabinofuranose and 3-*O*-β-L-arabinopyranose residues.

Keywords: *Spondias cytherea*; Anacardiaceae; Gum exudates; Heteropolysaccharide; Bidimensional NMR spectroscopy

1. Introduction

Species of the tree *Spondias* (Anacardiaceae) are widespread in the tropical region of the world.[1] *S. purpurea*, *S. mombin*, *S. purpurea* var. *lutea* and *S. dulcis*, located in Venezuela, produce gum exudates.[2] The polysaccharide isolated from these gums contains galactose, arabinose, xylose, rhamnose, and uronic acid residues, comprising glucuronic acid and its 4-*O*-methyl analogue.[3,4]

Previous studies have shown that the polysaccharides from *S. dulcis*,[5] *S. pinnata*[6] and *S. mangifera*[7] contain galactose, arabinose, and galacturonic acid.

This work shows relevant structural features of the polysaccharide from *S. dulcis* gum on the basis of chemical and 1D and 2D NMR spectroscopic studies.

2. Results

Analytical data of *Spondias dulcis* gum are presented in Table 1[1], and the sugar composition of the original gum and its degradation products appear in Table 2.[13]C Spectral data of the original polysaccharide are shown in Table 3. Bidimensional spectroscopy studies, HMQC, HMBC (Figs. 1 and 2), confirmed the signal assignments of 3-*O*- and 6-*O*-β-D-galactopyranose; 3-*O*-α-L-arabinofuranose, and 3-*O*-β-L-arabinopyranose; and those of the uronic acid residues present (Figs. 3–5). HMQC of the degraded gum B and a possible model for the backbone of the polysaccharide, appear in Figs. 6 and 7, respectively.

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[1] The D and L attributions were not established directly but follow those generally observed in polysaccharides from plant gums.

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Table 1
Analytical data [a,b] of *Spondias dulcis* gum exudates

Parameters	
Moisture (%)	9.4
Ash (%)	4.7
Nitrogen (%)	0.63
(N × 6.25)	3.94
Intrinsic viscosity (mL/g)	63
Specific rotation (°)	−7
Equivalent weight (g)	1273
Hence uronic acid (%)	14
Sugar composition after hydrolysis (%)	
Galactose	50
Arabinose	12
Mannose	6
Xylose	1
Rhamnose	17

[a] Corrected for moisture.
[b] Ref. 3.

3. Discussion

The gum from *Spondias dulcis*, clear brown in color, soluble in water, is levorotatory, as has been reported for others *Spondias* gums, except for *S. purpurea* gum which is dextrorotatory. The limit viscosity of *S. dulcis* gum is higher than that of other *Spondias* gums[5,7] and the nitrogen content is low, as reported for other *Spondias* gums.[3] The polysaccharide contains galactose as major component, along with arabinose, mannose, xylose and rhamnose as neutral sugars. The uronic acids are represented by glucuronic acid and its 4-*O*-methyl derivative (Table 1). This sugar composition differs from those published from other Indian specimen of *S. dulcis*,[5] *S. pinnata*[6] and *S. mangifera*[7] gums. Xylose and glucuronic acid were not detected in these gums.

Preparation of degraded gum A, by mild acid hydrolysis of the original gum from *S. dulcis* removed rhamnose, arabinose, xylose, and mannose residues. Degraded gum B, obtained by drastic oxidation of degraded gum A (0.25 M $NaIO_4$), is a (1→3)-β-galactan, the backbone of the structure. Also observed were residual uronic acid residues, which were difficult to remove (Table 2). Also prepared was the polysaccharide I, by a single Smith-degradation of the original gum. The sugar composition of the degraded products is given in Table 2.

Application of 1D and 2D NMR spectroscopy established interesting structural features of the polysaccharide from *S. dulcis* gum. ^{1}H NMR of the original polysaccharide showed the unequivocal signal of methyl protons of rhamnose (δ 1.03 ppm).[8] The ^{13}C NMR spectrum of this polymer (Table 3), shows the methyl resonances of rhamnose (16.92 ppm), 3-*O*- and 6-*O*-β-D-galactose,[9] and uronic acid residues.[10] The signal at 78.42 ppm that appears in the spectrum may be

Table 2
Sugar composition of the original polysaccharide of *S. dulcis* and its degradation products

Polymer	Gal	Ara	Man	Xyl	Rha	U.A.
Original gum	50	12	6	1	17	14
Degraded gum A	69	6	2	–	–	23
Degraded gum B	91	–	–	–	–	9
Polysaccharide I	54	26	6	–	–	14

U.A., uronic acid residues.

Table 3
^{13}C NMR spectral data [a] (ppm) of the polysaccharide from *Spondias dulcis* gum

Type of linkage	C-1	C-2	C-3	C-4	C-5	C-6	4-OMe
→1)β-D-Gal (3→ [b]	103.64 103.86	71.18	82.24	69.39	74.84	61.17	
→1)β-D-Galp (6→ [b]	102.68	70.08	72.53	–	73.11	68.72	
β-D-GlcA (1→ [c]	103.86	75.26	76.26	73.70	76.26	175.91 176.29	
4-*O*-Methyl-α-D-GlcA [c]	–	72.53	73.70	82.24	70.86	175.91 176.29	60.14

[a] Values relative to the signal of 1,4-dioxane (δ 66.67 ppm).
[b] Ref. 9.
[c] Ref. 10.

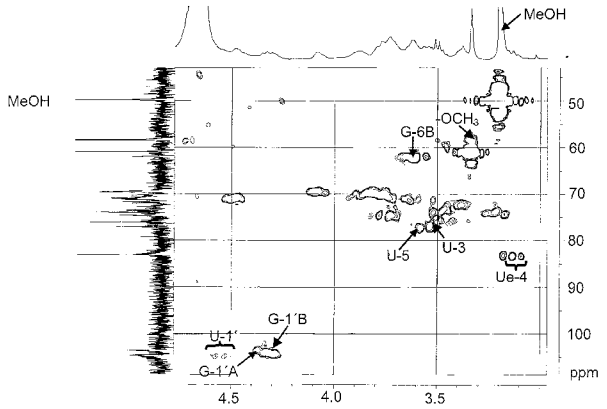


Fig. 1. HMQC of the original polysaccharide from *S. dulcis* gum. A = 3-*O*-β-D-galactose, B = 6-*O*-β-D-galactose, U = β-D-glucuronic acid, Ue = 4-OMe-α-D-glucuronic acid.

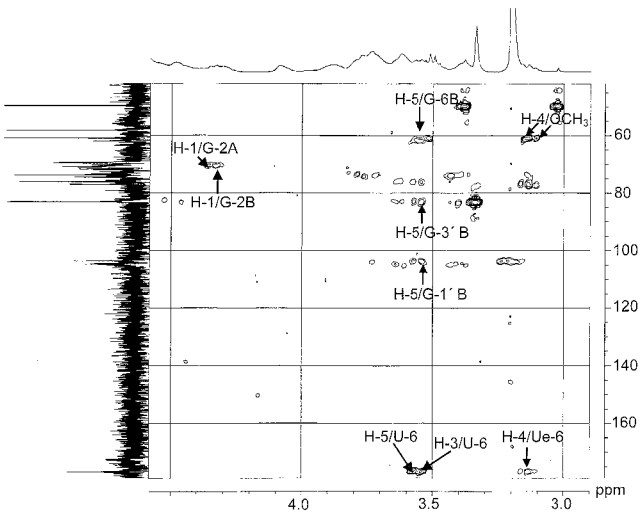


Fig. 2. HMBC of the original polysaccharide from *S. dulcis* gum. A = 3-*O*-β-D-galactose, B = 6-*O*-β-D-galactose, U = β-D-glucuronic acid, Ue = 4-*O*-methyl-α-D-glucuronic acid.

assignable to 3-*O*-β-D-galactose linked to terminal α-L-arabinofuranose residues.[9]

Bidimensional spectroscopy studies, by HMQC and HMBC (Figs. 1 and 2) confirmed the data shown in Table 3. Observed spectral correlations (by HMBC, Fig. 2) support the presence of 3-*O*-β-D-galactose residues in the gum structure (Fig. 3(A)); and showed that the signal of C-6 (61.73 ppm) of these residues is related, through two bonds, to the proton (3.54 ppm). This proton, according to HMQC (Fig. 1) is linked directly to C-5 of galactose (74.22 ppm) and it is related, through three bonds, to the anomeric carbon (104.09 ppm). In addition, C-3 of linked galactose is related, through three bonds, to the same proton. HMBC also showed the correlation, through four bonds, between the C-4 resonance (70.00 ppm) and the proton (4.32 ppm); this proton is linked directly to the anomeric carbon (Figs. 1 and 3(A)). Some resonances of 6-*O*-β-D-galactose were observed (Fig. 3(B)). The signal (70.00 ppm) due to C-2 of these residues, is related, through two bonds, to the proton that appears at 4.36 ppm (Fig. 2). This proton is linked directly to anomeric carbon (103.91 ppm), as shown by HMQC (Fig. 1), although the resonance of linked C-6 of galactose was not differentiated in the bidimensional spectra of the original gum.

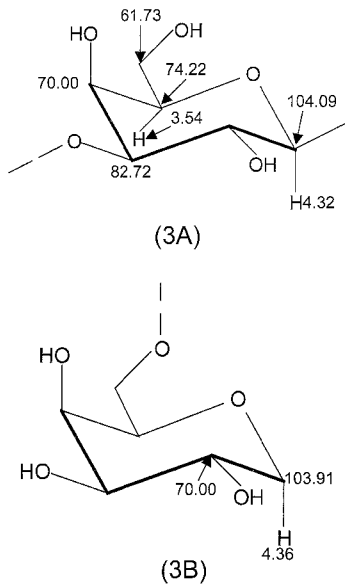


Fig. 3. Signal assignments of 3-*O*- and 6-*O*-galactose residues of the original polysaccharide from *S. dulcis* gum. A = 3-*O*-Gal; B = 6-*O*-Gal.

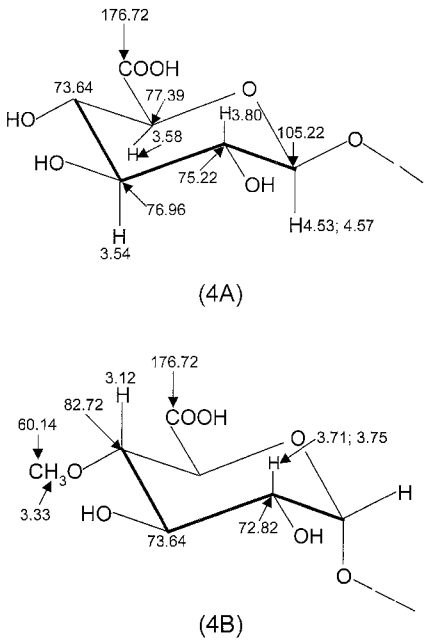
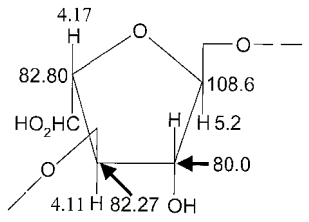
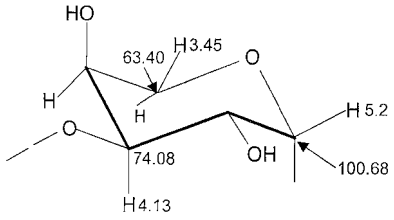


Fig. 4. Signal assignments of uronic acid residues. A = β-D-glucuronic acid; B = 4-*O*-methyl-α-D-glucuronic acid.



(5A)



(5B)

Fig. 5. Signal assignments of arabinose residues of degraded gum A from *S. dulcis* gum. A = 3-*O*-α-L-arabinofuranose; B = 3-*O*-β-L-arabinopyranose residues.

Also confirmed, by HMBC, were the resonances due to uronic acid residues, represented by glucuronic acid and its 4-*O*-methyl derivative (Fig. 4(A and B)). The resonance of C-6 of uronic acids (176.72 ppm) is related to three protons (3.12, 3.54, 3.58 ppm, Fig. 2). The two latter protons (3.54, 3.58 ppm) are linked directly to C-3 (76.96 ppm) and C-5 (77.39 ppm), respectively, of β-D-glucuronic acid residues (Fig. 1). The proton (3.58 ppm) linked to C-5 is related, through three bonds, to the anomeric carbon (105.22 ppm). This carbon, according to HMQC (Fig. 1) is linked to the proton which appears at two environments (4.53, 4.57 ppm). The signal (73.64 ppm) due to C-4 of these residues is related through three bonds, to the proton (3.80 ppm) linked directly to C-2 (75.22 ppm, Figs. 1 and 2). Signal assignments are shown in Fig. 4(A).

The unequivocal signal of methyl protons (3.33 ppm), linked directly to the carbon that appears at 60.14 ppm accords with the presence of 4-*O*-methyl-α-D-glucuronic acid (Fig. 4(B)). The methyl protons, according to HMBC (Fig. 2) are related, through three bonds, to the carbon (82.72 ppm) which has the methoxyl group appended. HMBC showed that the proton (3.12 ppm) linked directly to that carbon (82.72 ppm), is related, through three bonds, to C-6 (176.72 ppm, Figs. 1 and 2). It was also observed that the C-3 resonance (73.64 ppm) is related, through two bonds, to a proton in two environments (3.71, 3.75 ppm), which are directly linked to C-2 (72.82 ppm, Fig. 4(B)).

The resonances described for the uronic acid residues allowed differentiation of the β-D-glucuronic acid and its 4-*O*-methyl-α-derivative (Fig. 4(A and B)).

Bidimensional studies of degraded gum A, obtained by mild acid hydrolysis of original gum, showed the correlations discussed previously in the original gum (Figs. 1 and 2). Additional resonances accord with to the presence of 3-*O*-α-L-arabinofuranose and 3-*O*-β-L-arabinopyranose residues (Fig. 5).[9,11,12] The anomeric carbon (108.6 ppm) assignable to 3-*O*-α-L-arabinofuranose residues is linked directly to a proton (5.20 ppm) that appears at relatively low field. Also observed by HMQC were the secondary carbons and their protons. These resonances and those due to 3-*O*-β-L-arabinopyranose residues are shown in Fig. 5.

Degraded gum B, obtained by oxidation (0.5 M $NaIO_4$) of degraded gum A, represents a core of the structure, and is basically a β-(1→3) galactan, as reported for others *Spondias*[6,13] and for all the *Acacia* gums studied so far.[14–17]

Spectral data observed by HMQC (Fig. 6) confirmed that the structural backbone is predominantly constituted of 3-*O*-β-D-galactose residues and also showed the resonances due to 6-*O*-β-D-galactose. Also observed were the resonances of the proton (3.54 ppm), linked directly to C-3 (76.96 ppm) of β-D-glucuronic acid (Fig. 4(A)). The resonances of the proton (3.33 ppm) and carbon (60.14 ppm) of the methoxyl group and those due to the proton (3.12 ppm) linked directly to C-4 (82.72 ppm) confirmed the presence of 4-*O*-methyl-α-D-glucuronic acid (Fig. 4(B)). This spectral evidence indicates that degraded gum contains some remanent uronic acid residues which were not removed during the oxidation process; this has been observed for other gums.[14,17] The unequivocal signal of C-6 linked galactose (68.50 ppm) is observed it may be involved with uronic acid residues.

Signal assignments of the constituent residues of degraded gum B, the nucleus of the structure, are shown in Fig. 7.

Polysaccharide I, obtained from the original polysaccharides from *S. mombin* gum by a single Smith degradation showed spectral data that confirm those discussed previously.

4. Experimental

4.1. Origin and purification of gum samples

Gum specimens from *Spondias dulcis* Sol. ex Parkinson syn., *S. cytherea*, Sonnerat), known in Venezuela as Jobo de la India, were collected by the authors during the dry season (January–March, 1996) from trees growing in Maracaibo City, Zulia State, Venezuela, South America, after injuries were made at the trunk level.

The brown gum sample dissolved readily in H_2O and the solution was filtered through muslin, and Whatman

No. 1 and 42 filter papers, dialyzed against running tap water for 2 days, and the gum recovered by freeze-drying.

4.2. Analytical methods

The sugar composition of the neutral sugars was determined by high performance liquid chromatography (HPLC) and by a combination of p.c. and the phenol-H_2SO_4 method.[18] Paper chromatography was carried out on Whatman No. 1 and 3MM papers with the following solvent systems (v/v): (*a*) 1:5:3:3 benzene–butan-1-ol–pyridine–water (upper layer); (*b*) 3:18:1:4 AcOH–EtOH–formic acid–water and (*c*) 1:10:5 butan-1-ol–EtOH–0.1 M HCl. Before solvent (*c*) was used, the paper was pretreated with 0.3 M NaH_2PO_4 soln and allowed to dry. The nitrogen content was determined by the method of Kjeldahl.

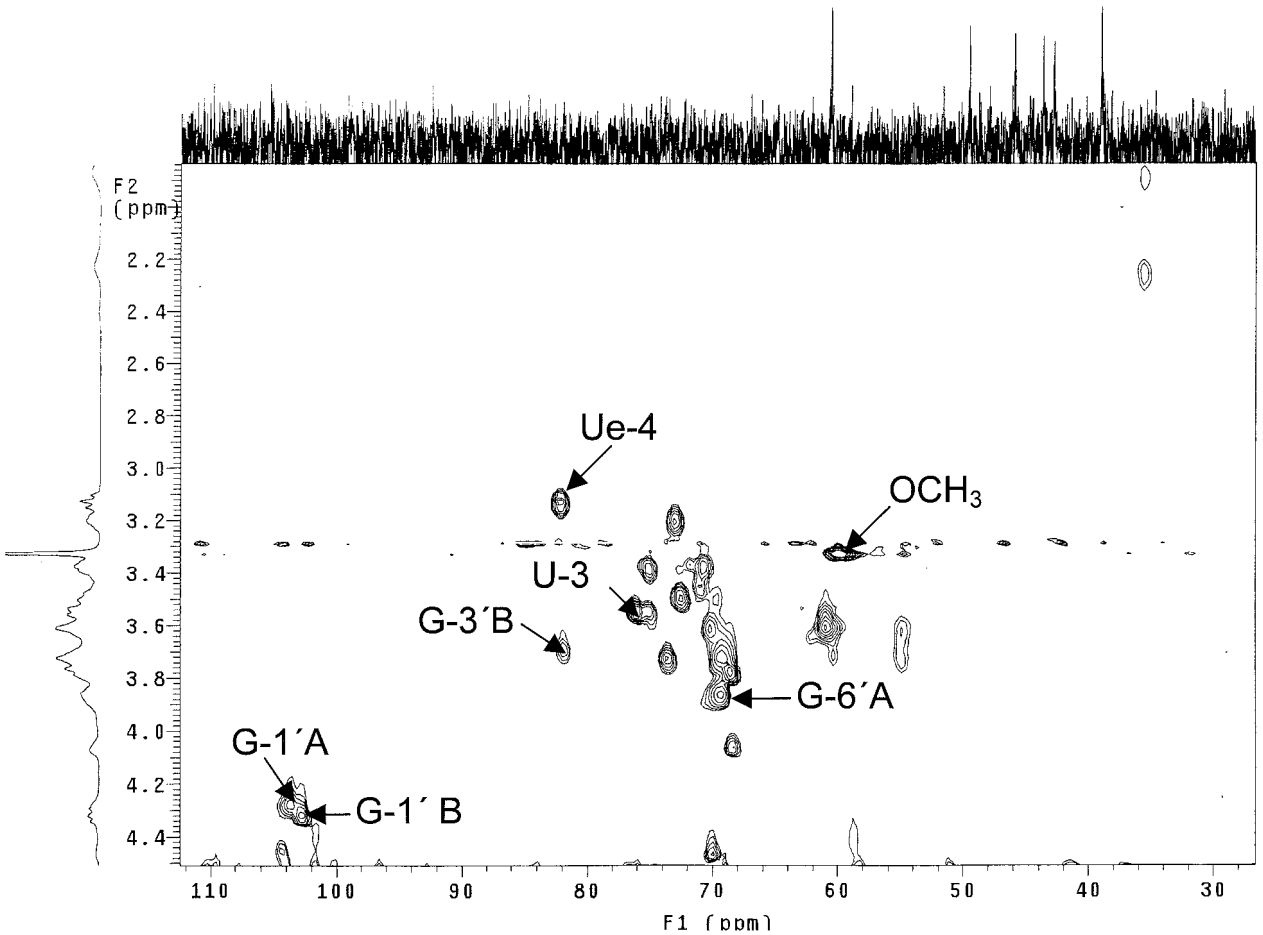


Fig. 6. HMQC of degraded gum B, obtained by oxidation of degraded gum A of *S. dulcis*. A = 3-*O*-β-D-galactose, B = 6-*O*-β-D-galactose, U = β-D-glucuronic acid.

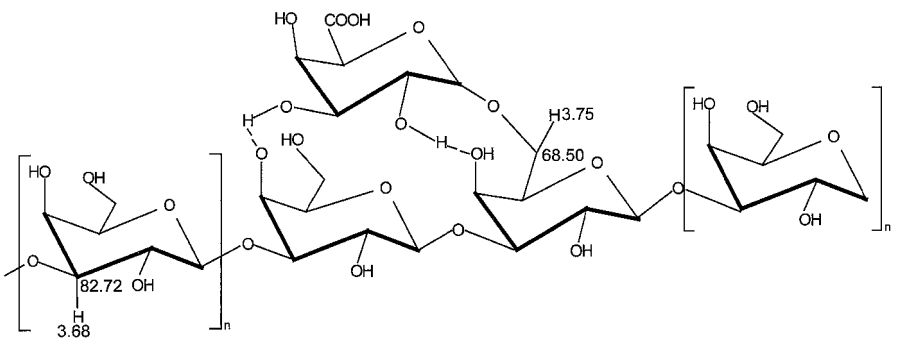


Fig. 7. A possible model for the backbone of the polysaccharide from *S. dulcis* gum.

The ^{13}C NMR spectra were recorded with a AM-200 spectrometer. Data points (6000–7000) were accumulated overnight at 37 °C with complete proton decoupling. The spectrum width was 5000 Hz and it was calibrated by the addition of methanol d_4 to the sample. The original gum (100 mg) was dissolved in deuterium oxide (1 mL).

Bidimensional spectroscopy was performed using Heteronuclear Multiple Quantum Coherence (HMQC) and Heteronuclear Multiple Bond Correlation (HMBC) with a Bruker AM-400 spectrometer.

4.3. Preparation and studies of degraded gums A and B

Purified gum (2.0 g) was hydrolyzed with 5 mM H_2SO_4 (290 mL) for 96 h at 100 °C. After cooling, neutralization, and filtration, the solution was dialyzed against distilled water for 24 h and then against running tap water for a further 48 h, and freeze-dried to obtain the degraded gum A (1.09 g). Preliminary small-scale experiments established suitable conditions for preparing degraded gum B. Degraded gum A (0.89 g) was dissolved in water (26 mL) and 0.5 M $NaIO_4$ (26 mL) was added. After 72 h in the dark, at room temperature (rt), the reaction was stopped by the addition ethylene glycol (0.12 mL). The product was reduced with $NaBH_4$ (0.52 g), dialyzed against running tap water for 48 h and hydrolyzed (2 M H_2SO_4), at rt, after dialysis and lyophilization degraded gum B was obtained (0.14 g).

4.4. Preparation and studies of polysaccharide I

Preliminary small-scale experiments indicated that 0.25 M $NaIO_4$ soln and an oxidation time of 96 h were required for *Spondias dulcis* gum. One Smith degradation was performed using the pure gum as the starting material (16 g) to afford polysaccharide I (4.08 g). The experimental conditions for the preparation and examination of this polymer were, in general, as previously described.[19]

Acknowledgements

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References

1. Hoyos, J. *Guia de arboles de Venezuela*, Monografía 32, Soc. Ciencias Naturales La Salle, Caracas, Venezuela, 1994, pp. 384.
2. Clamens, C.; Rincón, F.; Sanabria, L.; Vera, A.; León de Pinto, G. *Food Hydrocol.* **2000**, *14*, 253–257.
3. León de Pinto, G.; Martínez, M.; Mendoza, J.; Ocando, E.; Rivas, C. *Biochem. Syst. Ecol.* **1995**, *23*, 151–156.
4. León de Pinto, G.; Martínez, M.; Sanabria, L.; Rincón, F.; Vera, A.; Beltrán, O.; Clamens, C. *Food Hydrocol.* **2000**, *14*, 259–263.
5. Basu, S. *Carbohydr. Res.* **1980**, *81*, 200–201.
6. Ghosal, P. K.; Thakur, S. *Carbohydr. Res.* **1981**, *98*, 75–83.
7. Haq, S.; Chintalacharuvu, V. *Carbohydr. Res.* **1981**, *94*, 215–224.
8. León de Pinto, G.; Martínez, M.; Sanabria, L. *Food Hydrocol.* **2001**, *15*, 461–467.
9. León de Pinto, G.; Martínez, M.; Ludovic de Corredor, A.; Rivas, C.; Ocando, E. *Phytochemistry* **1994**, *37*, 1311–1315.
10. León de Pinto, G.; Martínez, M.; Rivas, C. *Carbohydr. Res.* **1994**, *260*, 17–25.
11. Mizutani, K.; Kasai, R.; Nakamura, M.; Tanaka, O.; Matsuura, H. *Carbohydr. Res.* **1989**, *185*, 27–38.
12. del Globol, C.; Gorin, P.; Jones, C.; Iacomin, M. *Phytochemistry* **1998**, *47*, 1207–1214.
13. León de Pinto, G.; Martínez, M.; Mendoza, J. A.; Avila, D.; Ocando, E.; Rivas, C. *Carbohydr. Res.* **1996**, *290*, 97–103.
14. Martínez, M.; León de Pinto, G.; Rivas, C.; Ocando, E. *Carbohydr. Polym.* **1996**, *29*, 247–252.
15. Anderson, D. M. W.; Creee, G. M. *Carbohydr. Res.* **1968**, *57*, 215–221.
16. Fincher, G. B.; Stone, B. A.; Clarke, A. E. *Annu. Rev. Plant Physiol.* **1983**, *34*, 47–70.
17. León de Pinto, G.; Martínez, M.; Ocando, E.; Rivas, C. *Carbohydr. Polym.* **2001**, *46*, 261–266.
18. Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. *Anal. Chem.* **1956**, *28*, 350–356.
19. Anderson, D. M. W.; Bell, P. C. *Anal. Chim. Acta* **1975**, *79*, 185–187.