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PII: S0144-8617(97)00153-7

Carbohydrate Polymers **35** (1998) 205–213

0144-8617/98/$19.00+0.00

The molecular characterization of the polysaccharide gum from *Laguncularia racemosa*

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(Received 7 July 1997; revised 26 September 1997; accepted 28 September 1997)

A polysaccharide isolated from the exudate of *Laguncularia racemosa*, (Combretaceae) has been investigated using Smith-degradation, methylation analysis, hydrolysis, and ^{13}C-NMR spectroscopy. The backbone of the structure is constituted of uronic acids, galactose and rhamnose. A complex pentasaccharide, constituted of these sugars, was isolated from the original gum and degradation products. This oligosaccharide is, probably, the main structural feature of the investigated polysaccharide. On the other hand, according to chemical and spectral evidence rhamnose is present, predominantly as internal residues. Arabinosyl (pyranosyl and furanosyl) residues and some galactosyl, glucuronic acid and 4-0-methyl-α-D-glucuronic acid residues are located in branches. © 1998 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

Laguncularia racemosa (''mangle blanco'') is a member of an ecosystem called ''manglares'', found extensively in Venezuela. Analytical data for some Combretaceae gums have been reported (Anderson et al., 1977; Anderson et al., 1990). The study of nine gum specimens from *Laguncularia racemosa*, was published recently (León de Pinto et al., 1993a). This work reports some structural features of the polysaccharide isolated from the gum of this species, which were obtained by a combination of chemical studies and ^{13}C-NMR spectroscopy.

MATERIALS AND METHODS

Origin and purification of the sample

Gum from *Laguncularia racemosa*, commonly known as ''mangle blanco'' was collected by Gutiérrez de Gotera, 1988, from trees growing in the location of Puerto Caballo and Puerto Mara, Zulia State, in the west of Maracaibo lake, Venezuela. The identification of voucher specimens was confirmed by Lic. Carmen Clamens, Universidad del Zulia. The gum exudate was purified as described previously (Martínez et al., 1992).

General methods

Standard methods for gum analysis were used (Anderson et al., 1968; Anderson et al., 1975). Paper chromatography was carried out on Whatman No. 1 and 3MM papers with the following solvent systems (v/v): (a) benzene, butan-1-ol, pyridine, water (1:5:3:3, upper layer); (b) acetic acid, ethyl acetate, formic acid, water (3:18:1:4); (c) acetic acid, ethyl acetate, formic acid, water (8:18:3:9); (d) butan-1-ol, ethanol, water (4:1:5); (e) ammonia (0.88), butan-2-one, water (1:200:17); (f) butan-1-ol, ethanol, 0.1 M hydrochloric acid (1:10:5). Before solvent (f) was used the paper was pretreated with 0.3 M sodium dihydrogen phosphate solution and allowed to dry.

G.l.c. of the methyl glycosides was performed with a Varian 2700 instrument fitted with a flame-ionization detector

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at nitrogen flow-rates of about 40 mL/min. The glass column (166 × 0.57 cm) used was 10% by weight of polyethylene glycol adipate on Chromosorb WHD at 190°C. Retention times are quoted relative to that of methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside for the methyl ethers. Optical rotations were measured with an Atago Polax-D polarimeter at 30°C. ^{13}C-NMR spectra were recorded in an AM-300 Bruker spectrometer in D_2O and the experimental conditions were described previously (León de Pinto et al., 1994a; León de Pinto et al., 1994c).

Neutral sugars were determined by the phenol–sulphuric acid method (Dubois et al., 1956) and uronic acids by direct titration with standard sodium hydroxide solution on exhaustively electrodialysed samples. Polysaccharides were methylated successively with dimethyl sulphate and sodium hydroxide, and with methyl iodide and silver oxide. Methanolyses were carried out under reflux for 6 h with methanolic 5% hydrogen chloride. The procedures for methylation of the oligosaccharides and polysaccharides have been reported (León de Pinto, 1991; León de Pinto et al., 1993b).

THE NEUTRAL AND ACID COMPONENTS

Purified gum (3 g) was hydrolysed with 0.5 M sulphuric acid (150 mL) for 8 h at 100°C. After cooling, and neutralization with barium carbonate, the solution was deionized with Amberilite IR-120(H^+) resin, concentrated and fractionated on a column (41 × 2.6 cm) of Duolite A-4 resin in the formate form. Elution with water and then 5% formic acid yielded the neutral and acidic fractions, respectively.

After concentration to a syrup, the neutral fraction was chromatographed in solvents (a) and (b) against authentic standards.

The acidic fraction was concentrated and, after removal of formic acid by the repeated addition of water followed by concentration to a syrup, paper chromatography was carried out in solvent (b) and (c). The acidic components were fractionated on Whatman 3MM papers in solvent (b) and they were hydrolyzed with 1 M sulphuric acid for 8 h at 100°C. The hydrolysates were chromatographed in solvents (a), (b) and (f). The major aldobiuronic acid was characterized by specific rotation, hydrolysis studies and methylation (Kuhn et al., 1955).

Isolation and identification of aldobiouronic acids by using formic acid

Purified gum (500 mg) was hydrolyzed with HCOOH (125 mL, 20%) for 5 h at 100°C. The formic acid was removed by repeated evaporation under reduced pressure. The hydrolysate was fractioned on a column (16 × 16 cm) of Duolite A-4 resin in the formate form. The experimental conditions used in the isolation and identification of the aldobiouronic acids were as described above.

Partial hydrolysis

The gum (150 mg) was treated with 0.25 M sulphuric acid (15 mL) for 1 hour at 100°C. The hydrolysate was studied by p.c. in solvents (a) and (b).

Autohydrolysis experiments

A solution of purified sample (1%) was heated at 100°C for 96 h; aliquots (10 mL) were withdrawn at various intervals and analyzed by p.c. The polymer was separated from the hydrolysate, dialysed and isolated by freeze-drying.

Preparation and examination 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 (León de Pinto, 1991; León de Pinto et al., 1993b). Degraded gum A (2.54 g) was obtained by mild acid hydrolysis (0.005 M H_2SO_4 at 100°C for 96 h) from purified gum (12.99 g). Drastic periodate oxidation (0.25 M) of degraded gum A (5.04 g) gave degraded gum B (150 mg). Preliminary small-scale experiments showed the suitable conditions for the preparation of degraded gum B; periodate consumption became constant within 96 h.

Smith-degradation studies

A series of two sequential Smith-degradations (periodate oxidation, reduction and acid hydrolysis) was performed with the pure gum as the starting material (60 g) and produced polysaccharide I (17.36 g). Polysaccharide I (14.3 g) gave polysaccharide II (0.13 g). The experimental conditions for these degradations were, in general, as described previously (León de Pinto, 1991; León de Pinto et al., 1993b). The preparation of each polysaccharide was repeated twice in order to check the yields and to have enough sample to complete the Smith-degradation processes.

RESULTS AND DISCUSSION

The polysaccharide from *Laguncularia racemosa* contains galactose, arabinose, rhamnose, glucuronic acid and its 4-*O*-methyl derivative (Table 1). Galacturonic acid, as traces, was shown by p.c. which has been reported previously for other Combretaceae gums (Anderson et al., 1977; Anderson et al., 1990). Partial hydrolysis studies of the original polysaccharide led to the isolation of two bioses. The main biose, [R_{gal} 0.28 (b); 0.61 (c)], has a low value of specific rotation (2°) with suggests a β-D-linkage; hydrolysis studies

Table 1. Sugar composition[a] of *Laguncularia racemosa* polysaccharide and its degradation products

Polymer	Yield (%)	$[\alpha]_D$ (deg.)	Constituent sugars (%)			
			Gal	Ara	Rha	UA
Original gum		+61	22	33	7	38
Autohydrolysis polymer	40.0	+61	17	25	12	46
Degraded gum A	19.5	+63	22	23	13	42
Degraded gum B	2.90	+17	24	—	40	36
Polysaccharide I	29.0	+63	13	20	23	44
Polysaccharide II	0.94	—	15	8	35	42

[a] Corrected for moisture UA = uronic acids.

and chromathography of this disaccharide in solvents (a), (b) and (f) revealed that galactose and glucuronic acid were present. Methylation analysis indicated the presence of 2,3,4-tri-*O*-methyl-D-glucuronic acid (T 2.26, 3.01), 2,3,5-tri-*O*-methyl-D-galactose (T 4.12, 4.70) and 2,3,4-tri-*O*-methyl-D-galactose (T 6.28). These results identified the biose as 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose. The other biose, R_{gal} 0.62 (b), $[\alpha]_D$ + 90°, showed, after hydrolysis, by chromatography the presence of galactose and 4-*O*-methyl-α-D-glucopyranosyl uronic acid. According to hydrolysis studies and physical properties the disaccharide is 4-0-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose which showed the same chromatography behaviour in solvent (b) than a standard specimen. These aldobiuronic acids were also isolated by column chromathography of the acidic fraction obtained from the hydrolysates of purified gum (0.5 M sulphuric acid and 20% HCOOH), and during the preparation and hydrolysis studies of the autohydrolysis polymer and degraded gum A.

The methylation analysis of the original gum and its degradation products (Table 2) reveals terminal rhamnopyranosyl, the presence of terminal and 3-*O*-substituted arabinofuranosyl residues, terminal and 2-*O*-substituted arabinopyranosyl residues, 4-*O*-, 3-*O*-, 6-*O*- and 3,6-di-*O*-substituted galactopyranosyl residues; terminal and 4-*O*-glucopyranosyluronic acid residues. The methyl glycosides of rhamnose have very low retention time; therefore, they are difficult to be observed.

During the preparation of the autohydrolysis polymer and degraded gum A, arabinose, galactose and glucuronic acid were released. Rhamnose was detected as traces at the end of the autohydrolysis process (96 h). These results suggest that rhamnose is, predominantly, an internal residue, while some arabinose, galactose, glucuronic acid and its 4-*O*-methyl ether residues may be located in the branches of the gum structure. Partial hydrolysis of degraded gum A and the autohydrolysis polymer led to the isolation and further identification of the same aldobiuronic acids, described previously for the original gum.

Degraded gum B, obtained by drastic oxidation of degraded gum A (0.25 M $NaIO_4$), the core of the structure, is a heteroglycan, constituted of uronic acids, galactose and rhamnose (Gal:Rha:UA 2:1:2) (Table 1). This atypical backbone of the polysaccharide studied contrasts with the previous results observed in most of the analogous polymers studied so far (Anderson et al., 1968; Churms et al., 1981; Ghosal et al., 1981; Street et al., 1983; León de Pinto et al., 1994a), although there have been reported a glucuronoxylan in *Cercidium praecox* gum exudate (León de Pinto et al., 1994c), galactomannan in gums from seeds (Hansen et al., 1992) and galactorhamnan in microorganisms (Oxley et al., 1988). Partial hydrolysis of degraded gum B led to the disaccharide 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-β-D-galactose (R_{gal}0.14 (a); 0.56(b) and a fragment [R_{gal} 0.17 (a); 0.31(b)], constituted of galactose, rhamnose, glucuronic acid and 4-*O*-methyl-α-D-glucuronic acid.

During the preparation of polysaccharides I and II galactose, arabinose, rhamnose and uronic acids were eliminated and the same complex fragment described previously was

Table 2. Methyl glycosides of *Laguncularia racemosa* polysaccharide

Methylated sugar[a]	T (min)[b]	Type of linkage
2,3,4-Me_3-L-Rha	(0.56)	Rha (1 →
2,3,5-Me_3-L-Ara	(0.56)	Ara_f (1 →
2,3,4-Me_3-L-Ara	0.94	Arap (1 →
3,4-Me_2-L-Ara	1.46; 1.88	→ 2) Arap (1 →
2,5-Me_2-L-Ara	1.72; 3.26	→ 3) Araf (1 →
2,3,4-Me_3-D-GlcA[c]	2.18; 2.74	GlcA (1 →
2,3,6-Me_3-D-Gal	2.47; 3.64; (3.82); (4.34)	→ 4) Galp (1 →
2,4,6-Me_3-D-Gal	(3.82); (4.34)	→ 3) Galp (1 →
2,3,4-Me_3-D-Gal	5.79; 7.83	→ 6) Galp (1 →
2,3-Me_2-D-GlcA[c]	9.37	→ 4) GlcA (1 →
2,4-Me_2-D-Gal	13.41; 14.29	→ 3) Galp (1 → 6

[a] The same *O*-methyl sugars were observed in degraded gum A and polysaccharide I. [b] Retention times relative to methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside. [c] As methyl ester methyl glycoside. Figures in parentheses indicate *T* values of components that were not completely resolved.

Table 3. Carbon-13 data[a] of the fragment isolated from *Laguncularia racemosa* gum and its degradation products

Type of linkage	C-1	C-2	C-3	C-4	C-5	C-6	4-OMe
→ 3)β-D-Galp(1 → [b]	104.1	71.4	82.1	69.9	74.9	60.7	
	103.0	71.7	82.0	69.2	75.2	60.1	
→ 6)β-D-Galp(1 → [c]	102.8	70.1	71.9	67.7	72.8	68.6	
	103.0	-	72.2	—	72.6	68.4	
β-D-GlcA(1 → [d]	104.7	75.5	77.1	73.2	77.5	177.5	
	104.9	75.4	76.9	73.2	76.9	177.0	
4-O-Me-α-D-GlcA(1 → [c]	99.1	71.8	73.3	82.6	70.8		59.9
	99.0	71.8	73.2	—	70.9		60.1

[a] Values relative to the signal of 1,4-dioxane (66.67 ppm). The unequivocal signal of methyl of rhamnose (16.67 ppm) was observed. [b] Martínez et al., 1996. [c] León de Pinto et al., 1995. [d] León de Pinto et al., 1996.

isolated. Partial hydrolisis studies of this fragment led to the isolation of 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose and 4-*O*-(4-0-methyl-α-D-glucopyranosyl uronic acid)-D-galactose, which were also isolated from hydrolysis studies of the original polysaccharide, autohydrolysis polymer and from degraded gum A. The configuration of the sugars involved in its constitution was corroborated by the specific rotations.

The ^{13}C-NMR spectrum of the complex fragment, described above, showed unequivocal signal due to the methyl group of rhamnose (16.67 ppm) (Agrawal, 1992; León de Pinto et al., 1993b; León de Pinto et al., 1994a); the resonances were attributed to 3-*O*- and 6-*O*-substituted galactopyranosyl residues (Martínez et al., 1996; León de Pinto et al., 1995) and those due to glucuronic acid (León de Pinto et al., 1996) and its 4-*O*-methyl derivative (León de Pinto et al., 1995) (Table 3).

The interpretation of the spectral and chemical evidence suggests a possible structure for the fragment (Fig. 1). The presence of this oligosaccharide in the degradation products of *L. racemosa* gum (degraded gum B, polysaccharide I and II) may indicate that it is the main feature of the structure investigated. The two aldobiuronic acids, described above, were also present in the original polysaccharide and degraded gum B (structure core).

Degraded gum B, the backbone of the structure studied, a heteroglycan, shows a spectrum, Fig. 2, that contains signals due to galactose, rhamnose, glucuronic acid and its 4-*O*-methyl derivative (Table 4,Table 5). It is worth noting that the chemical shifts of galactose and rhamnose are very close, although there are signals unequivocally assigned to the methyl group of rhamnose (16.67; 16.93 ppm) (Agrawal, 1992; León de Pinto et al., 1993a; León de Pinto et al., 1994a). There are resonances of 3-*O* (82.4 ppm) (Martínez et al., 1996), 6-*O*- (68.71 ppm) (León de Pinto et al., 1995) and 4-*O*-substituted-β-D-galactose residues (78.46 ppm) (Machytka et al., 1994). The resonances due to uronic acid residues (Table 5) are according to methylation analysis (Table 2) and the two aldobiuronic acids described previously.

The spectra of degraded gum A (Fig. 3) show interesting evidence. Fig. 3a contains, at high field, the resonances due to the methyl group of rhamnose (16.60; 16.86 ppm) and an acetyl group (20.68; 23.25 ppm) (Fang et al., 1991; León de Pinto et al., 1994a). There are also signals attributed to a methoxyl group (59.7; 59.8 ppm) (León de Pinto et al., 1995) of the 4-*O*-methyl-α-D-glucuronic acid residues in many environments (León de Pinto et al., 1995) and C-6 of galactosyl residues (68.60; 68.98 ppm) in two environments (Fig. 1) present in the polysaccharide. The resonances of 6-*O*- (68.6; 68.98 ppm), 4-*O*- (78.5 ppm) and 3-*O*- (81.1; 81.9; 82.3 ppm) substituted galactose residues are well defined (Table 3). The anomeric carbon region (Fig. 3b), shows the presence of reducing sugars (91.67 ppm) (León

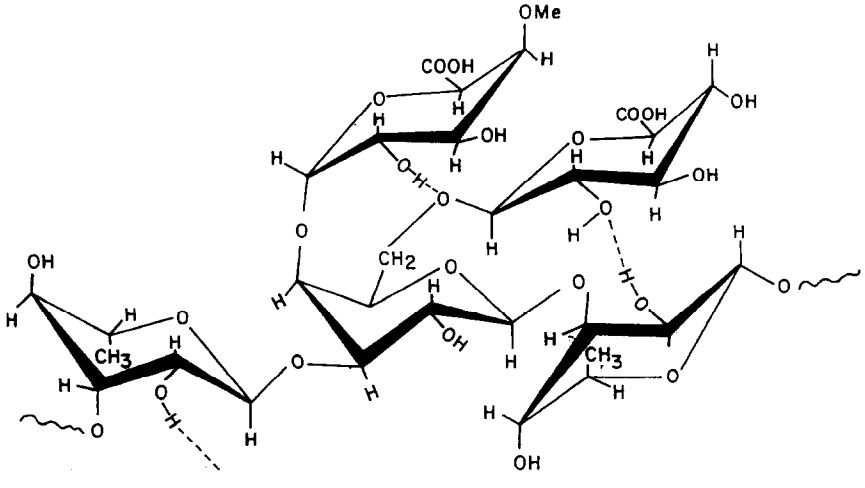


Fig. 1. Possible structural model of the pentasaccharide isolated from the degraded products of *L. racemosa* polysaccharide.

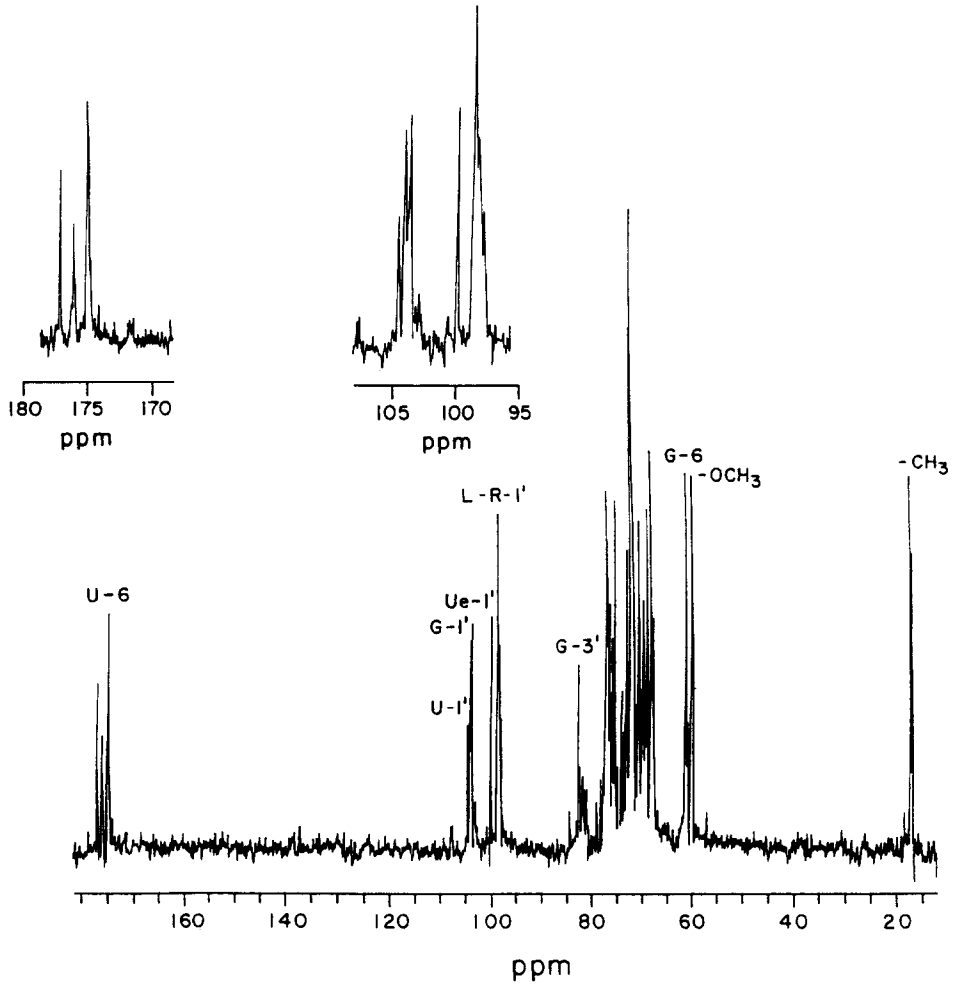


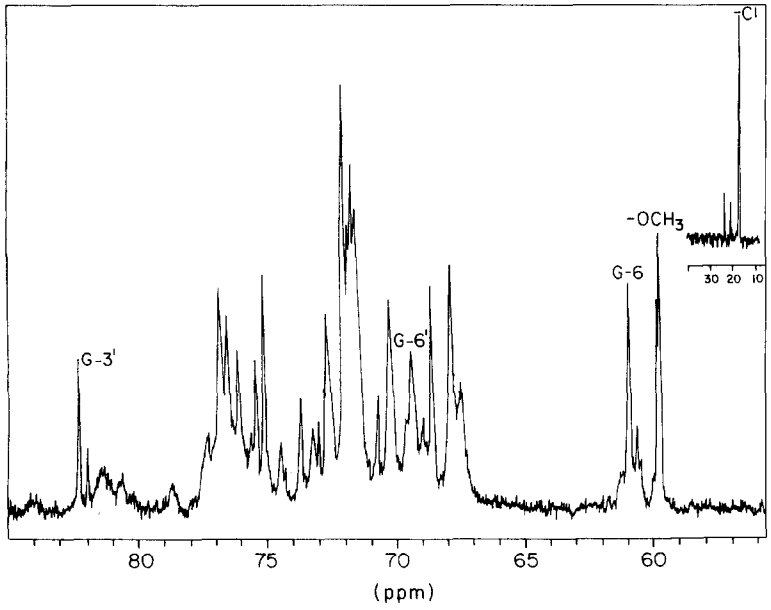
Fig. 2. ^{13}C-NMR spectrum of degraded gum B of *L. racemosa* gum. G = β-D-galactose; R = α-L-rhamnose; U = uronic acids; U_e = 4-*O*-methyl-α-D-glucuronic acid; ′ = carbon involved in the glycosidic linkage.

de Pinto, 1991; Agrawal, 1992), 3-*O*-α-rhamnose (98.32 ppm) (Oxley et al., 1988), 4-*O*-methyl-α-D-glucuronic acid (99.6 ppm) (León de Pinto et al., 1993b; León de Pinto et al., 1994c), terminal and 2-*O*-β-L-arabinopyranose (100.37; 100.96 ppm) (León de Pinto et al., 1994a), 6-*O*- (102.66 ppm) (León de Pinto et al., 1995) and 3-*O*-substituted-β-D-galactosyl residues (103.6;103.8 ppm) (Martínez et al., 1996) and β-D-glucuronic acid (104.2; 104.3 ppm) (León de Pinto et al., 1996). The signals that appear at low field (Fig. 3c), indicate that the uronic acid residues contain an acetyl group (174.68 ppm) (Fang et al., 1991; León de Pinto et al., 1994a) and that terminal β-D-glucuronic acid residues (176.79 ppm) and α-D-glucuronic acid are substituted by metals (181.53 ppm) (León de Pinto et al., 1994c). The resonances of α-L-arabinofuranosyl residues were not observed in this spectrum as they were removed

Table 4. ^{13}C-NMR data[a] of β-D-galactose residues in *Laguncularia racemosa* polysaccharide 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]		104.1	71.4	82.1	69.9	74.9	60.7
	d.g.B	103.8	71.8	82.4	69.1;69.5	75.2	61.0
	d.g.A	103.6;103.8	71.5;71.7;71.8	81.1;81.9;82.3	69.4	75.1	60.6;60.9
	II	104.1	70.9	81.9	69.9	74.9	60.8;61.3;61.6
→ 6) β-D-Galp (1 → [c]		102.8	70.0;70.3	71.9	67.6	72.8	68.1;68.9
	d.g.B	103.0	70.3	72.1	67.7	72.8	68.7
	d.g.A	102.7	70.2	72.0	67.5;67.9	72.7	68.6;68.9
	II	102.4	70.0	72.4	67.1	72.9	68.4;68.9

[a]Values relative to the signal of 1,4-dioxane (66.67 ppm). [b]Martínez et al., 1996. [c] León de Pinto et al., 1995. Some signals of 3-*O*- and 6-*O*-galactose residues were overlapped in the spectrum of the original gum. d.g.B. = degraded gum B; d.g.A = degraded gum A; II = polysaccharide II.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-OCH3
G-6
G-6'
G-3'
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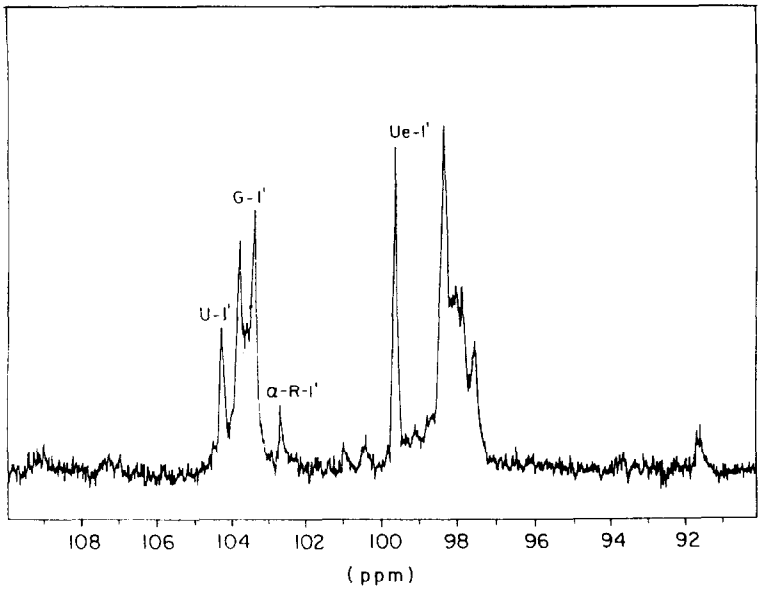
75
70
65
60
(ppm)


Ue-1'
G-1'
U-1'
α-R-1'
108
106
104
102
100
98
96
94
92
(ppm)

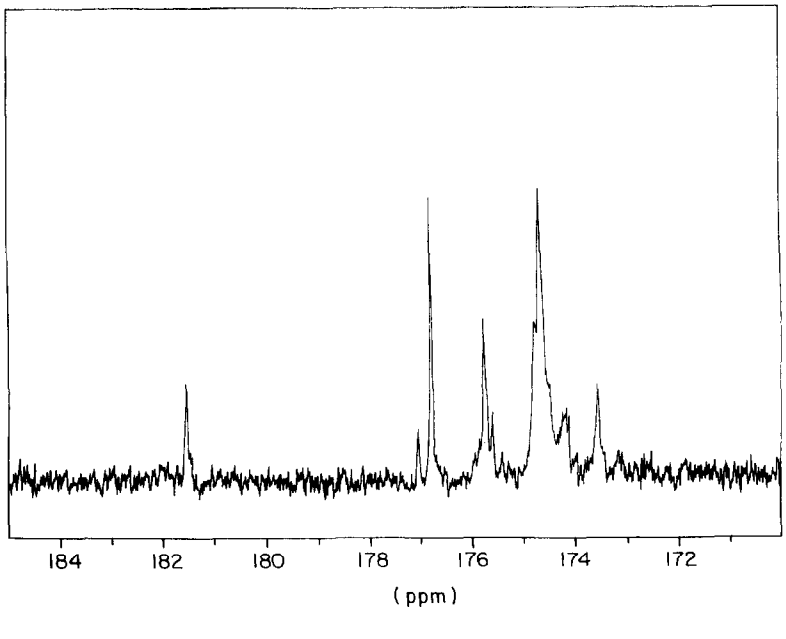

184
182
180
178
176
174
172
(ppm)

Table 5. ^{13}C-NMR data[a] of uronic acids residues of *Laguncularia racemosa* polysaccharide 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]		99.01;99.18	71.8	73.3	82.6	70.8		59.9
	d.g.B	99.7	72.1	-	82.4	70.8		59.8;59.9
	d.g.A	99.6	72.0	73.0;73.2	81.9	70.8		59.7;59.8
	o.g.	99.4	71.7	72.8	81.9	—		59.7
	II	99.7	72.4	72.9	81.9	70.9		60.8
β-D-GlcA (1 → [c]		104.7	75.5	77.1	73.3	77.5	177.5	
	d.g.B	104.3	75.5;75.7	76.2	—	76.6;76.9	174.8;175.8;176.9	
	d.g.A	104.2	75.1;75.4;75.6	76.2	73.0;73.2	76.5	175.7;176.9	
	o.g.	103.6[d]	74.9	76.3	72.8	76.3	176.8	
	II	104.1	74.9	77.2	72.9	77.2	176.6;177.6	

[a]Values relative to the signal of 1,4-dioxane (66.67 ppm). [b]León de Pinto et al., 1995. [c]León de Pinto et al., 1996. [d]This is a wide peak. d.g.B = degraded gum B; d.g.A = degraded gum A; o.g. = original gum; II = polysaccharide II.

during preparation of the degraded gum A. Although, there is spectral evidence of terminal (100.37 ppm) and 2-*O*-β-L-arabinopyranosyl residues (100.96 ppm) that is supported by methylation analysis (Table 2).

The ^{13}C-NMR spectrum of the original gum is complex (Fig. 4). The resonances of 6-*O*- and 4-*O*-substituted galactose are not well resolved. Resonances of terminal and 3-*O*-linked-α-L-arabinofuranosyl residues were unequivocaly assigned (León de Pinto et al., 1994a) (Table 6), and some signals due to internal arabinopyranose residues were present (100.96 ppm). There are signals that appeared at high field, attributed to the methyl group of rhamnose (16.9 ppm) (Agrawal, 1992; León de Pinto et al., 1993a; León de Pinto et al., 1993b) and acetyl groups (20.2; 20.5; 23.1 ppm) (Fang et al., 1991; León de Pinto et al., 1994a). These last signals are related with the peaks which appeared centered at 173.3 ppm The location of the acetyl groups in the structure of the original polysaccharide must be investigated. The signal at low field (181.0 ppm) is due to C-6 of α-D-glucuronic acid substituted by metals (León de Pinto et al., 1994c).

The ^{13}C-NMR spectra of polysaccharides I and II,

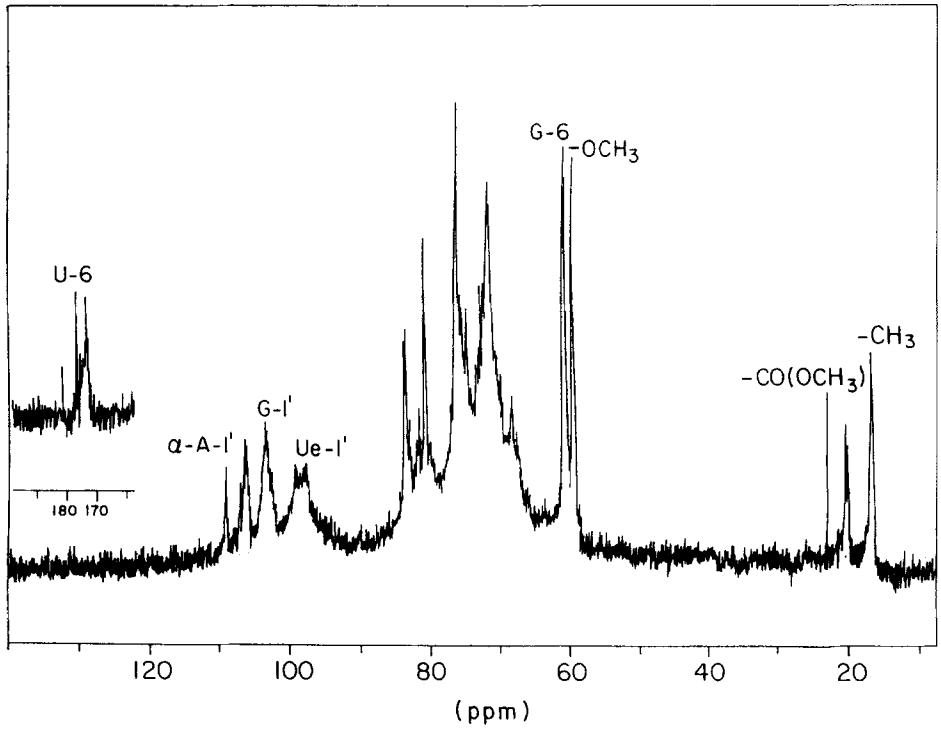


Fig. 4. ^{13}C-NMR of *L. racemosa* gum exudate. A = α-L-arabinofuranose; G = β-D-galactose; U = uronic acids; U_e = 4-*O*-methyl-α-D-glucuronic acid; ' = carbon involved in the glycosidic linkage.

Fig. 3. (a) ^{13}C-NMR spectrum of degraded gum A of *L. racemosa* (10–85 ppm). G = β-D-galactose; ' = carbon involved in the glycosidic linkage. (b) ^{13}C-NMR spectrum of degraded gum A of *L. racemosa* (92–108 ppm). G = β-D-galactose; R = α-L-rhamnose; U = β-D-glucuronic acid; U_e = 4-*O*-methyl-α-D-glucuronic acid; ' = carbon involved in the glycosidic linkage. (c) ^{13}C-NMR spectrum of degraded gum A of *L. racemosa*. (172–184 ppm).

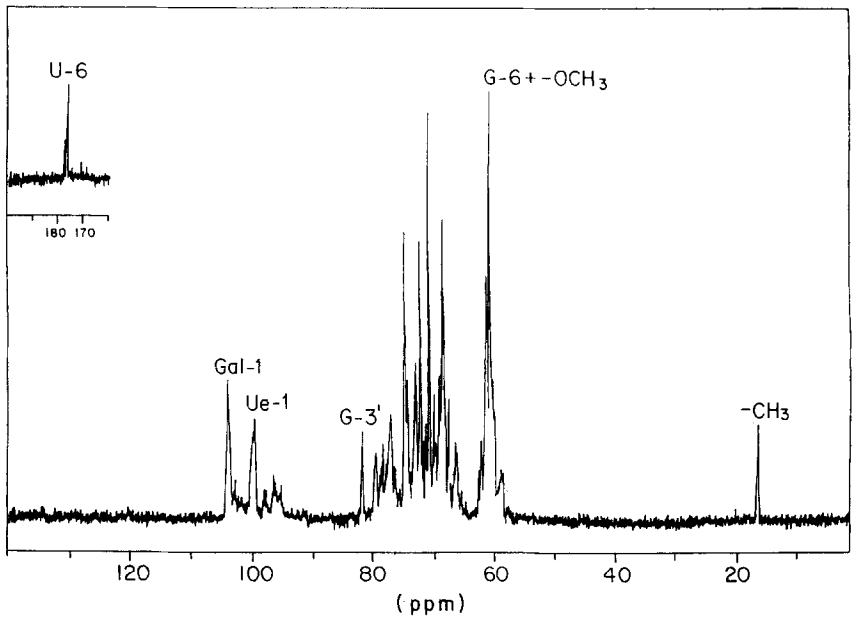


Fig. 5. ^{13}C-NMR spectrum of polysaccharide II of *Laguncularia racemosa*. G = β-D-galactose; U = uronic acids; U_e = 4-*O*-methyl-α-D-glucuronic acid; ′ = carbon involved in the glycosidic linkage.

Table 6. ^{13}C-NMR data[a,b] of α-L-arabinofuranose residues of *Laguncularia racemosa* gum

Type of linkage	C-1	C-2	C-3	C-4	C-5
α-L-Araf (1 → [c]	110.9	82.3	76.5	84.9	62.0
	109.6	81.0	—	83.8	61.0
→ 3)α-L-Araf (1 → [c]	108.2	80.7	83.2	83.6	62.0
	106.6;107.3	80.0	83.2	83.8	61.0

[a]Values relative to the signal of 1,4-dioxane (66.67 ppm). [b]These signals and those due to β-L-arabinopyranose were observed in the spectrum of polysaccharide I but those of β-L-arabinopyranose appear in the spectrum of degraded gum A. [c]León de Pinto et al., 1994a.

obtained by the successive Smith-degradation of the original polysaccharide, are quite similar except that the spectrum of polysaccharide I contains the resonances due to α-L-arabinofuranosyl (61.40; 108.00 ppm) and terminal β-L-arabinopyranosyl residues (64.40; 100.00 ppm) (León de Pinto et al., 1994a). These resonances are according to methylation analysis. There are also signals attributed to reducing sugars (88.74; 90.50; 91.17; 94.48; 97.99 ppm) (Agrawal, 1992; León de Pinto et al., 1993b). Polysaccharide II produced a well resolved ^{13}C-NMR spectrum (Fig. 5). There are unequivocal signals due to 3-*O*- and 6-*O*-substituted galactosyl residues, Table 4. The signals at 78.0 and 78.3 ppm may indicate the presence of 4-*O*-substituted galactosyl residues (Machytka et al., 1994) which is supported by the isolation and characterization of the disaccharide 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose and the resonances due to 4-*O*-methyl-α-D-glucuronic acid (Table 5). In addition, there are signals attributed to 4-*O*-methyl- and terminal β-D-glucuronic acid residues which were supported by methylation analysis.

Chemical and spectral studies of the polysaccharide of *L. racemosa* and its degradation products indicate that an oligosaccharide (Fig. 1) is a principal structural unit of the studied polysaccharide. The backbone of the polysaccharide, constituted of uronic acids, galactose and rhamnose, is an atypical feature for this kind of polysaccharide. Arabinosyl (pyranosyl and furanosyl) residues, some galactosyl, glucuronic acid and 4-*O*-methyl glucuronic acid residues are present in the branches of the gum structure.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support that the Centro de Investigaciones en Química de los Productos Naturales has received from the CONDES (Consejo de Desarrollo Científico y Humanístico), University of Zulia, Venezuela.

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