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Structural investigation of the polysaccharide of *Spondias mombin* gum

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Abstract

The polysaccharide, isolated from *Spondias mombin* gum exudate, was subjected to acid hydrolysis and Smith degradation processes in order to investigate its relevant structural features. Chemical and spectral evidence suggested the existence of small blocks of (1 → 3)-linked β-D-galactosyl residues, interspersed with 6-*O* substituted D-galactosyl residues, in the backbone of the investigated structure; these residues are also in the side-chains. In addition, there was arabinose, up to three units long, predominantly in the form of furanosyl residues (terminal, 2-*O*- and 3-*O*-linked). Arabinopyranosyl residues (terminal and 2-*O*-linked) as well as rhamnose and mannose are also present in minor amounts. Uronic acids, β-D-glucuronic acid and its 4-*O*-methyl analogue, are attached at the C-6 and C-4 positions of the galactose moieties in the backbone. These sugar acid residues were difficult to remove from the core. ^{13}C-NMR spectroscopy confirmed the results obtained by chemical methods, and showed the presence of reducing terminal sugar residues and internal acetyl groups.

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

1. Introduction

Few species belong to *Spondias* L. (Anacardiaceae) which are widespread in tropical regions of the world (Hoyos, 1989). *S. dulcis* and *S. pinnata* grow abundantly in India (Basu & Rao, 1981; Ghosal & Thakur, 1981) while *S. purpurea* and *S. mombin* are disseminated in Venezuela (Hoyos, 1989). The structural features of the polysaccharides isolated from the gums of those *Spondias* have been reported (Basu & Rao, 1981; Ghosal & Thakur, 1981; León de Pinto, Martínez, Mendoza, Ocando & Rivas, 1996).

This work deals with the structural investigation of one acidic polysaccharide isolated from *Spondias mombin* gum.

2. Materials and methods

2.1. Origin and purification of the gum exudate

The gum sample from *Spondias mombin* L., commonly known as "jobo", was collected by the authors from specimens located in Guama, Yaracuy State, central Venezuela, during the dry season (January–April) in 1995. The identification of voucher specimens was confirmed by Lic. Carmen Clamens, Universidad del Zulia. The gum was dissolved in cold water, filtered (muslin, Whatman No. 41 and 42 papers), dialysed for two days against tap water, and freeze-dried.

2.2. General methods

The specific rotations were measured with a Perkin–Elmer 343 polarimeter at 589 nm. The homogeneity of the original polysaccharide was tested on a column (30 cm) of Fractogel TSK H.W.-65 (S), using 0.1 M sodium chloride as eluent. 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) butan-1-ol, ethanol, 0.1 M hydrochloric acid (1:10:5). Before solvent (c) was used the paper was pretreated with 0.3 M sodium dihydrogen phosphate solution and allowed to dry. Neutral sugars were determined by the phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers & Smith, 1956) and uronic acids by direct titration with standard sodium hydroxide solution on exhaustively electrodialysed samples.

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Table 1
Analytical data of the original polysaccharide of *Spondias mombin* gum and its degradation products

Polymer	Yield (%)	Sugar constituents (%)				
		Gal	Ara	Man	Rha	U.A
Original gum[a]	33	43	30	7	3	17
Degraded gum A	17	69	6	2	–	23
Degraded gum B	Low[b]	74	–	–	–	26
Polysaccharide I	30	54	26	6	–	14
Polysaccharide II	30	61	16	9	–	14
Polysaccharide III	47	60	5	–	–	35

[a] Obtained from the crude gum.
[b] The yield was very low. U.A. = uronic acids as glucuronic acid and its 4-*O*-methyl derivative.

2.3. Methylation analysis

The dry sample (1–5 mg) was dissolved in Me_2SO (50–220 μl) and potassium methylsulphynil carbanion (200 μl) was added. The solution was cooled and methyl iodide (150 μl) was added at low temperature. The methylated sample was treated as was reported (Harris, Henry, Blakeney & Stone, 1984) and was hydrolysed with 2 M TFA (0.3 ml) for 1 h at 121°C. The mixture of the methylated monomers was reduced with borohydride (1 ml) in 2 M ammonia for 60 min at 60°C and then was acetylated with ethyl acetate (1 ml) and anhydride acetic acid (3 ml) using 1-methylimidazole (200 μl) as catalyst. The alditol acetates were extracted with dichloromethane and its mixture was stored at −20°C in a screw-capped glass vial prior to gas chromatography. The retention times (T_r) of the alditol acetates of methylated sugars, were in reference to that of 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methyl arabinitol (T_r 15.24), chosen because this peak appeared in all chromatograms. Gas chromatograpy–mass spectrometry was done in a Fisons Voyager system (GC–MS). Gas chromatograph was equipped with a 25 m × 0.25 mm I.D. SGL-1701 column coated with a 0.25-μm film of OV-1701. The carrier gas was helium at a flow rate of 1 ml/min. The injector and transfer line temperatures were set to 250 and 280°C, respectively. The oven temperature was raised from 50 to 250°C at 5°C/min, then held at 250°C for 5 min.

2.4. ^{13}C-NMR spectra

The spectra of the original polysaccharide and its degradation products were recorded with a Bruker AM-300 spectrometer in D_2O and the experimental conditions were described previously (León de Pinto, Martínez, Ludovic de Corredor, Rivas & Ocando, 1994a; León de Pinto, Martínez & Rivas, 1994b).

2.5. 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 Amberlite IR-120 (H^+) resin, concentrated and fractionated on a column (41 × 2.6 cm^2) of Duolite A-4 resin in the formate form. Elution with water and then 5% formic acid yielded 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 solvents (b) and (c). The aldobiouronic acids were fractionated on Whatman 3MM papers in solvents (b) and they were hydrolysed with 1 M sulphuric acid for 8 h at 100°C. The hydrolysates were chromatographed in solvents (a), (b) and (c).

2.6. Isolation and identification of aldobiouronic acids by using formic acid

Purified gum (500 mg) was hydrolysed with HCOOH (125 ml, 20%) for 5 h at 100°C. 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.

2.7. Partial hydrolysis

The gum (150 mg) was treated with 0.25 M sulphuric acid (15 ml) for 1 h at 100°C. The hydrolysate was studied by paper chromatography in solvents (a) and (b).

2.8. 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), dialysed in distilled water (80 ml) and the dialysate analysed by paper chromatography. The polymer was isolated by freeze-drying (83%).

2.9. 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 (León de Pinto et al., 1994a,b; Martínez, León de Pinto, Rivas & Ocando, 1996). Degraded gum A (0.68 g) was obtained from purified gum (4.0 g) by mild acid hydrolysis (5 mM H_2SO_4, 100°C for 96 h). 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.

Table 2
Methylation analysis of the gum from *S. mombin* and its degradation products

Alditol acetate[a]	T_r[b]	Molar proportion				Type of linkage
		A	B	C	D	
2,3,5-Me_3-Ara	1.00	21.79	25.05	23.59	11.67	Araf (1 →
2,3,4-Me_3-Rha	1.04	0.35	0.66	–	–	Rhap (1 →
2,3,4-Me_3-Ara	1.07	0.84	3.67	–	–	Arap (1 →
3,5-Me_2-Ara	1.16	21.11	5.63	12.35	10.58	→ 2)Araf(1 →
2,5-Me_2-Ara	1.18	8.44	17.68	5.49	8.43	→ 3)Araf(1 →
3,4-Me_2-Ara	1.23	0.96	3.87	2.65	–	→ 2)Arap(1 →
2,3,4,6-Me_4-Gal	1.30	0.78	–	1.77	4.09	Galp(1 →
2,4,6-Me_3-Gal	1.43	0.94	1.73	29.25	28.56	→ 3)Galp(1 →
2,3,4-Me_3-Gal	1.51	1.26	0.51	0.80	5.54	→ 6)Galp(1 →
2,6-Me_2-Gal	1.53	0.43	0.93	2.35	–	→ 3,4)Galp(1 →
4,6-Me_2-Gal	–	–	0.46	1.08	–	→ 2,3)Galp(1 →
2,4-Me_2-Gal	1.64	12.18	10.32	9.45	10.25	→ 3,6)Galp(1 →
2-Me-Gal	1.70	2.56	4.89	0.84	–	→ 3,4,6)Galp(1 →
4-Me-Gal	1.76	1.63	0.75	0.51	–	→ 2,3,6)Galp(1 →

[a] Verified by mass spectrometry.
[b] T_r relative to 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methyl arabinitol. A: original gum, B: Autohydrolysis polymer, C: polysaccharide I, D: polysaccharide II. It was detected, –GlcA as methyl ester methyl glycoside in other methylation experiment.

2.10. Preparation and studies of polysaccharides I–III

A series of three Smith degradations was performed using the pure gum as the starting material (50 g) to afford polysaccharide I (15.2 g). This polymer (13.0 g) yielded polysaccharide II (3.93 g), and the latter (2.0 g) yielded polysaccharide III (0.93 g). The experimental conditions for the preparation and examination of these polymers were, in general, as previously described (León de Pinto et al., 1994a,b; Martínez et al., 1996).

3. Results and discussion

The original polysaccharide, obtained from the purified gum of *Spondias mombin*, was found to be composed of galactose, arabinose, mannose, rhamnose, glucuronic acid and its 4-*O*-methyl ether (Table 1). These sugar constituents are also present in *S. purpurea* gum (León de Pinto et al., 1996); the polysaccharides from *S. dulcis* and *S. pinnata* gums contain galactose, arabinose and galacturonic acid (Basu & Rao, 1981; Ghosal & Thakur, 1981). Galacturonic acid was not observed in the polysaccharide from *S. mombin* gum.

Partial hydrolysis studies of the original polysaccharide, followed by paper chromatography in solvent (a) gave three fractions which were characterized by their specific rotation values, and by hydrolysis studies and methylation analysis.

Partial hydrolysis of the first fraction, Rgal 0.19 (a), gave arabinose, galactose and an oligomer Rgal 0.28 (a), $[\alpha]_D$ + 1° which corresponded to 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose. The second fraction, Rgal 0.28 (a) $[\alpha]_D$ + 1° showed the same characteristics of the aldobiouronic acid, obtained from partial hydrolisis of the first fraction. The third fraction, Rgal(a) 0.75, $[\alpha]_D$, + 6°, gave galactose and arabinose by acid hydrolysis (2 M H_2SO_4 for 4 h).

Neutral and acidic components, separated by column chromatography of the formic acid hydrolysate of the original polysaccharide, corroborated the presence of the neutral sugars shown in Table 1, and led to the isolation of two aldobiouronic acids, characterized as 6-*O*-β-(D-glucopyranosyluronic acid)-D-galactose, Rgal 0.28 (a), $[\alpha]_D$ + 1° and 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose, Rgal (a) 0.62, $[\alpha]_D$ + 94°.

Methylation analysis of the alditol acetates from the original gum and its degraded products (Table 2) revealed that the structure of the investigated polysaccharide contains 3-*O*-, 6-*O*-mono-, 3,6- and 3,4-di-*O*-substituted galactosyl residues; terminal, 2-*O*- and 3-*O*-L-arabinofuranosyl; terminal and 2-*O*-L-arabinopyranosyl; D-glucuronosyl acid, and L-rhamnopyranosyl as terminal residues. The alditol acetates were analysed by mass spectrometry; the main diagnostic ions of the permethylated alditol acetates were observed (Björndal, Lindberg & Svensson, 1967; Björndal, Lindberg, Pilotti & Svensson, 1970).

The preparation of the autohydrolysis polymer and degraded gum A were monitored; aliquots were withdrawn at various intervals and analysed by paper chromatography. It was separated and characterized two oligosaccharides, Rgal 0.28(a); 0.75(a), which were isolated from partial hydrolysis studies of the original polysaccharide. Arabinose and rhamnose were also removed; the lability of these residues has been observed in other gums (León de Pinto, Alvárez, Martínez, Rojas & Leal, 1993; León de Pinto et al., 1994a).

Degraded gum B, obtained by periodate oxidation (0.25 M) of degraded gum A, consisted of a β-(1 → 3)-galactan (Table 1). Removal of uronic acid residues by periodate oxidation of this polymer was difficult, which

Table 3
^{13}C-NMR spectral data of β-D-galactose residues (values relative to the signal of 1,4-dioxane (δ 66.67 ppm)) in *Spondias mombin* gum exudate and its degradation products

Type of linkage	Polymer	C-1	C-2	C-3	C-4	C-5	C-6
→ 3)β-D-Gal*p*(1 → [a]	d.g.A.	103.5	71.61	81.78	69.08	74.55	60.19
	o.g.	103.22[b]	71.25	81.80	69.79	74.91	60.84
	I	103.90	71.09	81.30	69.17	75.04	60.82
	III	103.8	70.90	81.80	68.22	74.53	60.74
→ 6)β-D-Gal*p*(1 → [a]	d.g.A.	102.47	70.00	72.21	–	73.00	68.10
	o.g.	102.67	70.10	72.36	67.99	72.80	68.39
	I	103.90[c]	70.26	72.09	67.44	73.25	68.43
	III	103.95	70.27	72.58	–	73.10	68.52

[a] León de Pinto et al. (1994a).
[b] This signal is overlapped with that due to C-1′ of β-D-glucuronic acid.
[c] This signal is overlapped with those due to C-1′ of 3-*O*-galactose and β-D-glucuronic acid residues. d.g.A. = degraded gum A, o.g. = original gum, I and III = polysaccharides I and III.

may be related to steric hindrance and or the inter-residue hydrogen bonding. The success of the Smith degradation depends on ensuring that all potentially vulnerable diols have been oxidized (Aspinall, 1982).

The original polysaccharide isolated from *S. mombin* was subjected to three successive Smith degradations giving polysaccharides I–III. Rhamnose was removed in the preparation of polysaccharide I, while mannose was eliminated after three degradations. These results suggest that rhamnose is present as terminal residues while mannose may be located as internal residues in the original polysaccharide.

Removal of uronic acids from polysaccharides I–III was difficult as was shown in the preparation of degraded gum B (Table 1).

Methylation analysis (Table 2) shows that the preparation of degraded products from the original polysaccharide led to removal of periodate vulnerable residues (*O*-6- and *O*-4-linked). The original polysaccharide has a high proportion of 3,6-di-*O* as compared to 3,4-di-*O*-substituted residues. The relative proportion of (1 → 3)-linked galactosyl residues increases when the original polysaccharide is degraded. Polysaccharide III does not show the presence of 3,4-di-*O*- but does contain 3-*O*-, 6-*O*- and 3,6-di-*O*-substituted galactosyl residues (5:1:2). This relative proportion suggests that its structure consists of small blocks of Gal*p* (1 → 3) interspersed with Gal*p* (1 → 6). This finding is in agreement with the features reported for *Acacia tortilis* gum (Gammon, Churms & Stephen, 1986). The isolation of the aldobiouronic acid 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose during the preparation of the original polysaccharide supports the presence of 3,6-di-*O*-substituted galactosyl residues in the *S. mombin* polysaccharide. The gradual removal of arabinosyl residues during the preparation of polysaccharides I–III suggests that the low proportion present in polysaccharide III may be released in the preparation of polysaccharide IV; therefore, arabinose may exist in chains up to four units in the side-chains of the original polysaccharide. Arabinose exists predominantly as arabinofuranose (2-*O*-, 3-*O*-linked). The ratio of these linkages varies from 2.5 in the original gum to 1.2 in the polysaccharide III, which indicates that both

Table 4
^{13}C-NMR spectral data of uronic acid residues (values relative to the signal of 1,4-dioxane (δ 66.67 ppm)) in *Spondias mombin* gum exudate and its degradation products

Type of linkage	Polymer	C-1	C-2	C-3	C-4	C-5	C-6	4-OMe
β-D-GlcAp(1 → [a]	d.g.A.	103.98	75.35	75.92	73.40	75.92	175.59[b]	
	o.g.	103.22[c]	75.69	76.18	73.90	76.50	175.50	
	I	103.90[d]	75.58	76.50	73.25	76.50	–	
	III	103.82	74.53	76.30	73.09	76.23	175.07	
4-*O*-Me-α-D-GlcAp(1 → [a]	d.g.A.	100.00	71.61	73.40	81.88	70.84	–	59.81
	o.g	99.73	71.82	73.90	82.21	70.98	–	59.83
	I	100.00	–	73.25	82.00	70.93	–	60.99
	III	99.89	–	73.09	81.79	70.87	–	60.94

[a] León de Pinto et al. (1994b).
[b] The signals due to acetyl group (C-1: 173.2; $-CH_3$: 20.68 ppm) are observed in all the spectra.
[c] This signal is overlapped with that due to C-1 of 3-*O*-galactose.
[d] This signal is overlapped with that due to C-1 of 3-*O*- and 6-*O*-galactose residues. d.g.A. = degraded gum A, o.g. = original gum, I and III = polysaccharides I and III.

Table 5
^{13}C-NMR spectral data of L-arabinose residues (values relative to the signal of 1.4-dioxane (δ 66.67 ppm)) in *Spondias mombin* gum exudate and its degradation products

Type of linkage	Polymer	C-1	C-2	C-3	C-4	C-5
α-L-Araf(1 → [a,b]	o.g.	109.22	81.22	76.47	83.91	61.14
	II	109.03	81.79	76.29	83.77	60.97
→ 3)-α-L-Araf(1 → [a,b,c]	o.g.	106.66	80.29	83.91	83.91	62.00
	II	107.26	–	83.77	83.77	62.89

[a] León de Pinto et al. (1994a).
[b] The same resonances are observed in the spectraum of polysaccharides I.
[c] There are possibly resonances of 2-*O*-α-L-arabinofuranose. o.g. = original gum, II = polysaccharide II.

linkages exist in the same proportion in this structure. On the other hand, the side-chains have a higher proportion of terminal arabinofuranosyl residues than arabinopyranosyl residues (26:1). These few residues disappeared in the preparation of polysaccharide I, but the 2-*O*-linked arabinopyranosyl residues were removed in the second Smith degradation (Table 2).

The gum polysaccharide from *S. mombin* and its degradation products, in D_2O, gave well-resolved ^{13}C-NMR spectra, whose interpetration was made on the basis of chemical results (Tables 1 and 2) and earlier reports (León de Pinto et al., 1993; León de Pinto et al., 1994a,b). The spectrum of the original polysaccharide from *S. mombin* gum contained signals attributed to 3-*O*- and 6-*O*-galactose residues, terminal and 3-*O*-α-L-arabinofuranose residues, and to uronic acid residues (Tables 3–5). There are also resonances due to C-5 (62.54 ppm) of terminal β-arabinopyranose residues (León de Pinto et al., 1994a). The C-1 signal (100.30 ppm) could have arisen from the 2-*O*-linked β-arabinopyranosyl unit of the side-chains; an anomalous slight upfield shift has been reported for C-2 of the arabinopyranosyl residues on glycosidation (Mizutani, Kasai, Nakamura, Tanaka & Matauura, 1989). Assignment of these resonances was supported by methylation analysis (Table 2). There are also possibly resonances due to 2-*O*-α-arabinofuranose residues.

The signals due to 6-*O*-galactose residues seem to be unequivocal (Table 3); the C-6 involved in this linkage appears in many environments (68.22, 68.34, 68.41 ppm) (León de Pinto et al., 1994a). In addition, the spectrum contains resonances due to methoxyl groups probably linked to neutral sugars (54.83, 57.13 ppm) (Vogt, Stephen & Jackson, 1990) as well as those attributed to C-3 of β-D-galactose residues linked to terminal α-L-arabinofuranose (Cartier, Chambat & Joselau, 1987) and/or C-4 linkage of β-D-galactose (78.21, 78.52 ppm) (Vogt et al., 1990);

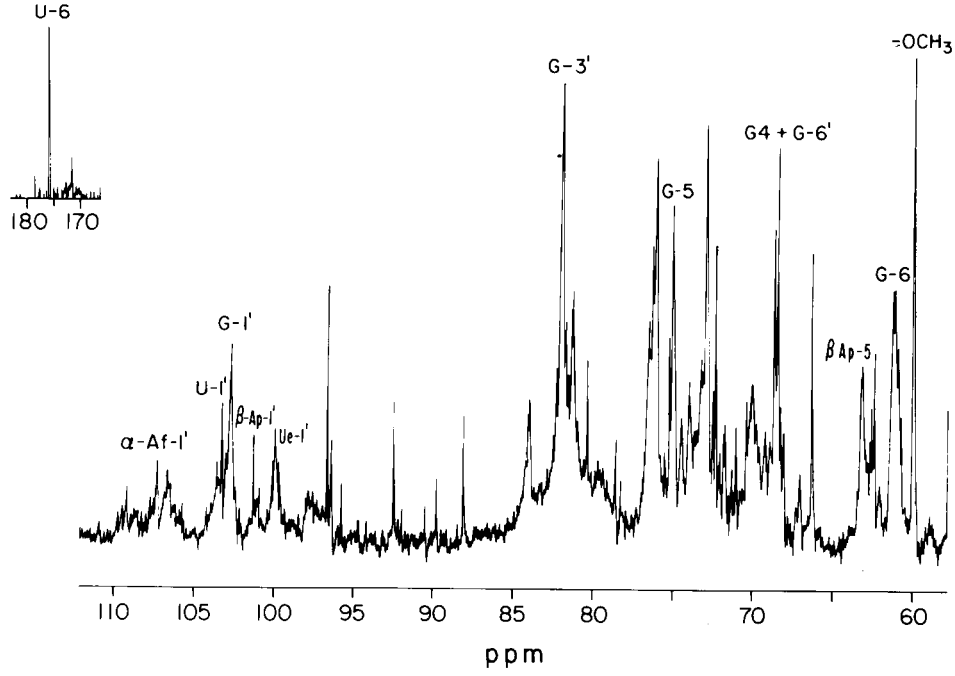

Fig. 1. ^{13}C-NMR spectrum of degraded gum A of *S. mombin*. G = β-D-galactopyranose. U = Uronic acids. Ue = 4-*O*-methyl-α-D-glucuronic acid. A_f = α-L-arabinofuranose. A_p = β-L-arabinopyranose. $'$ = carbon involved in the glycosidic linkage.

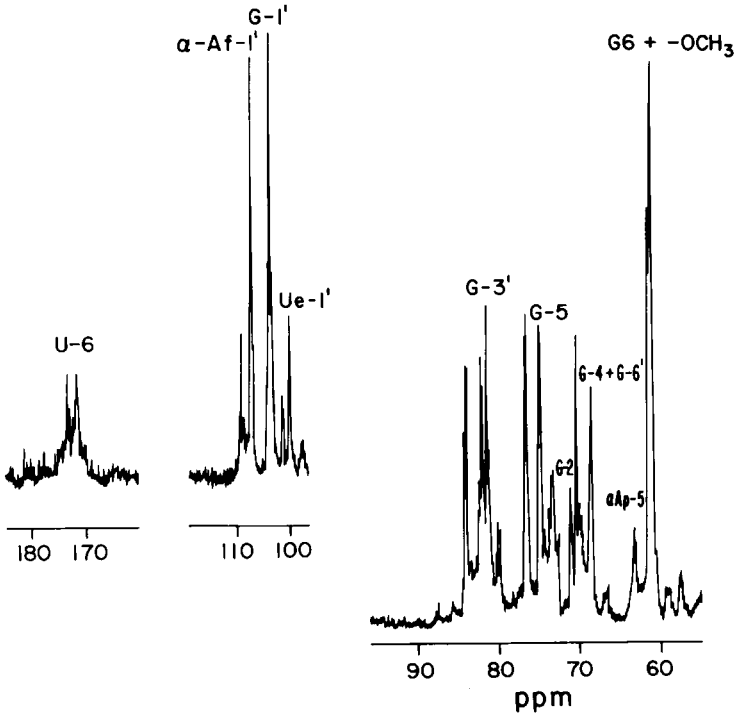

Fig. 2. ^{13}C-NMR spectrum of polysaccharide I of *S. mombin*. G = β-D-galactopyranose. U = Uronic acids. Ue = 4-*O*-methyl-α-D-glucuronic acid. A_f = α-L-arabinofuranose. A_p = β-L-arabinopyranose. $'$ = carbon involved in the glycosidic linkage.

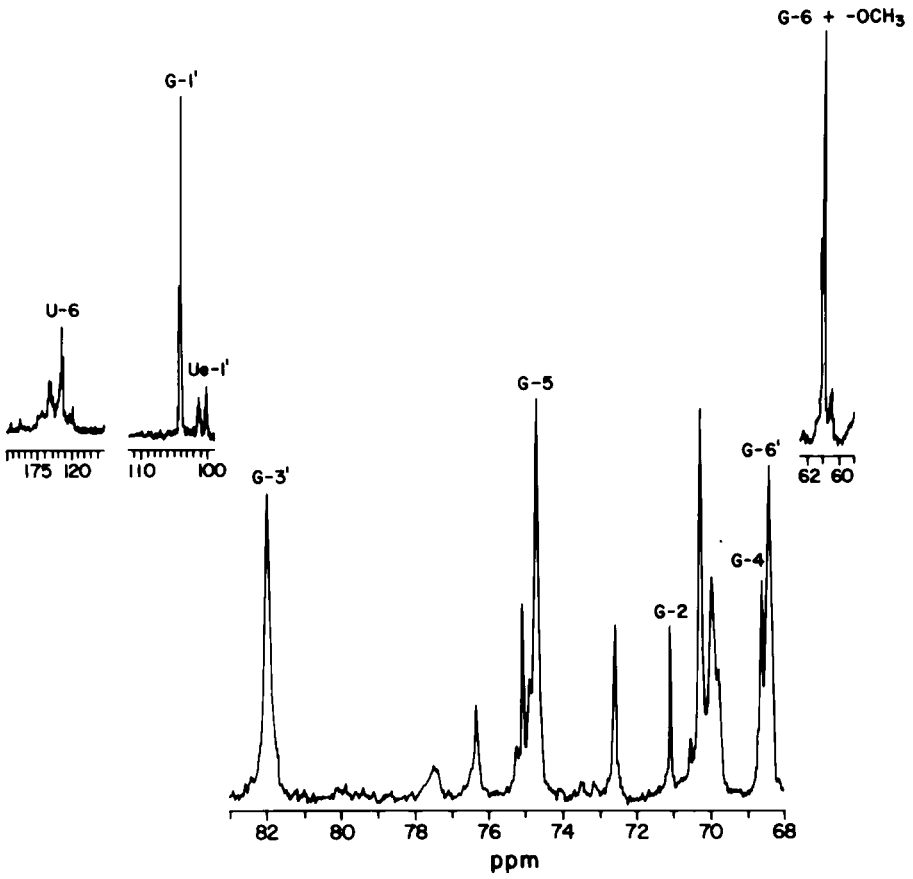

Fig. 3. ^{13}C-NMR spectrum of polysaccharide III of *S. mombin*. G = β-D-galactopyranose. U = Uronic acids. Ue = 4-*O*-methyl-α-D-glucuronic acid. A_f = α-L-arabinofuranose. A_p = β-L-arabinopyranose. $'$ = carbon involved in the glycosidic linkage.

assignment of these signals was supported by methylation analysis (Table 2) and by the isolation of 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose. There are also the signals due to acetyl groups (173.00, 20.50, 21.00 ppm) (León de Pinto, Gutiérrez de Gotera, Martínez, Ocando & Rivas, 1995; León de Pinto, Martínez, Bolaño de, Rivas & Ocando, 1998), reducing sugars (in the range from 88.00 to 98.00 ppm) (Martínez et al., 1996) and the unequivocal signal of the methyl group of rhamnose (17.00 ppm) (León de Pinto et al., 1998).

The spectrum of degraded gum A (Fig. 1) showed most of the resonances observed in the spectrum of the original polysaccharide (Tables 3 and 4). The signals (54.83, 57.13 ppm) due to methoxyl group linked to neutral sugar residues (Vogt et al., 1990) are still present and there are resonances due to terminal and 3-*O*-α-arabinofuranose residues (Table 5); resonances due to terminal and β-L-arabinopyranose residues were absent. Although there are some signals due probably to C-1 of 2-*O*-β-L-arabinopyranosyl residues (100.30, 100.36 ppm) (Mizutani et al., 1989). The absence of the signals assigned to C-4-linked β-D-galactose (78.21, 78.52 ppm) may be related to the removal of the aldobiouronic acid 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose and α-L-arabinofuranosyl residues, during the preparation of degraded gum A. The anomeric region of this spectrum is simpler than that of the original polysaccharide; signals due to terminal reducing sugar residues (88.00, 98.00 ppm) are absent. Therefore, they may belong to arabinose residues predominantly which were eliminated in the preparation of degraded gum A. The resonances of the acetyl group (173.20, 21.00 ppm) are still present in the spectrum.

Polysaccharide I, obtained from the original polysaccharide of *S. mombin* by a single Smith degradation, exhibited a spectrum (Fig. 2) which contains resonances described in the previous spectra (Tables 3–5).There were observed signals assigned to terminal and 3-*O*-α-L-arabinofuranosyl and 2-*O*-β-L-arabinopyranosyl residues (Table 5), according to methylation data (Table 2). Signals attributed to acetyl groups were also present in that spectrum.

The spectrum of polysaccharide III (Fig. 3) is quite similar to that of polysaccharide II except that signals due to α-arabinose were not observed. Both spectra showed resonances due to 3-*O*- and 6-*O*-galactosyl residues as well as those corresponding to uronic acid moieties (Tables 3 and 4). The signals attributed to 2-*O*-β-L-arabinopyranosyl residues disappeared in the spectra of polysaccharide II, but those due to 3-*O*-α-L-arabinofuranose were still present, suggesting that they are internal residues. Some signals due to internal β-L-arabinopyranosyl residues i.e. C-1 (101.32 ppm), C-2 (66.25 ppm) and C-5 (61.87 ppm) (Mizutani et al., 1989) were still present. The resonances due to acetyl groups (173.24. 21.25 ppm) (León de Pinto et al., 1995; León de Pinto et al., 1998) described in the above spectra, were also observed in the spectra of polysaccharides II and III. The location of this group within the polysaccharide structure must be further investigated.

Chemical and spectral evidence suggest the existence of small blocks of (1→3)-β-D-galactosyl residues interspersed with D-galactosyl residues substituted at both O-3 and O-6 in the backbone of the investigated structure. Uronic acid residues, linked to O-6 and O-4 of galactose, were difficult to remove from the nucleus. Arabinose chains of up to four units may exist in the side-chains; these residues exist predominantly in the furanose ring form (terminal, 2-*O*-, and 3-*O*-linked). Arabinopyranose (terminal and 2-*O*-substituted) were also present with rhamnose and some uronic acids present as terminal residues.

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