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Note

Structural studies of *Melicocca bijuga* gum exudate

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INTRODUCTION

Melicocca bijuga (Sapindaceae) a native plant of tropical America, is highly disseminated in Venezuela. It exudes a clear gum which is very soluble in water. Earlier studies on *Acacia senegal*[1] and *Acacia xanthophloea*[2] gum exudates established the importance of carbon-13 NMR, in combination with chemical methods, in the structural study of these complex polymers. The spectra of *M. bijuga* gum and its degradation products reported here were analyzed in relation to relevant model compounds.

EXPERIMENTAL

Origin and purification of gum sample.—Gum from *Melicocca bijuga* ("mamón") was collected in Maracaibo, Venezuela, South America in 1986. The gum (92 g) dissolved readily in cold water (3.1 L). The very clear solution was passed through Whatman No. 1 and 42 filter papers and dialyzed against running tap-water for two days; the gum was recovered by freeze-drying.

General experimental methods.—Standard methods of gum analysis were used[3–5]. The solvent systems used in paper chromatography were (*a*) 3:18:1:4 AcOH–EtOAc–HCO_2H–H_2O; (*b*) 1:5:3:3 (upper layer) benzene–1-butanol–pyridine–H_2O; (*c*) 10:5:1 EtOH–0.033 M H_3PO_4–1-butanol; and (*d*) 4:1:5 (upper layer) 1-butanol–EtOH–H_2O. Before using solvent (*c*), papers were dipped in 0.3 M NaH_2PO_4 solution and air-dried. Optical rotations at equilibrium were measured at room temperature in an Atago-Polax D polarimeter, using solutions (0.7–1.0%)

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of the samples in water. GLC was carried out with a Varian 2700 instrument fitted with a flame-ionization detector with N_2 flow rates of 40 mL/min. The glass column (168 × 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. ^{13}C NMR spectra were recorded with a Brüker WP-80-DG carbon-13 NMR spectrometer. Data points (6000–7000) were accumulated overnight at 37°C and with complete proton decoupling. The spectral width was 5000 Hz and they were calibrated by the addition of 1,4-dioxane to the samples. The polysaccharides (100–200 mg) were dissolved in D_2O (1 mL).

Sugar analysis.—The samples (100 mg) were hydrolyzed with 0.5 M H_2SO_4 for 18 h at 100°C; the hydrolyzates were cooled, neutralized ($BaCO_3$), deionized (Amberlite IR-120 resin, H^+ form) and concentrated to syrups on a rotary evaporator under diminished pressure at 30°C. Chromatography were carried out on Whatman No. 1 and 3MM papers with solvent systems (*a*) and (*b*). Sugars were determined by the phenol–H_2SO_4 method[6].

Identification of neutral and acidic sugars.—Purified gum (300 mg) was hydrolyzed with 0.5 M H_2SO_4 (20 mL) for 8 h as 100°C. The hydrolyzate was treated as described for sugar analysis and was fractionated on a column (16 × 6 cm) of Duolite A-H resin in the formate form. Elution with water (700 mL) and then with formic acid (5%; 500 mL) yielded the neutral and acidic fractions, respectively. After concentration to a syrup, paper chromatography was performed with solvents (*a*) and (*b*).

Methylation of the gum.—The pure gum (300 mg) was methylated[7] to give a product (140 mg) that was subjected to Purdie treatment[8] to give fully methylated derivatives. The product was methanolyzed and examined by GLC.

Preparation and examination of degraded gum A.—Purified gum (7.2 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. The dialyzate was concentrated and chromatographed in solvents (*a*) and (*b*). Degraded gum A was hydrolyzed with 0.25 and 0.5 M H_2SO_4 under the conditions just given[5].

Preparation and examination of degraded gum B.—Preliminary periodate oxidation experiments showed that 72 h was sufficient to oxidize degraded gum A. The uptake of periodate was monitored volumetrically[9]. Degraded gum A (1.5 g) was dissolved in water (44 mL) and 0.5 M $NaIO_4$ (50 mL) was added. After 72 h in darkness at room temperature the reaction was stopped by the addition of ethylene glycol (1.8 mL). The solution was treated as described previously[5]. Degraded gum B was isolated as the freeze-dried product and was studied by acid hydrolysis followed by PC.

Smith-degradation studies.—Preliminary small-scale experiments indicated that 0.25 M $NaIO_4$ solution and an oxidation time of 72 h were required for *M. bijuga*

gum. The oxidation was monitored by measuring the release of formic acid with time[10]. A series of three sequential Smith degradations was performed with the pure gum as starting material (50 g) to obtain polysaccharide I (11.0 g). Polysaccharide I (11.0 g) gave polysaccharide II (6.2 g), and polysaccharide II (1.5 g) gave polysaccharide III (0.6 g). The experimental conditions for these degradations were, in general, as described previously[5]. All of the polysaccharides were studied by acid hydrolysis followed by PC.

RESULTS AND DISCUSSION

The gum polysaccharide from *Melicocca bijuga* and its degradation products, in deuterium oxide, gave well-resolved ^{13}C NMR spectra.

Polysaccharide III, obtained by three successive Smith degradations of the original gum, contained only galactose (Table I). The ^{13}C NMR spectrum of this polymer, (Fig. 1), is very similar to that reported for the (1 → 3)-β-galactan in related polysaccharides[1,2]. Signal assignments attributed to this polymer (Table II), obtained by reference to methyl-3-*O*-methyl-β-D-galactopyranoside[11], permitted interpretation of the more-complex spectra. The highest-intensity peak (61.8 ppm) is attributable to the hydroxylated C-6 of galactose[12].

Polysaccharide II, obtained by two successive Smith degradations of the original gum, gave a ^{13}C NMR spectrum, very similar to that of polysaccharide III. It shows, in addition to the signals assigned to the (1 → 3)-β-galactan, signals (104.6 and 69.9 ppm) attributable to the β-(1 → 6) linkage[12–14] (Table II). This result is according to the chemical evidence, galactose was removed in the preparation of polysaccharide III. The multiplicity of peaks observed for C-3 atoms of galactose (73.5 and 74.30 ppm; 82.10 and 82.90 ppm) suggests two different environments available to the C-3 atoms.

Polysaccharide I, obtained by one Smith degradation of the original gum, gave a ^{13}C NMR spectrum (Fig. 2), more complex than the previous ones. It exhibits, in addition to the signals due to β-D-galactose (Table II), resonances assigned to α-L-arabinofuranose residues (Table III) and to uronic acid residues (Table IV). The anomeric linkage-region shows peaks corresponding to β-galactose (103.6,

TABLE I

Sugar composition [a] of *Melicocca bijuga* gum and its degradation products

Polymer	Gal	Ara	Rha	Uronic acid [b]
Original gum	43	15	17	25
Degraded gum A	63	6		31
Degraded gum B	100			
Polysaccharide I	67	22		11
Polysaccharide II	84	6		10
Polysaccharide III	100			

[a] Corrected for moisture. [b] β-D-Glucuronic acid and its α-4-methyl ether.

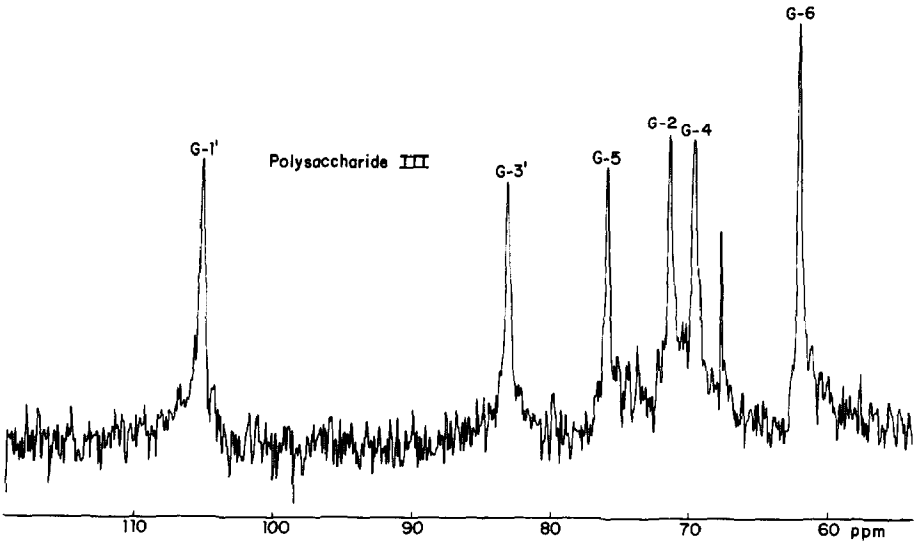

Fig. 1. ^{13}C NMR spectrum of polysaccharide III.

TABLE II

Comparison of ^{13}C NMR signals [a] of galactose in *Melicocca bijuga* gum

Model compounds	Polymer	C-1	C-2	C-3	C-4	C-5	C-6
Methyl-3-*O*-methyl-β-D-galactopyranoside [b]		103.9	69.8	82.0	69.2	75.1	61.2
β-(1 → 3) galactan [c]		102.9	70.1	71.6	69.1	73.9	59.7
				(80.9)			
	Original	103.2	71.0	72.8	69.8	75.2	62.1
	gum			(79.9)			(69.8)
				(82.6)			
				(82.9)			
	Degraded	103.2	70.9	72.6	69.4	76.0	61.4
	gum A	103.6	71.1	72.8	69.5	76.4	61.7
				(82.7)			(69.8)
		103.6					
	Polysaccharide	104.2	71.0	73.4	69.3	75.5	61.7
	I	104.7		73.7		75.9	(69.9)
				(82.1)			
				(82.9)			
	Polysaccharide	104.6	71.1	73.5	69.3	75.8	61.8
	II			74.3			(69.9)
				(82.1)			
				(82.9)			
	Polysaccharide	104.9	71.1	73.3	69.9	75.6	61.8
	III			(82.9)			

[a] Values relative to the signal of 1,4-dioxane (67.4 ppm relative to the signal of Me_4Si). [b] Ref. 11. [c] Ref. 2. Figures in parentheses are assigned to C-3 and C-6 linked galactose residues.

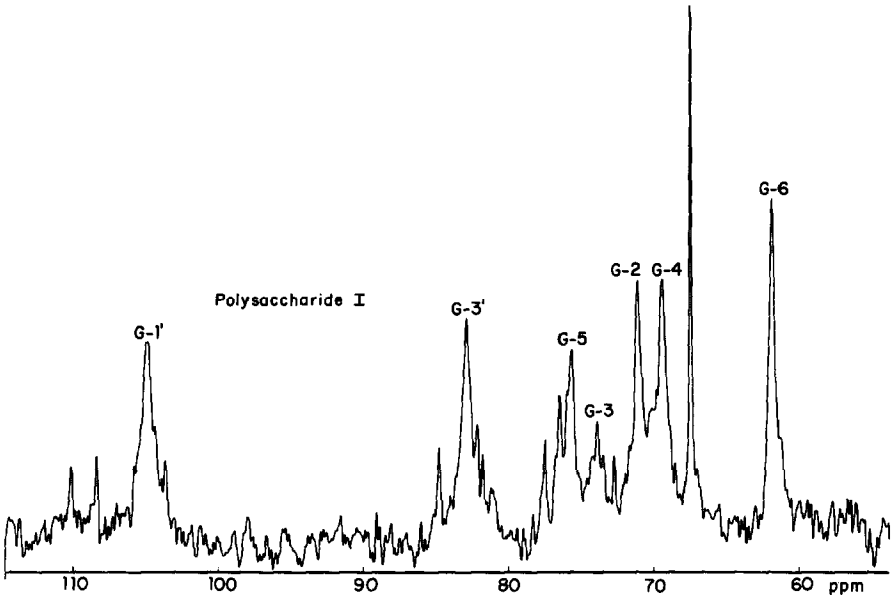

Fig. 2. ^{13}C NMR spectrum of polysaccharide I.

104.2, and 104.7 ppm)[11,15] and α-arabinofuranose (108.3 and 110.1 ppm)[15,16] residues. Peaks at 69.9 and 82.9 ppm indicate the presence of 6-*O*- and 3-*O*-linked galactose residues, respectively[13,14]. Therefore, this spectral evidence suggests the existence of β-(1 → 6) (103.6 and 69.9 ppm) and β-(1 → 3) (104.2, 104.7, and 82.9 ppm) linkages, as observed in the spectrum of polysaccharide II. The signals (108.3 and 110.8 ppm) assigned to α-arabinofuranose, suggest the existence of two environments for C-1 of α-L-arabinofuranosyl residues.

Degraded gum A, obtained by mild acid hydrolysis of the original gum, afforded a ^{13}C NMR spectrum (Fig. 3), that shows carbon resonances of galactose and uronic acid residues (Tables II and IV), respectively. The anomeric region shows

TABLE III

Comparison of ^{13}C NMR signals [a] of α-L-arabinofuranose in *Melicocca bijuga* gum and polysaccharide I

Model compound	Polymer	C-1	C-2	C-3	C-4	C-5
Methyl α-L-arabino-furanoside [b]		110.9	82.3	76.5	84.9	62.0
	Original	107.4	(84.7)	76.9	84.7	62.1
	gum	108.2	81.9	77.4	84.9	
			82.2			
	Polysaccharide	108.3	(84.7)	76.4	84.7	61.7
	I	110.1	81.7	77.3		

[a] Values relative to the signal of 1,4-dioxane (67.4 ppm relative to the signal of Me_4Si). [b] Ref. 11. Figures in parentheses are assigned to C-2 linked α-L-arabinofuranose.

TABLE IV

^{13}C NMR data [a] of uronic acid residues in *Melicocca bijuga* gum and its degradation products

Model compounds	Polymer	C-1	C-2	C-3	C-4	C-5	C-6	4-OMe
β-D-Glucuronic acid [b]		97.4	75.5	77.1	73.3	77.5	177.5	
4-*O*-Methyl-α-D-glucuronic acid [c]		(99.7)	72.2	73.3	82.70	70.8		61.1
	Original gum	104.4	75.9		73.8		176.0	
		104.7			74.2			
		101.5 [d]		73.8 [d]	82.9 [d]	70.9 [d]		60.8 [d]
				74.2 [d]				
	Degraded gum A	104.6	74.4		73.8		176.0	
					74.1			
		101.6 [d]	72.5 [d]	73.8 [d]	82.7 [d]			60.8 [d]
				74.1 [d]				

[a] Values relative to the signal of 1,4-dioxane (67.4 ppm relative to the signal of Me_4Si). [b] Ref. 18. [c] Ref. 17. [d] Signals due to 4-*O*-methyl-α-D-glucuronic acid.

evidence for three different kinds of linkage. These resonances were assigned to 4-*O*-methyl-α-D-glucuronic acid (101.6 ppm)[17], β-D-galactose (103.2 and 103.6 ppm)[11,15], and β-D-glucuronic acid (104.6 ppm)[18].

The original gum of *Melicocca bijuga* gave, as expected, a complex spectrum (Fig. 4). The gum contains galactose, arabinose, rhamnose, glucuronic acid, and its 4-*O*-methyl analogue (Table I). Nevertheless, all signals were assigned (Tables II–IV) by reference to spectra of the degraded polymers. The spectrum of the original gum exhibited two signals at the extreme ends of the spectrum, which can

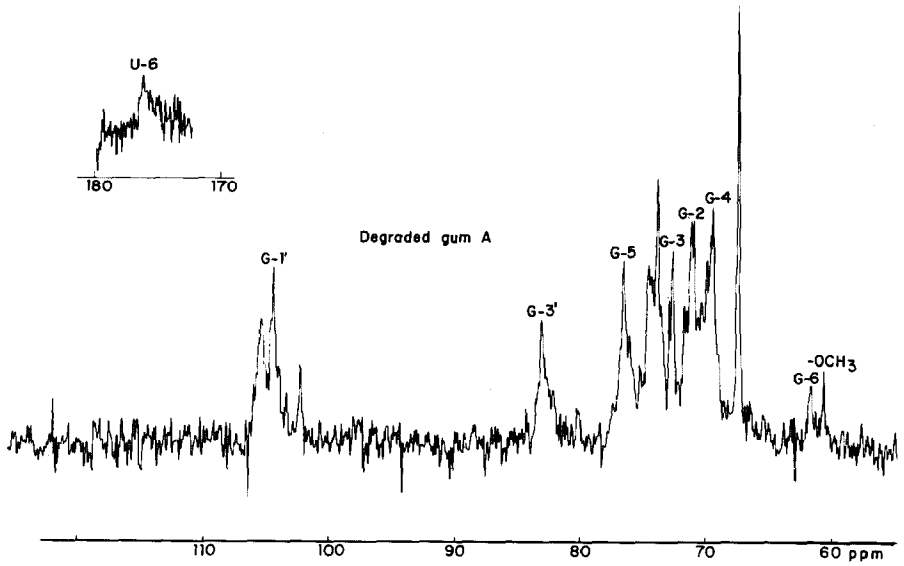

Fig. 3. ^{13}C NMR spectrum of degraded gum A.

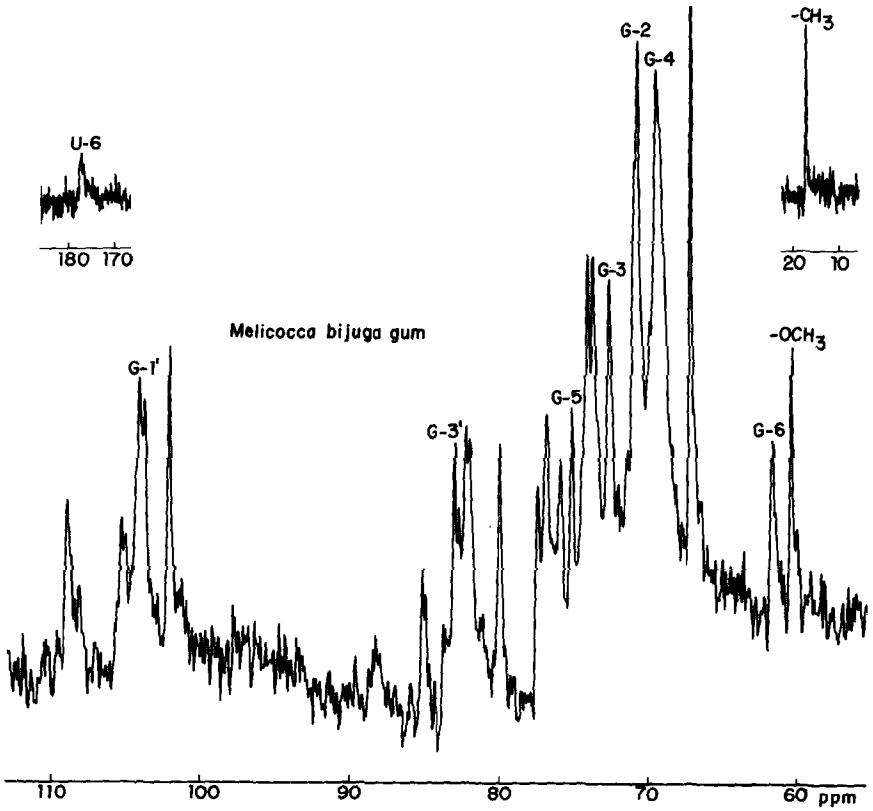

Fig. 4. ^{13}C NMR spectrum of *Melicocca bijuga* gum.

be assigned unambiguously to C-6 of rhamnopyranose (17.0 ppm)[1,11] and uronic acid residues (176.0 ppm)[18]. This spectrum (Fig. 4), shows four well-defined regions, as observed in related polymers[1,2]. The first region has peaks assignable to methoxyl groups (60.8 ppm)[17] and to C-6 of galactose (62.1 ppm)[11]. The second region (67–78 ppm) shows resonances of the hydroxylated ring carbon atoms. The group of peaks (79–85 ppm) attributable to carbons involved in the glycosidic linkage[19] and from arabinofuranose residues[11], belongs to the third region. Anomeric resonances (100–110 ppm), well separated from signals produced by the other nuclei, indicate the presence of at least seven different types of linkage. There are three overlapping peaks in the spectrum of the original gum, namely, the first due to C-6 of galactose and C-5 of α-arabinofuranose (62.1 ppm); the second, assigned to 4- and 6-linked galactose (69.8 ppm), and finally the overlapping peak (84.7 ppm) attributable to 4- and 2-linked arabinofuranosyl residues, as demonstrated by methylation analysis (Table V). ^{13}C Resonances, except for that of C-6 of rhamnose, were not observed in the spectrum of the original gum, probably because of overlapping of the signals of galactose and rhamnose atoms[20].

TABLE V

Methylation analysis of *Melicocca bijuga* gum

Methyl ethers [a]	T [b] (min)	Linkage
2,3,4-Me_3-L-Rha	0.47	L-Rha*p*-(1 →
2,3,5-Me_3-L-Ara	0.68	L-Ara*f*-(1 →
3,5-Me_2-L-Ara	1.05; (2.42)	→ 2)-L-Ara*f*-(1 →
2,3,4,6-Me_4-D-Gal	1.67	Gal*p*-(1 →
2,3,6-Me_3-D-Gal	(2.97), (3.91), (4.52)	→ 4)-Gal*p*-(1 →
2,4,6-Me_3-D-Gal	(3.91) (4.52)	→ 3)-Gal*p*-(1 →
2,3,4-Me_3-D-Gal	5.68; 6.01	→ 6)-Gal*p*-(1 →
2,4-Me_2-D-Gal	12.23; 13.91	→ 3,6)-Gal*p*-(1 →
2,3,4-Me_3-D-GlcA [c]	(2.42); 2.80	GlcA-(1 →

[a] Relative to methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside. [b] Ref. 5. [c] As methyl ester glycoside. Figures in parenthesis indicate T values of components that were not completely resolved.

The downfield displacements of the resonances of C-3 (+9.2 ppm) and C-6 (+8.1 ppm) atoms of galactose involved in the interglycosidic linkages (Table II) are comparable to the downfield shifts observed on 3-O[2,19] and 6-O[2,14,19] substitution of analogous compounds. The multiplicity of signals due to C-3 atoms of galactose in all the spectra, except in the spectrum of the galactan, suggests different environments for those atoms within the molecule of the polymers. Dearabinosylation, as reported for other arabinogalactans[2,15], enhanced the signals of the galactan and resolved the signal at 69.9 ppm (Table II). The spectrum of the original gum (Fig. 4), exhibits a resonance (79.9 ppm) assignable to C-3 of galactose linked to terminal arabinose residues[15].

The resonances of the arabinose residues (Table III), were assigned on the basis of literature data[11] and chemical results. Removal of arabinose in the preparation of degraded gum A led to the loss of the resonances due to those residues (Fig. 3) observed in the spectrum of the original gum (Fig. 4). The anomeric resonances at 107.4 and 108.2 ppm in the spectrum of the original gum, assigned to C-1 of α-arabinofuranose residues, appeared at lower field (108.3 and 110.1 ppm) in the spectrum of polysaccharide I. Steric hindrance may be responsible for this behavior. These anomeric resonances, which appeared in both spectra, were assigned to (1 → 2) internal- (107.4 and 108.3 ppm) and terminal- (108.2 and 110.1 ppm) α-arabinofuranose residues[21]. These results are in agreement with those obtained by methylation analysis (Table V). Loss of arabinofuranosyl residues on the preparation of polysaccharide I by Smith degradation of the original gum (Fig. 4), is in accord with a simultaneous increase in the intensity of the signals (110.1 and 84.7 ppm), in the spectrum of polysaccharide I. The absence of a signal at 79.9 ppm in this spectrum (Fig. 2), is probably related to the removal of α-arabinofuranose residues linked to C-3 of galactose[15], as observed in the spectrum of degraded gum (Fig. 3). Chemical and spectroscopic results reveal that most of α-arabinose residues in the original gum are probably (1 → 2)-linked and some are terminal residues linked to O-3 of galactose. The L-arabinose chains are at least

three units long, because three Smith degradations were sufficient to eliminate all of the arabinose residues (Table I).

The two aldobiouronic acids present in the original gum and in degraded gum A were 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose (R_{Gal} 0.28 (*a*), $[\alpha]_D^{20}$ −4°) and 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose (R_{Gal} 0.62 (*a*), $[\alpha]_D^{20}$ +91°). The resonances assignable to uronic acids (Table IV), are in accord with the chemical results. The signals at 60.8 and 82.9 ppm are unequivocally assignable to methoxyl and C-4 of 4-*O*-methyl α-D-glucuronic acid[17]. The anomeric signal (104.6 ppm) of C-1 of β-D-glucuronic acid appears at lower field than that assigned (101.5 ppm) to its 4-methyl ether[17].

This study shows that *M. bijuga* gum consists mainly of a (1 → 3)-β-D-galactan core with (1 → 6)-β-D-galactopyranosyl branches and side chains of α-L-arabinofuranosyl residues predominantly (1 → 2)-linked. β-D-Glucuronic acid and its α-4-methyl ether are probably linked to O-6 and O-4 of galactose, respectively.

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