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Relevant structural features of the polysaccharide from *Pithecellobium mangense* gum exudate

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Dedicated to Dr D.M.W. Anderson, who worked heartily in gum chemistry.

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Abstract

Pithecellobium mangense (Leguminosae), disseminated in north-east of Venezuela, produces a clear gum very soluble in water. The polysaccharide, isolated from this gum, contains galactose, arabinose and glucuronic acid and its 4-*o*-methyl derivative. Smith degradation analysis indicated that the backbone structure is that of a β-(1 → 3) galactan. Methylation analysis revealed the presence of 3,6-di-*o*-substituted galactosyl units, indicating a branched structure. Arabinosyl side chains, up to four units long, were present as α-L-arabinofuranosyl (terminal and 3-*o*-linked) and β-L-arabinopyranosyl (3-*o*-linked) residues. Two aldobiouronic acids, 6-*o*-β-D-glucopyranosyluronic acid-D-galactose and 4-*o*-(4-*o*-methyl-α-D-glucuronopyranosyl)-D-galactose, were isolated and characterized.

Keywords: *Pithecellobium mangense*; Structural analysis; Gum exudate; ^{13}C NMR spectroscopy

1. Introduction

Plants of the genus *Pithecellobium* (Leguminosae), most of which grow in southeast Asia, are well known as rich sources of tannins and related compounds (Lee, Morimoto, Nonaka & Nishioka, 1992). *Pithecellobium mangense*, disseminated in tropical regions of Central and South America, is located in northeast of Venezuela (Torrecilla, 1997). This species produces a clear gum very soluble in water. The analytical data of this gum have been published (León de Pinto, Martínez, Gutiérrez de Gotera, Vera & Rivas, 1995). This work deals with structural studies of the polysaccharide isolated from the *P. mangense* gum.

2. Materials and methods

2.1. Origin and purification of the gum exudate

The gum from *P. mangense* (Jacquin) Mc. Bride (Syn. *Chloroleucon mangense*) (Jacquin) Britton & Rose var. *mangense*) (Torrecilla, 1997). was collected from many specimens in a close area of a county located 20 km west of Maracaibo, Zulia State, Venezuela, South America. This species, known as "quebrajacho" in Venezuela, was identified by Dr Lourdes de Guevara, a botanical taxonomist of La Universidad Central de Venezuela. The gum was collected two weeks, after injiures were made at trunk level. The sample cleaned by hand (5 g), was dissolved in distilled water (100 ml). The solution was filtered through muslin and then through whatman No. 1 and No. 2 filter papers, dialysed for two days against tap water, and freeze-dried.

2.2. General methods

Standard methods for gum analysis were used (Anderson & Cree, 1968; Anderson & Bell, 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) and (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

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Table 1
Analytical data of *P. mangense* gum and its degradation products

Polymer	α_D (°)	Gal	Ara	UA[a]
Original gum[b]	−5	55	27	18
Degraded gum A	−7	73	10	17
Degraded gum B	–	83		17
Polysaccharide I	–	54	28	18
Polysaccharide II	−7	68	22	10
Polysaccharide III	–	67	21	12

[a] UA = uronic acids as glucuronic acid and its 4-*o*-methyl derivative.
[b] Corrected for moisture.

hydroxide solution on exhaustively electrodialysed samples. The specific rotations were measured with a Perkin–Elmer 343 polarimeter at 589 nm.

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 reported (Harris, Henry, Blakeney & Stone, 1984) and hydrolyzed with 2 M TFA (0.3 ml) for 1 h at 121°C. The mixture of the methylated monomers was reduced with 0.5 M borohydride (1 ml) in 2 M ammonia for 60 min at 60°C and then was acetylated with ethyl acetate (1 ml) and acetic anhydride (3 ml) using 1-methylimidazole (200 μl) as catalyst. The alditol acetates were extracted with dichloromethane and 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 referenced to 1,4-di-*o*-acetyl-2,3,5-tri-*o*-methylarabinitol (T_r 15.24).

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. Experimental conditions were described previously (León de Pinto, Martínez, Ludovic de Corredor, Rivas & Ocando, 1994a).

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) 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 3 MM 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)–(c).

2.6. 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.

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 p.c. in solvents (a) and (b).

2.8. 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; Martínez, León de Pinto, Rivas & Ocando, 1996). Degraded gum A (1.2 g) was obtained from purified gum (2 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 $NaIO_4$) of degraded gum A.

2.9. Preparation and studies of polysaccharides I–IV

A series of four polysaccharides were prepared by using controlled Smith-degradation which consists in periodate oxidation (0.125 M $NaIO_4$), borohydrate reduction and hydrolysis acid at room temperature. The pure gum as starting material (40 g) led to obtain polysaccharide I (11.2 g). This polymer (10 g) yielded polysaccharide II (4.25 g), and the latter (3 g) yielded polysaccharide III (900 mg) which led to polysaccharide IV. The experimental conditions for the preparation and examination of these polymers were, in general, those previously described (León de Pinto et al., 1994a; Martínez et al., 1996).

3. Results and discussion

The polysaccharide isolated from *P. mangense* consists of galactose (60%) arabinose (21%) and uronic acid (19%), Table 1. The absence of rhamnose was confirmed by

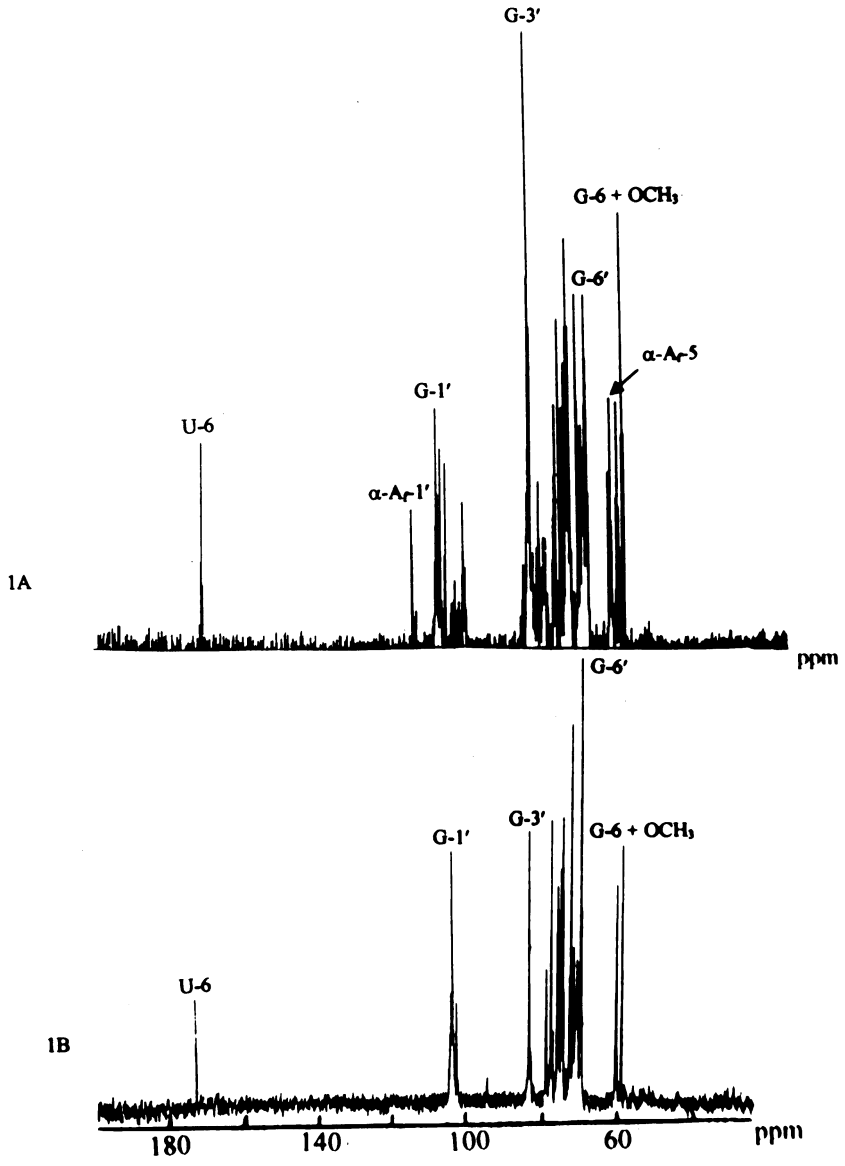

Fig. 1. ^{13}C NMR spectra of the original gum (1A) and degraded gum A (1B) from *P. mangense*. G = β-D-galactopyranose. U = uronic acids, ' = carbon involved in the glycosidic linkage.

chromatography and ^{13}C NMR spectroscopy; isolation of neutral and acidic components by column chromatography confirmed the presence of the above neutral sugars. The uronic acids were represented by glucuronic acid and its 4-*o*-methyl derivative.

Partial acid hydrolysis of the original gum led to two oligosaccharides which, based on chromatographic behaviour, hydrolysis studies and methylation analysis, were identified as aldobiouronic acids. The first oligosaccharide, R_{gal} 0.27 (a), $[\alpha]_D + 1°$, was 6-*o*-β-D-glucopyranosyluronic acid-D-galactose and the second one, R_{gal} 0.67(a), $\alpha_D + 91°$, corresponded to 4-O-(4-O-methyl-alpha-D-glucupyranosyluronic acid)-D-galactose.

The preparation of degraded gum A with dilute acid removed arabinofuranosyl residues preferently. The acidic vulnerability of these residues has been reported in many gums (León de Pinto et al., 1995; León de Pinto, Martínez, Beltrán, Torres & Rivas, 1997). Partial acid hydrolysis of degraded gum A led to obtain the same aldobiouronic acids described above for the original gum.

Degraded gum B, obtained from oxidation (0.25 M $NaIO_4$) of degraded gum A, represents a core of the structure, which is basically a β-(1 → 3) galactan. This structural feature is very common in gums from *Acacia* (Anderson & Cree, 1968; Fincher, Stone & Clarke, 1983; León de Pinto, 1991) and in other gums (León de Pinto et al., 1994a; León de Pinto, Martínez, Bolaño, Rivas & Ocando, 1998a).

The preparation of the polysaccharide I, having the original gum as starting material and the successive Smith

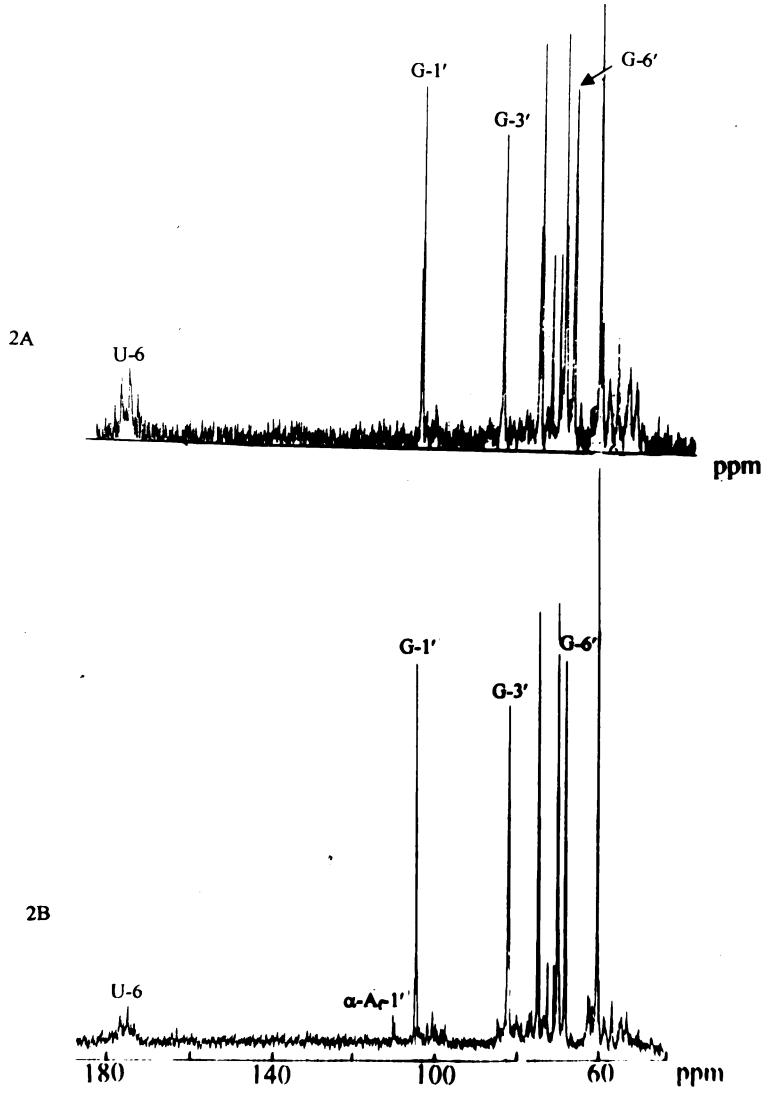

Fig. 2. ^{13}C NMR spectra of the polysaccharide III (2A) and degraded gum B (2B) from *P. mangense*. G = β-D-galactopyranose. U = uronic acids. Af = α-L-arabinofuranose. ′ = carbon involved in the glycosidic linkage.

degradation to prepare polysaccharides II and III showed a gradual removal of arabinose, Table 1. The relative high yield of these polysaccharides suggests stability to oxidative cleavage (León de Pinto, et al., 1998a).

Polysaccharide IV has a structure very close to that of degraded gum B; there are traces of uronic acids, Table 1. The length of the arabinose side-chains may be up to four units, because four successive Smith-degradations were required to remove the arabinosyl residues from the structure. The presence of uronic acids in the degraded polysaccharides may be associated with the resistance of those residues to Smith degradation. It has been observed that the fragments resulting from the oxidative scission of hexuronic acids are more resistant to hydrolysis than the corresponding fragments from neutral sugars (Aspinall, 1983).

The low values of the specific rotation of the original polysaccharide and its degradation products, Table 1, suggest the existence of β-linkages predominantly in the structure (León de Pinto, Gutiérrez de Gotera, Martínez, Ocando & Rivas, 1998b) which may be related with galactosyl, arabinopyranosyl and glucuronosyl acid residues.

Signal assigments of ^{13}C NMR spectra of the original polysaccharide and its degradation products, Figs. 1 and 2, were made using appropriate models, taking into consideration methylation analysis, Table 2, hydrolysis studies and previous results from other gums (León de Pinto et al., 1994a; León de Pinto, Martínez & Rivas, 1994b; León de Pinto et al., 1998a; León de Pinto, Martínez, Gutiérrez de Gotera, Rivas & Ocando, 1998c).

^{13}C NMR spectrum of the original gum from *P. mangense*, Fig. 1A, contains the resonances due to galactose, arabinose and uronic acid residues. There are some signals, Tables 3 and 4, Fig. 1A which were assigned unequivocally, i.e. the signal at lowest field (175.69 ppm) corresponds to C-6 of uronic acids (León de Pinto et al.,

Table 2
Methylation analysis of the gum from *P. mangense* gum

Alditol acetate	Tr[a]	Type of linkage
2,3,5-Me_3-Ara	1.00	Araf (1 →
2,3,4-Me_3-Ara	1.07	Arap(1 →
2,5-Me_2-Ara	1.18	→ 3)Araf(1 →
2,3,4,6-Me_4-Gal	1.30	Galp(1 →
2,4,6-Me_3-Gal	1.43	→ 3)Galp(1 →
2,3,4-Me_3-Gal	1.51	→ 6)Galp(1 →
2,4-Me_2-Gal	1.64	→ 3)Galp(1 → ↓ 6

[a] Tr relative to 1,4-di-*o*-acetyl-2,3,5-tri-*o*-methyl arabinitol. It was detected GlcA(1 → as methyl ester methyl glycoside in other methylation experiments.

1994b); the anomeric region contains the resonances assignable to reducing sugars (96–97 ppm) (León de Pinto et al., 1997), β-L-arabinopyranose (101.42 ppm), 6-*o*-β-D-galactopyranose (102.58; 102.81 ppm), 3-*o*-β-D-galactopyranose (103.69 ppm) (León de Pinto et al., 1994a; León de Pinto et al., 1998a,c); terminal (109.36 ppm) and 3-*o*-α-L-arabinofuranose (108.53 p.p.m.) residues (León de Pinto et al., 1994a). The signal of C-1 of β-D-glucuronic acid is overlapped with that of 3-*o*-β-D-galactopyranose. The anomeric region signals, except those due to reducing sugars, are related to C-3 (82.00; 82.47 ppm) and C-6 (68.61 ppm) of β-D-galactopyranose involved in 1,3 and 1,6 glycosidic linkages; C-3 (83.63; 83.91 ppm) of α-L-arabinofuranose and C-3 (74.59 ppm) of β-L-arabinopyranose residues (León de Pinto et al., 1994a). The two last assignments were related to the resonances due to C-5 of β-L-arabinopyranose (62.88; 63.47 ppm) and α-L-arabinofuranose residues (61.04; 61.29 ppm) (León de Pinto, 1994a). There is also a signal (79.86 ppm) (León de Pinto et al., 1998c) assignable to galactose 3-*o*-linked to terminal α-L-arabinofuranosyl residues in the spectrum of the original gum, Fig. 1A, which disappeared in the spectrum of degraded gum A, Fig. 1B, as expected, because these residues were removed during the preparation of degraded gum A. This spectrum, simpler than that of the original gum, 1A, confirmed the presence of 3-*o*- and 6-*o*-galactosyl residues, β-D-glucuronic acid and 4-*o*-methyl-α-D- glucuronic acid, Tables 3 and 4. The signal C-1 (101.54 ppm) in this spectrum revealed the presence of β-L-arabinopyranosyl residues.

^{13}C NMR spectrum of degraded gum B, Fig. 2A, quite simple, shows the resonances due to 3-*o*- and 6-*o*-galactose, Table 3. There are also observed signals due to C-1 and C-6 of uronic acid residues, which corroborates, as was observed by chemical methods, that these residues, as traces, were difficult to remove from the structure. The spectrum of polysaccharide III, Fig. 2B, is very similar to that exhibited for degraded gum B, Fig. 2A, as was expected according to chemical results. They showed unequivocal resonances of 3-*o*-β-D-galactopyranose residues.

The spectrum of polysaccharide I contains signals attributed to 3-*o*-, and 6-*o*-galactose; β-D-glucuronic acid and the α anomer of its 4-*o*-methyl derivative, terminal and 3-*o*-α-L-arabinofuranose and 3-*o*-β-L-arabinopyranose.

Chemical and spectral evidence suggest that *P. mangense* gum has a β-(1 → 3) galactan backbone; there is also evidence of 6-*o*-β-D-galactopyranose. Methylation analysis support the presence of 3,6-di-*o*-substituted galactose, α-L-arabinofuranose (terminal and 3-*o*-linked) and β-L-arabinopyranose (3-*o*-linked) in the whole gum structure; uronic

Table 3
^{13}C NMR spectral data (values relative to the signal of 1,4-dioxane (δ 66.67 ppm)) of the carbons involved in glycosidic linkages of neutral sugars of *P.mangense* gum and its degradation products

Type of linkage	Polymer	C-glycon	C-aglycon
→ 3)β-D-Gal*p*(1 → [a]	A	103.69	82.00
			82.47
	B	103.31	81.98
		103.90	
	C	103.88	81.91
	D	103.89	81.91
→ 6)β-D-Galp(1 → [a]	A	102.58	68.61
		102.81	
	B	102.90	68.56
	C	103.88[b]	68.33
			68.51
	D	103.89[b]	68.36
α-L-Araf(1 → [a]	A	109.36	
	D	109.15	
→ 3)α-L-Araf(1 → [a]	A	108.53	83.63
	D	108.51	83.50
→ 3)β-L-Arap(1 → [a]	A	101.42	74.60
	B	101.54	74.63
	C	101.48	74.62

[a] León de Pinto et al., 1994a.
[b] This signal is overlapped with that due to C-1' of 3-*o*-β-D-galactopyranose. A = original gum, B = degraded gum A, C = degraded gum B, D = polysaccharide III.

Table 4
^{13}C NMR unequivocal data (values relative to the signal of 1,4-dioxane (δ 66.67 ppm)) of uronic acids of *P. mangense* gum and its degradation products

Type of linkage	Polymer	C-1	$-OCH_3$
β-D-Glcp(1 → [a]	A	103.69[b]	
	B	104.10	
	C	104.19	
	D	104.21	
4-*o*-Me-α-D-GlcA(1 → [a]	A	99.12	59.92
		99.29	
	B	99.30	59.86
	D	99.37	60.40

[a] León de Pinto et al., 1994a,b.
[b] This signal is overlapped with that due to C-1 of 3-*o*-β-D-galactopyranose. A = original gum. B = degraded gum A. C = degraded gum B. D = polysaccharide III.

acids are present as glucuronic acid and its 4-*o*-methyl analogue.

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