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Chemical and spectroscopic studies of the gum polysaccharide from *Acacia macracantha*

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The polysaccharide isolated from *Acacia macracantha* gum, a Venezuelan Gummiferae spp., contains galactose, arabinose, rhamnose, glucuronic acid and its 4-*O*-methyl analogue. ^{13}C-NMR spectroscopy, in combination with chemical methods, was applied in this study. A complex fragment, constituted of galactose, arabinose and glucuronic acid (3:2:1), was obtained from the original polysaccharide and its degradation products. This fragment appears to be an important structural feature of the gum. The backbone of the structure (degraded gum B) is a β-(1→3) galactan, although glucuronic acid and arabinose residues are still present in the core of the structure. Stereochemistry of the molecule may work against periodate oxidation of these sugar residues. Arabinose may be present as internal residues and as short side-chains while glucuronic acid exists mainly as a terminal residue. Copyright © 1996 Published by Elsevier Science Limited.

INTRODUCTION

Studies of the polysaccharide gums from *Acacia* spp. in Bentham Series 4 (Gummiferae) have demonstrated that the main skeletal chains are composed of D-galactopyranose residues that are decomposed by periodate. The presence in the galactan core of a high proportion of D-galactopyranosyl residues that are vulnerable to Smith-degradation was demonstrated by the very considerable extent of cleavage of the main skeletal chain that evidenced when the gum polysaccharides from *A. nubica* (Anderson & Cree, 1968), *A. drepanolobium* (Anderson & Dea, 1968), *A. seyal* (Anderson *et al.*, 1968), *A. karoo* (Churms *et al.*, 1983) and *A. xanthophloea* (León de Pinto, 1991; León de Pinto & Martínez, 1995) were subjected to two or more successive Smith-degradations. These structural studies indicated that long chains of (1→3)-linked D-galactopyranosyl residues are not a dominant structure feature of these polysaccharides. The presence of small blocks of periodate-resistant, (1→3)-linked-D-galactopyranosyl residues has been reported in the galactan core of the gum polysaccharide of *A. karoo* (Churms *et al.*, 1983). Analytical data of *A. macracantha* and *A. tortuosa* gums have been published recently (Martínez *et al.*, 1992; León de Pinto *et al.*, 1993a). This paper reports some structural features of the polysaccharide gum of *Acacia macracantha* Humb et Bonpl, ex Willd in Willd, using chemical methods and ^{13}C-NMR spectroscopy.

EXPERIMENTAL

Origin and purification of gum sample

Gum from *A. macracantha*, Gummiferae spp., known in Venezuela as "cuji hediondo", was collected in the location of "El Mecocal", Zulia State (east of Lake Maracaibo), Venezuela, by the authors in February–March, 1989. The identification of voucher specimens was confirmed by Dr Lourdes Cárdenas de Guevara, a botanical taxonomist of the Universidad Central de Venezuela. A voucher specimen is deposited at the Botanical Garden, Maracaibo, Venezuela. The gum exudate, very soluble in water, was purified as described previously (Anderson & Pinto, 1980; Martínez *et al.*, 1992).

General experimental methods

Standard methods for gum analysis were used (Anderson *et al.*, 1967; Anderson & Dea, 1968). The solvent systems used in paper chromatography were (v/v): (a) 3:18:1:4 AcOH–EtOAc–HCO_2H–H_2O; (b) 1:5:3:3 (upper layer) 1-butanol–EtOH–H_2O; (c) 10:5:1 EtOH–0.1M HCl–1-butanol; and (d) 4:1:5 (upper layer) 1-butanol–EtOH–H_2O. Before using solvent (c), papers were dipped in 0.3M NaH_2PO_4 solution and air-dried. The nitrogen content was determined by the method of Kjeldahl. Experimental conditions for the optical rotation, glc and

^{13}C-NMR analyses have been reported previously (León de Pinto *et al.*, 1992, 1993b, 1994a,b,c).

Autohydrolysis experiments

A solution of the purified sample (5%) was heated at 100°C for 120 h; portions (10 ml) were withdrawn at various intervals, concentrated and analyzed by pc. The polymer was 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, 1992, 1993b, 1994a,b,c). Degraded gum A (1.54 g) was obtained from purified gum (4 g) by mild acid hydrolysis. Preliminary small-scale experiments showed that 120 h were required for the preparation of degraded gum B by periodate oxidation of degraded gum A.

Smith-degradation studies

A series of four sequential Smith-degradations was perfomed with the pure gum as the starting material to obtain polysaccharide I (33%), polysaccharide II (20%), polysaccharide III (19%) and polysaccharide IV (13%). The experimental conditions for these degradations were reported previously (León de Pinto, 1991, 1992, 1993b, 1994a,b,c). The preparation of polysaccharides I–III was repeated in order to check the yields and to have enough sample to complete the Smith-degradation process.

RESULTS AND DISCUSION

The gum polysaccharide from *A. macracantha*, contained galactose, arabinose, rhamnose and uronic acids (β-D-glucuronic acid and its α-4-methyl ether), Table 1. Partial acid hydrolysis, followed by paper chromatography, did not show the presence of galactooligosaccharides, therefore, the probability of a long chain of galactose is low, which has been observed in other Gummiferae *Acacia* gums (Anderson *et al.*, 1967; Anderson & Dea, 1968; Churms *et al.*, 1983; León de Pinto, 1991; León de Pinto & Martínez, 1995). A complex fragment, constituted of galactose, arabinose and glucuronic acid (3:2:1), was isolated and characterized. Methylation analysis of this oligosaccharide showed 3-*O*-substituted galactose (T=3.47); 3,6-di-*O*-substituted galactose (T=11.37; 14.01), 2-*O*-substituted arabinopyranose (T=1.47), terminal arabinopyranose (T=0.97) and terminal glucuronic acid (T=2.36, 2.82). A possible structural model, Fig. 1, is given for this fragment.

Neutral and acid components, separated by column chromatography, showed the presence of the neutral sugars mentioned previously, and 6-*O*-β-D-glucopyranosyluronic acid-D-galactose. This aldobiuronic acid was characterized by its chromatography behavior, hydrolysis studies and methylation analysis. Studies of the formic acid hydrolyzate corroborated the previous results.

The interpretation of the methylation analysis of the original gum, Table 2, suggests the presence of 3-*O*-, 6-*O*-mono, and 3,6-di-*O*-substituted galactose residues; terminal and 2-*O*-substituted arabinopyranose residues; terminal arabinofuranose and 4-*O*- and terminal glucuronic acid residues.

The relatively high nitrogen content found for *A. macracantha* gum (4.98%), Table 1, is comparable with that reported for *A. tortuosa* gum (6.02%) (León de Pinto *et al.*, 1993a); and higher than those reported for *A. tortilis* (1.9%) (Gammon *et al.*, 1986) and *A. robusta* (2.8%) gums (Churms et al., 1986), but lower than the highest values reported for *A. erioloba* (9.0%) and *A. hebeclada* (9.4%) gums (Churms *et al.*, 1986).

Autohydrolysis of the original gum and the preparation of degraded gum A released arabinose and rhamnose. On the other hand, galactose and the complex fragment, isolated in the partial hydrolysis of the origi-

Table 1. Analytical data[a] of *A. macracantha* gum and its degradation products

Polymer	Yields, %	[α][b], degrees	Sugar composition[c], %				Gal:Ara
			Gal	Ara	Rha	U.A.	
Original polysaccharide[d]	66	−11	43	30	5	22	1.4
Autohydrolysis polymer	31	−47	47	23	6	24	2.0
Degraded gum A	38	−38	61	12	–	27	5.1
Degraded gum B	13	−55	66	21	–	13	3.1
Polysaccharide I	33	−28	55	19	6	20	2.9
Polysaccharide II	20	−53	60	12	-	28	5.0
Polysaccharide III	19	−50	60	13	-	27	4.6
Polysaccharide IV	13	n.d.	65	21	-	14	3.1

[a]Corrected for moisture. [b]In water. [c]Relative to the total sugar content. [d]Nitrogen content = 4.98%; hence protein (N * 6.25) = 31.12%. n.d. = not determined.

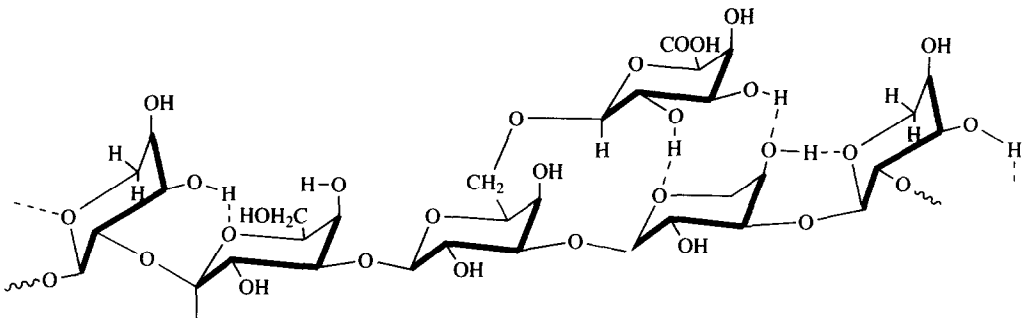

Fig. 1. Suggested conformation of the structural fragment on the basis of methylation analysis and NMR assignments.

Table 2. Methylglycosides[a] of *A. macracantha* gum exudate

O-methyl sugar	*T* (min)	Type of linkage
2,3,5-Me_3-Ara	0.64	Ara*f*(1→
2,3,4-Me_3-Ara	0.97	Ara*p*(1→
3,4-Me_2-Ara	1.46	→2)Ara*p*(1→
2,3,6-Me_3-Gal	2.97,(3.91),(4.77)	→4)Gal*p*(1→
2,4,6-Me_3-Gal	(3.91),(4.77)	→3)Gal*p*(1→
2,3,4-Me_3-Gal	5.83,7.73	→6)Gal*p*(1→
2,4-Me_2-Gal	10.30,12.23,13.9	→3,6)Gal*p*(1→
2,3,4-Me_3-GlcA[b]	2.23,2.80	Glc*Ap*(1→
2,3-Me_2-GlcA[b]	9.52	→4)Glc*Ap*(1→

[a]Relative to methyl 2,3,4,6-*tetra*-*O*-methyl-β-D-galactopyranoside. [b]As methyl ester methyl glycoside.

nal gum, were released during the preparation of degraded gum A. Mild acid hydrolysis of this polymer showed arabinose, galactose and uronic acid residues. The susceptibility of arabinose residues to autohydrolysis and mild acid hydrolysis is according to the liberation of α-L-arabinofuranose residues, predominantly, as has been observed in other analogous polymers (León de Pinto, 1991; León de Pinto *et al.*, 1992, 1993b, 1995).

Degraded gum B, obtained by drastic periodate oxidation (0.25 M) of degraded gum A, consists of galactose, arabinose and glucuronic acid (5:2:1), Table 1. The preparation of this polymer was repeated three times under different experimental conditions in order to check its sugar composition. Hydrolysis studies, chromatographic separation and methylation analysis revealed that the core of the gum is a β-(1→3) galactan, as has been reported for many *Acacia* gums (Anderson *et al.*, 1967; Anderson & Dea, 1968; León de Pinto, 1991) and analogous polymers (León de Pinto *et al.*, 1992, 1993b). Although the presence of arabinose and glucuronic acid in the structure of the polysaccharide core may be related to steric hindrance that prevents their removal by periodate oxidation, as was demonstrated by the isolation of the complex fragment, Fig. 1, in the partial hydrolysis of this polymer.

A. macracantha gum was subjected to four successive Smith-degradations, giving polysaccharides I–IV, Table 1. Methylation analyses of these degraded polysaccharides confirmed the results observed in the original gum, Table 2. Partial hydrolysis studies of these polymers led to the isolation of the above fragment, Fig. 1, which may explain the high yields obtained for the degraded polysaccharides, Table 1. The oligosaccharide, Fig. 1, isolated in the original gum and its degradation products, appears as the relevant structural feature of the gum polysaccharide studied. This atypical finding contrasts with the results reported for gums from other Gummiferae spp. (Anderson *et al.*, 1967; Anderson & Dea, 1968; León de Pinto, 1991).

The gum polysaccharide from *A. macracantha* and its degradation products, in deuterium oxide, gave well-resolved ^{13}C-NMR spectra. Signal assignments of these spectra have been made on the basis of chemical evidence and of previous results (León de Pinto, 1991; León de Pinto *et al.*, 1992, 1993b, 1994a,b).

The ^{13}C-NMR spectrum of degraded gum B, Fig. 2, contains the resonances assigned unequivocally to 3-*O*- and 6-*O*-substituted galactose residues, Table 3. The two environments observed for C-1 (103.9; 104.3 ppm),

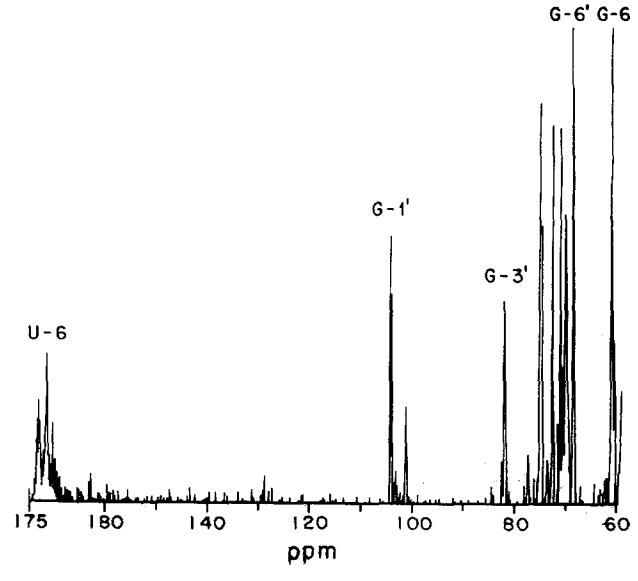

Fig. 2. ^{13}C-NMR spectrum of degraded gum B of *A. macracantha* gum. G = β-D-galactose. ' = carbon atom involved in the glycosidic linkage. U-6 = C-6 of uronic acids.

Table 3. Carbon-13 NMR data[a,b] of β-D-galactose residues in *A. macracantha* gum exudate and its degradation products

Type of linkage	Polymer	C-1	C-2	C-3	C-4	C-5	C-6
→3)Gal*p*(1→[c]		105.0	71.2	83.0	69.3	75.6	61.8
	o.g.	103.8	71.4	81.4	69.2	74.1	61.0
		104.2	71.9	82.0	69.3	75.0	61.2
				82.1	69.8	75.1	
				82.3	69.9		
	d.g.A	103.4	71.5	81.6	69.1	75.0	60.3
		103.7	71.7	81.9	69.8	75.2	60.9
		104.3					
	d.g.B	103.9	71.1	82.1	69.9	74.7	60.5
		104.3	71.6			75.1	60.9
	I	103.9	70.9	81.9	69.8	74.9	60.4
							60.9
→6)Gal*p*(1→[d]		103.3	70.4	72.5	67.8	73.0	68.1
	o.g.	102.4	70.5	72.5	66.3	73.0	68.4
		102.6		72.6		73.7	68.5
				72.8			68.6
							68.8
	d.g.A	102.6	70.1	72.4	66.5	73.6	68.5
				72.9			68.5
	d.g.B	102.0	70.2	72.6		72.6	68.4
							68.6
	I	103.9		72.4	66.6	68.5	

[a]Values relative to the signal of 1,4-dioxane (δ 66.67 ppm). [b]The same signals were observed in the spectrum of polysaccharide II. o.g. = original gum; d.g.A = degraded gum A; d.g.B = degraded gum B; I = polysaccharide I. [c]León de Pinto *et al.* (1993b). [d]León de Pinto *et al.* (1994a).

C-2 (71.1; 71.6 ppm), C-5 (74.7; 75.1 ppm) and C-6 (60.5; 60.9 ppm) of 3-*O*-substituted galactose residues may be related to the two types of galactose residues shown in the structural fragment, Fig. 1, which was isolated by partial hydrolysis of this polymer. There are resonances attributed to C-1 (101.21 ppm) and C-5 (60.45 ppm) of internal β-L-arabinopyranose residues (León de Pinto *et al.*, 1994a). The last signal, of high intensity, overlaps with the C-6 of the galactose residues. There are resonances due to β-D-glucuronic acid and its α-4-methyl ether, the presence of which was shown by chemical methods, Table 1.

The ^{13}C-NMR spectrum of degraded gum A, Fig. 3, obtained by mild acid hydrolysis of *A. macracantha* gum, was as complex as those corresponding to degraded gum B, discussed previously. Partial dearabinosylation enhanced some signals and led to a better resolution of the resonances, as has been

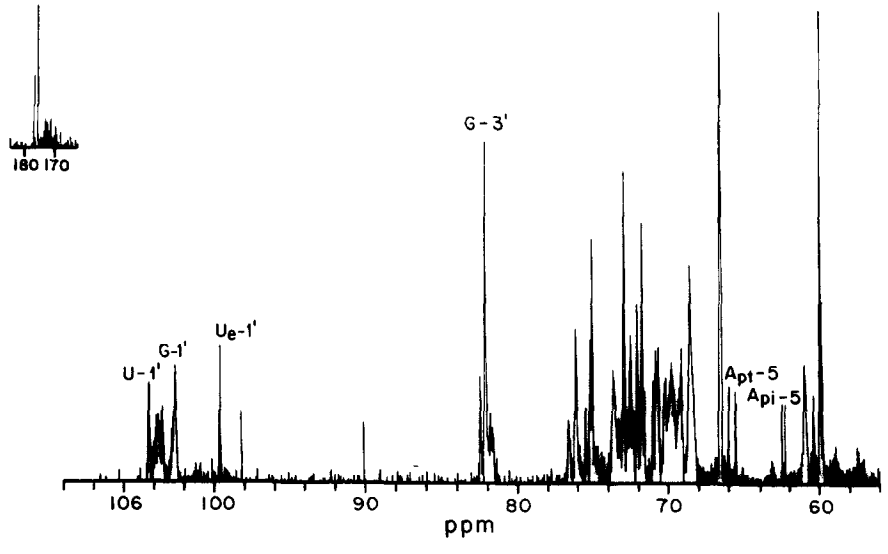

Fig. 3. ^{13}C-NMR spectrum of degraded gum A of *A. macracantha* gum. A_{pt} = terminal β-L-arabinopyranose. A_{pi} = internal β-L-arabinopyranose. U_e = 4-*O*-methyl-α-D-glucuronic acid residues. U = β-D-glucuronic acid.

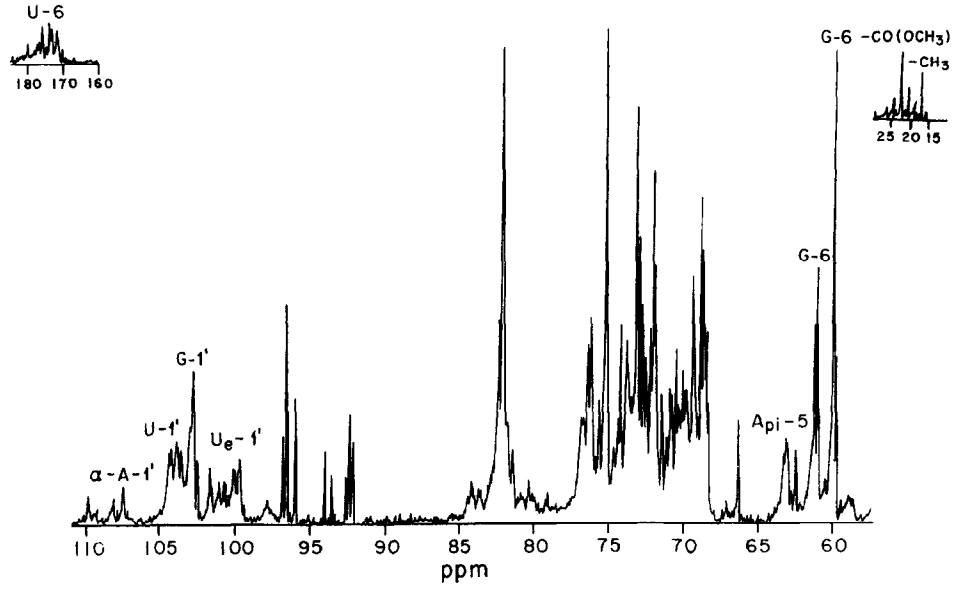

Fig. 4. ^{13}C-NMR spectrum of *A. macracantha* gum exudate. A = α-L-arabinofuranose.

reported for other arabinogalactans (León de Pinto *et al.*, 1992, 1993b, 1994a). The anomeric region (90–105 ppm) showed signals due to the terminal reducing residues (90 ppm) (Bock & Pedersen, 1983); 4-*O*-methyl-α-D-glucuronic acid (98.08, 99.49 ppm) (León de Pinto *et al.*, 1994b); 6-*O*- (102.56 ppm) (León de Pinto *et al.*, 1994a) and 3-*O*-substituted (103.4, 103.69 ppm) (León de Pinto, 1991; León de Pinto *et al.*, 1993b) galactose residues and β-D-glucuronic acid residues (104.26 ppm) (León de Pinto *et al.*, 1994b). The expansion of the resonances in the range (68–77 ppm) led to improved signal assignments. There are signals due to C-5 of terminal (65.47; 65.52; 65.93 ppm) (Bock & Pedersen, 1983) and internal β-L-arabinopyranose residues (62.24; 62.43 ppm) (León de Pinto *et al.*, 1994a). The anomeric carbon-atoms of 6-*O*-substituted galactose and β-L-arabinopyranose residues (102.56 ppm) may overlap.

The spectrum of the original gum of *A. macracantha*, Fig. 4, contains the resonances of the sugar residues, described previously, Tables 2–4. The anomeric region showed many terminal reducing sugars (92–96 ppm). Spectral evidences, supported by methylation analysis, revealed the presence of terminal α-L-arabinofuranose and 2-*O*- linked-β-L-arabinopyranose residues. On the other hand, there are unequivocal signals of acetyl groups (20.45; 22.30 ppm) (Agrawal, 1992; León de Pinto *et al.*, 1994b) and methyl of rhamnose (16.83 ppm) (León de Pinto *et al.*, 1994c). The multiplicity of the resonances due to C-6 of uronic acid residues may indicate different environments for these sugar residues within the molecule.

Table 4. Carbon-13 NMR data[a,b] of uronic acid residues in *A. macracantha* gum exudate and its degradation products

Type of linkage	Polymer	C-1	C-2	C-3	C-4	C-5	C-6	4-OMe
4-*O*Me-α-D-GlcA(1→[c]		99.7	72.2	73.3	82.7	70.8		61.1
	o.g.	99.6	72.1	73.0	82.9	70.7		59.8
			72.2			70.8		59.9
	d.g.A	99.5	72.4	72.9	82.4	70.6		59.7
						70.7		59.9
						70.9		
	d.g.B	101.2	72.5	72.5	82.0	70.5		59.1
	I	99.9	72.5	73.0	81.9	70.9		60.3
Glc*Ap*(1→[c]		104.0	75.5	77.1	73.3	77.5	177.5	
	o.g.	104.1	75.6	76.1	73.0	76.3	173.5	
	d.g.A	104.1	75.4	76.0	72.9	76.5	175.7[d]	
							176.8[d]	
	d.g.B	104.3	75.1	77.5	72.5	77.5	171.6	
	I	103.9	75.0	76.5	73.0	76.5	173.0	

[a]Values relative to the signal of 1,4-dioxane (δ66.67 ppm). [b]The same signals were observed in the spectrum of polysaccharide II.[c]León de Pinto *et al.* (1994b). [d]Averaged values.

The interpretation of the spectra of the polysaccharides I–II confirmed the evidence observed in the other spectra.

This work shows, by chemical and spectroscopy studies, the presence of a complex fragment, which may exist as a relevant feature of the structure of the gum polysaccharide obtained from *A. macracantha* gum. The relatively high nitrogen content of the Gummiferae gum studied is an interesting feature and may indicate the existence of a direct polysaccharide–protein covalent linkage. This is now being investigated.

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