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THE POLYSACCHARIDE GUM FROM *ACACIA TORTUOSA**

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Key Word Index—*Acacia tortuosa*; Leguminosae; gum exudates; polysaccharide structure; ^{13}C NMR spectroscopy.

Abstract—The polysaccharide gum obtained from Venezuelan *Acacia tortuosa* contains galactose, arabinose, xylose as traces, glucuronic acid and its 4-*O*-methyl analogue. ^{13}C NMR spectroscopy, in combination with chemical methods, was applied in this study. The preparation of degraded products, *via* hydrolytic- and Smith-degradations, indicated interesting structural features of the polysaccharide. The core of the structure is essentially a branched β-(1 → 3) galactan. Arabinose, glucuronic acid and its 4-*O*-methyl analogue could not be totally removed from the core. Arabinose (as furanose and pyranose residues) exist as terminal and 3-*O*-linked, while xylose, glucuronic acid and its 4-*O*-methyl derivative are probably terminal residues. The high content of proteinaceous material in *A. tortuosa* gum must be investigated in order to determine whether there are many polysaccharide-protein covalent linkages. © 1997 Elsevier Science Ltd

INTRODUCTION

Many papers of analytical data and structural studies of *Acacia* gum exudates have been published [1–4]. The Venezuelan species are all included in the series Gummiferae, Vulgares and Fillicineae [5]. Comparison between the analytical data of *A. macracantha* and *A. tortuosa* gums, Venezuelan Gummifereae species have been reported [6, 7]. The present work deals with the structural study of *Acacia tortuosa* gum.

RESULTS AND DISCUSSION

The polysaccharide isolated from *A. tortuosa* gum contains galactose, arabinose, xylose as traces and uronic acid residues (Table 1). Partial hydrolysis, followed by paper chromatography showed an oligosaccharide, Rgal 0.33 (a) which was isolated and characterized by hydrolysis, chromatographic studies and methylation analysis as 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose. Neutral galactosaccharides were not isolated which may be related to the low probability of long chains of galactose in the structure of the investigated polysaccharide [3].

Studies of the sulphuric (0.5 M) and formic (10%) acids hydrolysates, led to the separation of neutral and acid components by column chromatography. The presence of galactose, arabinose, glucuronic acid and 4-*O*-methyl-α-D-glucuronic acid was detected.

Methylation analysis of the original gum (Table 2) showed the presence of 3-*O*-, 6-*O*-, 3,6-*O*-D-galactose residues, terminal and 3-*O*-L-arabinofuranose and 3-*O*-L-arabinopyranose residues, together with terminal glucuronic acid.

Degraded gum A, prepared by mild hydrolysis, consisted mainly of galactose, arabinose and uronic acid (Table 1). During the preparation of degraded gum A, galactose, arabinose, xylose and glucuronic acid were removed. The unusual presence of xylose in *Acacia* gum was confirmed as terminal residues by its isolation during the preparation of this polymer. Partial hydrolysis of degraded gum A led to the isolation of 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, which was characterized previously in the partial hydrolysate of the original gum.

Degraded gum B, obtained by drastic periodate oxidation (0.25 M) of degraded gum A, is essentially an acidic branched β-(1 → 3) galactan. Arabinose, glucuronic acid and its 4-*O*-methyl analogue were not totally removed from the core. This finding, observed previously in *A. macracantha* gum [8] and other gums [9, 10], may be related to steric hindrance due to hydrogen bonding between those sugars that work against their removal by periodate oxidation. The iso-

* Dedicated to Dr D. M. W. Anderson, who has investigated *Acacia* gums for many years.

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Table 1. Sugar composition of *Acacia tortuosa* gum and its degradation products

Polymer	Yield, %	Sugars, %[a] Galactose	Arabinose	Xylose	Uronic acids
Original gum		69	13	<1	18
Degraded gum A		72	7	—	21
Degraded gum B	45	62	9	—	29
Polysaccharide I	30	58	20	—	22
Polysaccharide II	38	63	17	—	20
Polysaccharide III	20	60	19	—	21

[a] Corrected for moisture.

Table 2. Methylation analysis* of *Acacia tortuosa* gum exudate

O-methyl sugars	T (min)	Type of linkage
2,3,5-Me_3-Ara	0.65	Araf(1→
2,4-Me_2-Ara	(2.20); 2.36	→3)Arap(1→
2,5-Me_2-Ara	1.26; (2.20)	→3)Araf(1→
2,3,6-Me_3-Gal	2.98; (3.90); (4.75)	→4)Galp(1→
2,4,6-Me_3-Gal	(3.90); (4.75)	→3)Galp(1→
2,3,4-Me_2-Gal	5.82; 7.01	→6)Galp(1→
2,4-Me_2-Gal	12.20; 14.0	→3,6)Galp(1→
2,3,4-Me_2-GlcA†	2.42; 2.80	GlcAP(1→

* Relative to methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside.

† As methyl ester methyl glycoside.

lation and characterization of the bioses 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose and 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose confirmed the presence of these uronic acids in the core.

Acacia tortuosa gum was subject to the three successive Smith-degradations, giving the polysaccharides I–III. The high yields obtained for the degraded products (Table 1), as has been reported for other Gummiferae [3, 4] and Vulgares gums [11], may indicate relatively low proportions of end-groups and suggest a low degree of branching of their structure. On the other hand, the high proportion of uronic acid residues in polysaccharide I and the small variation of sugar composition in the different polysaccharides (Table 1), as a possible consequence of incomplete removal of neutral and acidic components, may be related to the relatively high yields obtained for the polysaccharides.

The gum polysaccharide from *A. tortuosa* and its degradations products gave well-resolved ^{13}C NMR spectra in deuterium oxide. Signal assignments of these spectra have been made on the basis of chemical evidence and previous studies [9, 10, 12, 13].

The ^{13}C NMR spectrum of degraded gum B was very complex and showed resonances due to 3-*O*- and 6-*O*-β-D-galactose residues [12, 13], β-D-glucuronic acid and its 4-*O*-methyl analogue [9] (Tables 3 and 4). It is worth noting the multiple environments for C-6 of 3-*O*-β-D-galactose residues (δ 60.5 and 61.0), C-6 of β-D-glucuronic acid (δ 175.7 and 176.8) and for the methoxyl group of the 4-*O*-methyl ether (δ 59.0, 59.8, 59.9 and 60.1). The isolation and characterization of the oligosaccharides 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose and 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose, the sugar composition, the methylation analysis (Tables 1 and 2) support the spectral evidences for the backbone of the polysaccharide gum from *A. tortuosa*.

^{13}C NMR spectra of degraded gum A and polysaccharides I–III contained signals due to β-D-galactose and uronic acid residues, observed in the spectrum of degraded gum B (Tables 4 and 5). There are unequivocal resonances due to terminal and 3-*O*-L-arabinofuranose residues and some signals for 3-*O*-β-L-arabinopyranose residues in the spectra of polysaccharides I and II [9, 12, 13]. Resonances due to acetyl groups (δ 21.0, 22.0, 171.5 and 173.2) were observed in the spectra of degraded gum B, degraded gum A and polysaccharide I [9]. The location of the acetyl groups in the structure of the polysaccharide gum studied was not investigated.

Chemical and spectroscopic evidences support the existence of an acidic branched β-(1 → 3) galactan in the backbone of the structure of the polysaccharide gum of *A. tortuosa*. Removal of uronic acid residues was difficult. Arabinose (as furanose and pyranose) exist as terminal and 3-*O*-linked units, while xylose is a terminal residue. The absence of rhamnose in this gum, corroborated by chemical and spectroscopic studies, may be useful for distinguishing this gum from the related *A. macracantha*, a Venezuelan Gumniferae species [6]. On the other hand, the relatively high nitrogen content reported for *A. tortuosa* gum (6.02%) [7] is an interesting feature that must be investigated in order to determine whether there are any polysaccharide–protein covalent linkages.

EXPERIMENTAL

General. Standard methods for gum analysis were used [9]. The solvent systems used for PC were (a) HoAc–EtOAc–HCO_2H–H_2O, 3:18:1:4, (b) *n*-BuOH–EtOH–H_2O, 1:5:3:3 (upper layer), (c) EtOH–0.1 M HCl–*n*-BuOH, 10:5:1 and (d) *n*-BuOH–EtOH–H_2O, 4:1:5 (upper layer). Before using solvent (c), papers

Table 3. ^{13}C NMR spectral data*,† of β-D-galactose residues in *Acacia tortuosa* gum exudate

Type of linkage	Polymer	C-1	C-2	C-3	C-4	C-5	C-6
→3)β-D-Galp(1→‡		103.8	71.1	82.1	68.8	75.3	61.3
					69.0		
	Degraded gum B	103.5	71.8	82.0	69.3	75.1	60.3
			72.1				61.0
	Degraded gum A	103.5	71.8	82.0	69.3	75.1	61.0
				82.4	69.9		
	Original gum	102.6	71.8	82.1	§	75.1	§
	I	103.9	71.1	82.0	69.8	74.7	61.0
			71.2		69.9	74.8	
						75.1	
	II	103.9	71.3	80.1	69.7	75.2	61.8
→6)β-D-Galp(1→‡		103.3	70.4	72.8	67.8	73.0	68.1
	Degraded gum B	103.0	70.7	72.6	§	73.0	68.6
	Degraded gum A	102.6	70.7	72.6	67.0	73.0	68.6
	Original gum	102.6	70.0	73.0	§	73.0	68.5
	I	101.2	70.5	72.6	66.7	72.6	68.4
							68.6

* Values relative to the signal of 1,4-dioxane (δ 66.67).
† Same signals observed in spectrum of polysaccharide III.
‡ Ref. [10].
§ Signals not well resolved.

Table 4. ^{13}C NMR data*,† of β-D-glucuronic acid residues in *Acacia tortuosa* gum and its degradation products

Type of linkage	Polymer	C-1	C-2	C-3	C-4	C-5	C-6
β-D-GlcA (1→‡		104.7	75.5	77.1	73.3	77.5	177.5
	Degraded gum B	104.3	75.1	76.2	73.7	76.7	175.7
							176.8
	Degraded gum A	104.3	75.1	76.2	73.6	76.7	171.5
							173.2
							175.7
							176.8
	Original gum	102.6	75.1	76.1	§	76.1	173.9
							175.7
							176.5
							176.9
	I	104.2	75.1	76.3	73.1	77.5	171.5
							173.3
	II	103.9	75.2	76.3	73.2	77.3	172.8
						77.5	174.2
						77.7	175.9
						77.9	

* Values relative to signal of 1,4-dioxane (δ 66.67) I = polysaccharide I; II = polysaccharide II.
† Spectrum of polysaccharide III shows signals at δ 181.45 and 181.51 due to β-D-glucuronic acid substituted by metals.
‡ Ref. [9].
§ Signal not observed.

were dipped in 0.3 M NaH_2PO_4 soln and air-dried. Determinations of opticals rotations, conditions for GC and ^{13}C NMR analyses are reported elsewhere [9, 10].

Origin and purification of gum. Gum from *A. tortuosa* (L.) Willd ('úveda'), was collected in the location of 'Los Puertos de Altagracia', Zulia State (east of lake Maracaibo), Venezuela, by the authors in February–March, 1994. Identification of voucher specimens was confirmed by Dra. Lourdes Cárdenas de Guevara, a botanical taxonomist of the Universidad Central de Venezuela. The gum exudate, very soluble in H_2O, was purified as described previously [6].

Preparation and examination of degraded gums A and B. Unless otherwise stated, the experimental procedures used for the prepn and examination of degraded gums A and B were the same as those described previously [12, 13]. Preliminary small-scale expts showed that 96 hr were required for the prepn of degraded gum B.

Table 5. ^{13}C NMR spectral data*,† of 4-*O*-methyl-D-glucuronic acid in *Acacia tortuosa* gum and its degradation products

Type of linkage	Polymer	C-1	C-2	C-3	C-4	C-5	4-OMe
4-OMe-D-GlcA(1→‡		99.7	72.2	73.3	82.7	70.8	61.1
	Degraded gum B	99.6	72.1	73.7	82.4	70.7	59.0
						70.9	59.8
							59.9
							60.1
	Degraded gum A	99.6	72.0	73.6	82.4	70.7	59.8
						70.9	59.9
							60.0
	Original gum	99.6	§	§	82.1	70.0	60.0

* Values relative to signal of 1,4-dioxane (δ 66.7).
† Same resonances observed in spectra of polysaccharides I, II, III.
‡ Ref. [9]
§ Signals not well resolved.

Smith-degradation. A series of three sequential Smith-degradations was performed with the pure gum as the starting material to obtain polysaccharide I (30%), polysaccharide II (38%) and polysaccharide III (20%). The experimental conditions for these degradations were, in general, as described previously [12, 13]. The prepn of polysaccharides I–III was repeated in order to check the yields and to have enough sample to complete the sequential Smith-degradation processes.

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