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Chemical and spectroscopic studies of *Cercidium praecox* gum exudate

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Abstract

The structure of the polysaccharide from *Cercidium praecox* (R&P) Harms gum exudate has been studied by Smith degradation, by sugar and methylation analyses, and by ^{13}C NMR spectroscopy. The results showed a (1 → 4)-xylan core. Some xylose residues are substituted at O-2 by α-D-glucuronic acid and 4-*O*-methyl-α-D-glucuronic acid residues. β-D-Glucuronic acid is present, probably as terminal residues. The arabinose is present as α-L-furanose and β-L-pyranose.

Key words: Cercidium praecox; Leguminosae; Glucuronoxylan; Gum exudate; ^{13}C NMR spectroscopy

1. Introduction

Cercidium praecox (R&P) Harms, Leguminosae, the only Venezuelan species of the genus *Cercidium*, exudes a clear gum that is very soluble in water. The chemical composition of this gum [1] differs significantly from that corresponding to the majority of the Leguminosae gums studied so far [2], and the structural features of this gum are here studied by ^{13}C NMR spectroscopy, in combination with chemical data [3,4].

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2. Experimental

Origin and purification of gum sample.—Gum from *Cercidium praecox*, commonly known as "yabo", was collected by Gladys León de Pinto between March and July, 1984, from trees growing in a very localized area in Quisiro, Zulia State in northwestern Venezuela, near lake Maracaibo. The crude gum was then purified as described previously [3].

General methods.—Standard methods for gum analysis were used [5,6]. Descending PC was performed on Whatman papers No. 1 and 3MM with: (*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–0.1 M HCl–1-butanol. Before using solvent *c*, papers were dipped in 0.3 M NaH_2PO_4 solution and air dried. GLC was performed with a Varian 2700 instrument fitted with a flame-ionization detector with an N_2 flow rate of 40 mL/min. The glass column (166 × 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. Optical rotations were measured with an Atago Polax-D polarimeter at 30°C. The ash composition of the original gum was determined by atomic absorption spectroscopy. The IR spectrum of the original gum, was recorded as a pellet in KBr. ^{13}C NMR spectra of the original gum and polysaccharides I and II were recorded with an AM-300 spectrometer in D_2O. The signal for 1,4-dioxane at 66.67 ppm served as the internal reference. The spectrum of degraded gum A was recorded under the same experimental conditions in a WP-80-DS spectrometer.

The procedures for partial and total hydrolysis, isolation and identification of neutral and acidic components, quantitative analysis of sugars, methylation of the oligosaccharides and polysaccharides have been reported [6–10].

Autohydrolysis experiments.—A solution of purified sample (5%) was heated for 96 h at 100°C; portions (10 mL) were withdrawn at various intervals and analyzed. The polymer was isolated by freeze-drying.

Isolation and identification of aldobiouronic acids by using formic acid.—Purified gum (200 mg) was hydrolyzed with HCO_2H (50 mL, 20%) for 5 h at 100°C. The formic acid was eliminated by successive evaporation under diminished pressure. The separation of neutral and acidic sugars was performed by column chromatography. The experimental conditions used in the isolation and identification of the aldobiouronic acids have been reported [6].

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 [3–6]. Degraded gum A (0.4 g) was obtained from purified gum (6.2 g). Preliminary small-scale experiments showed that 96 h were required for the preparation of degraded gum B.

Smith-degradation studies.—A series of three sequential Smith degradations was performed with the pure gum as the starting material (40 g) to afford polysaccharide I (15 g). Polysaccharide I (12 g) gave polysaccharide II (1.89 g), and the latter

(1.0 g) yielded polysaccharide III (0.34 g). The experimental conditions for these degradations were, in general, as described previously [3].

3. Results and discussion

The gum was obtained from a botanically authenticated tree of *Cercidium praecox* (R&P) Harms. The purified gum polysaccharide contained xylose, arabinose, and uronic acids (β-D-glucuronic acid and its α-4-methyl ether) (Table 1). The absence of galacturonic acid and galactose in this gum contrasts with the results reported for one Argentinian sample [2]. Neutral and acidic components were separated and identified by column chromatography. Xylose and arabinose were confirmed as the neutral sugars. The acidic components were isolated and further characterized by their chromatographic behavior, by hydrolysis studies, and by methylation analysis, as 4-*O*-(α-D-glucopyranosyluronic acid)-D-xylose, R_{Xyl} 0.33 (*a*) and 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose, R_{Xyl} 0.68 (*a*). The identification of the neutral and acidic sugars present in the gum were corroborated by the studies of the formic acid hydrolyzate. Partial hydrolysis of the original gum yielded the aldobiouronic acids just described and an oligosaccharide R_{Xyl} 0.75 (*b*) characterized by hydrolysis studies, methylation, and chromatography analyses as a (1 → 4)-xylobiose.

Autohydrolysis of the original gum and the preparation of degraded gum A released arabinose, xylose, and two oligosaccharides corresponding to 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-β-D-xylose, R_{Xyl} 0.69 (*a*), and a (1 → 4)-xylotetraose, R_{Xyl} 0.14 (*b*) [11], which were identified by hydrolysis studies, chromatography, and methylation analyses. Mild acid hydrolysis of degraded gum A showed the oligosaccharides observed in the original gum. Arabinosyl-substitute oligosaccharides could not be detected after mild acid treatment of the gum, indicating that the L-arabinose residues had been hydrolyzed [12].

Degraded gum B, the backbone of the structure, was obtained by drastic periodate oxidation (0.25 M) of degraded gum A. It consisted of xylose and uronic acid residues (Table 1). A possible structural model is shown (Fig. 1). The observed

Table 1
Sugar composition of *Cercidium praecox* gum and its degradation products

Polymer	Yield (%)	Sugars [a] (%)		
		Xylose	Arabinose	Uronic acids [b]
Original gum		59	16	25
Degraded gum A	6	70		30
Degraded gum B		89		11
Polysaccharide I	37	42	15	43
Polysaccharide II	16	53	12	35

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

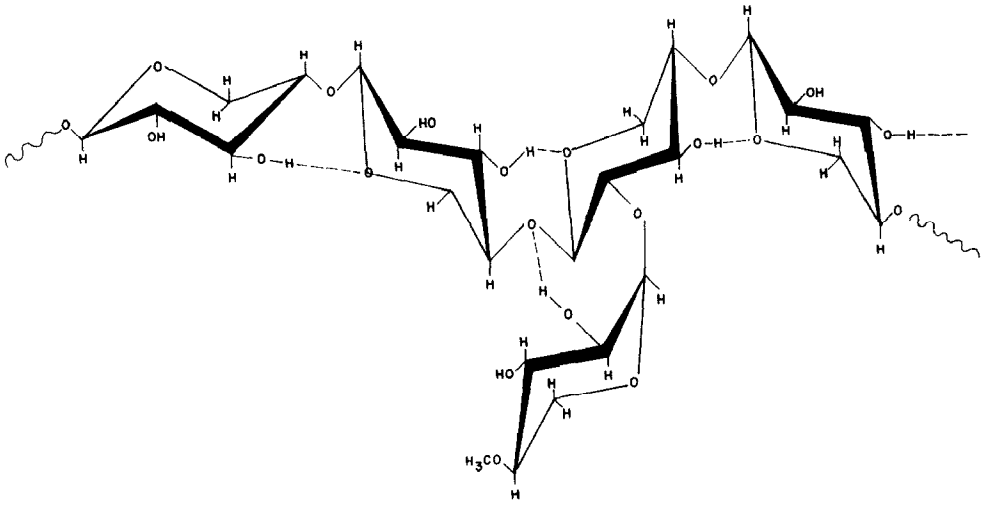

Fig. 1. Possible model for the core of *Cercidium praecox* gum. The (1 → 4)-xylan may be substituted either by α-D-glucuronic acid or its α-4-methyl ether.

protection against periodate oxidation of sugar residues in the core structure of the gum may arise from hydrogen bonding between the sugar residues [13].

The gum was submitted to three successive Smith degradations. A mixture of xylose-containing products, isolated from the partial hydrolyzates of polysaccharides I, II, and III were identified as β-(1 → 4)-linked xylobiose, R_{Xyl} 0.73 (*b*), xylotriose, R_{Xyl} 0.40 (*b*), and xylotetraose, R_{Xyl} 0.14 (*b*). The presence of these oligosaccharides was demonstrated by chromatographic behavior, hydrolysis studies, and methylation analyses.

The characterization of these oligosaccharides, hydrolysis studies, Smith-degradation processes, and methylation analysis [14] (Table 2) revealed that the core of *C. praecox* gum exudate consists of (1 → 4)-linked β-D-xylopyranosyl residues substituted at O-2 by α-D-glucuronic acid and its 4-methyl ether. Some structural features observed in *C. praecox* gum are similar to those reported for that of *Cercidium australe* gum [15].

The IR spectrum of the polysaccharide isolated from the original *C. praecox* gum shows ester carbonyl bands (1540–1640 cm^{-1}) [16] and hydrogen-bonded –OH (3100–3500 cm^{-1}); lactone groups were absent [15].

Signal assignments of ^{13}C NMR spectra were made on the basis of appropriate model compounds [17–19] which were chosen according to chemical evidence.

The *C. praecox* gum gave a well-resolved ^{13}C NMR spectrum (Fig. 2). The signals at lowest field (176.06, 176.81, and 186.34 ppm) may be assigned to C-6 of uronic acids [19,20]. The lowest downfield signal may be related to the substitution of α-glucuronic acid residues by metal ions [19]. The ash composition of the gum revealed the presence mainly of calcium (352 000 ppm), magnesium (483 000 ppm), and sodium (253 000 ppm); these values are comparable to those reported for other gums [2]. The ^{13}C NMR spectrum showed acetyl signals (20.58, 21.03, and

Table 2
Methylation analysis of original gum from *Cercidium praecox*

Glycosyl residue	T [a,b]	Position of OMe groups	Linkage
Ara*p*	0.98	2,3,4	Ara*p*-(1 →
	2.25; 2.38	2,4	→ 3)-Ara*p*-(1 →
Ara*f*	0.54; 0.75	2,3,5	Ara*f*-(1 →
Xyl*p*	0.45; 0.54	2,3,4	Xyl*p*-(1 →
	1.50; 1.67; 1.80	2,3	→ 4)-Xyl*p*-(→
	4.00; 6.20	2	→ 4)-Xyl*p*-(1 → 3 ↓
	3.50; 5.51	3	→ 4)Xyl*p*(1 → 2 ↓
GlcA [c]	2.38; 3.20	2,3,4	GlcA-(1 →
and			or
4-Me-GlcA [c]			4-Me-GlcA-(1 →

[a] Relative to methyl 2,3,4-tetra-*O*-methyl-β-D-glucopyranoside. [b] Ref 14. [c] As methyl ester methyl glycoside.

173.46 ppm) [21]. Resonances that appear in the range 60–110 ppm were attributed to xylose, arabinose, and uronic acid residues (Tables 3 and 4). The anomeric region (96–105 ppm) showed signals for α-D-glucuronic acid residues (96.55 and 96.72 ppm) [19], 4-*O*- (101.14 ppm) [17], and terminal (101.34 ppm) [17] xylose residues; and β-D-glucuronic acid (104.93 ppm) [22]. There is overlapping at

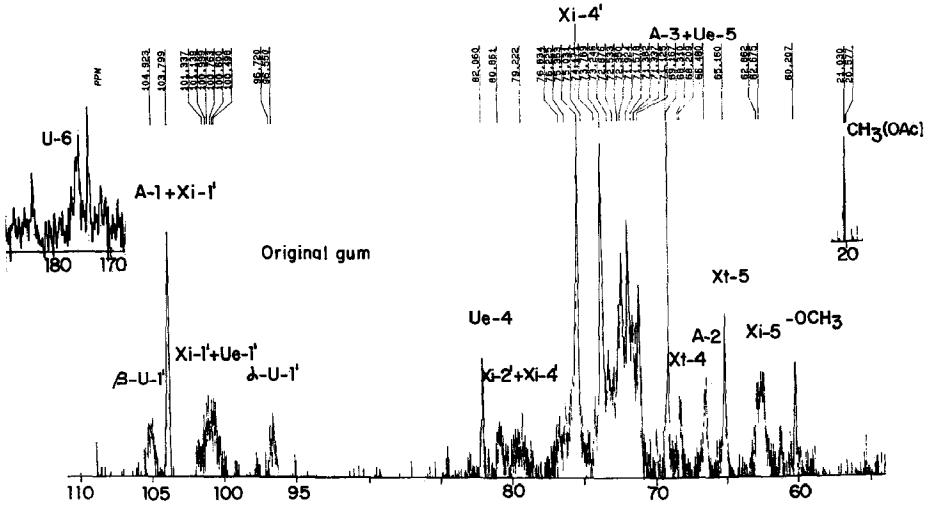

Fig. 2. ^{13}C NMR spectrum of *C. praecox* gum recorded with a Bruker AM-300: X_i, internal β-D-xylose; x_t terminal β-D-xylose; A,L-arabinose; U_e, 4-*O*-methyl-α-D-glucuronic acid; U, glucuronic acid. The same symbols appear in Figs. 3 and 4. 1,4-Dioxane (δ 66.67 ppm) was used as the external standard.

Table 3
^{13}C NMR data [a] of β-D-xylopyranose residues in *Cercidium praecox* gum and its degradation products

Type of linkage	Polymer	C-1	C-2	C-3	C-4	C-5
β-D-Xyl*p*-(1 → [b]		102.70	73.60	76.50	70.00	66.10
	II	101.73	73.18	76.49	68.75	65.31
	o.g.	101.34	73.25	76.63	68.21	65.16
					68.31	
	d.g.A	101.93	72.76	76.57	68.21	65.96
→ 4)-β-D-Xyl*p*-(1 → [b]		102.50	73.60	74.50	(77.20)	63.80
	II	101.73	72.86	73.80	76.50	62.84
	o.g.	101.34	72.53	73.77	(75.35)	62.87
	d.g.A	101.93	72.76	73.79	(76.57)	62.63
→ 4)-β-D-Xyl*p*-(1 → [c]		105.60	(79.20)	73.40	(78.20)	63.90
2	II	103.97	(78.80)	72.86	(78.65)	62.84
↓	o.g.	103.79	(79.22)	72.53	(79.22)	62.86
α-ether	d.g.A	103.93	(80.24)	72.76	(80.24)	62.63
→ 4)-β-D-Xyl*p*-(1 → [d]		102.00	(77.30)	73.10	(76.90)	63.40
2	II	101.73	(76.50)	72.18	(75.50)	62.84
↓	o.g.	101.14	(76.63)	72.35	(75.35)	62.67
α-GlcA	d.g.A	101.20	(76.57)	72.03	(75.55)	62.63

[a] Values relative to the signal of 1,4-dioxane (δ 66.67 ppm). [b] Ref 17. [c] Ref 18. [d] Ref 19. Figures in parentheses wre assigned to C-2 and C-4 of xylopyranose residues. II, polysacccharide II; o.g., original gum; d.g.A, degraded gum A.

Table 4
^{13}C NMR data [a] of uronic acid residues in *C praecox* gum and its degradation products

Type of linkage	Polymer	C-1	C-2	C-3	C-4	C-5	C-6	4-OMe
4-OMe-α-D-GlcA-(1 → [b]		99.70	72.20	73.30	82.70	70.80	176.0 [c]	61.10
	II	100.10	71.58	72.86	82.10	69.30	177.50	50.85
					82.40	69.49		60.55
								60.90
	o.g.	100.50	71.56	72.35	82.06	69.17	176.06	60.21
		100.60		72.53			176.81	
	d.g.A	101.93	71.24	72.76	82.28	69.24	177.20	59.83
β-D-GlCA-(1 → [d]		104.40	75.50	77.10	73.30	77.50	177.50	
	o.g.	104.93	74.77	75.35	72.88	75.35	176.06	
							176.81	
	d.g.A	105.02	75.55	76.12	72.76	76.57	177.20	
α-D-GlcA-(1 → [e]		98.50	72.60	72.90	77.50	71.80	172.9 [f]	
							185.5 [e]	
	II	96.75	72.18	72.86	76.50	71.58	174.29	
		97.62					174.66	
	o.g.	96.72	72.35	72.88	76.63	71.58	173.65	
		96.55						
	d.g.A	96.27	72.03	72.76	76.57	71.24	174.63	
		97.97						

[a] Values relative to the signal of 1,4-dioxane (δ 66.67 ppm). [b] Ref 18. [c] Ref 4. [d] Ref 22. [e] Ref 19. [f] Ref 20. II, polysaccharide II; o.g., original gum; d.g.A, degraded gum A.

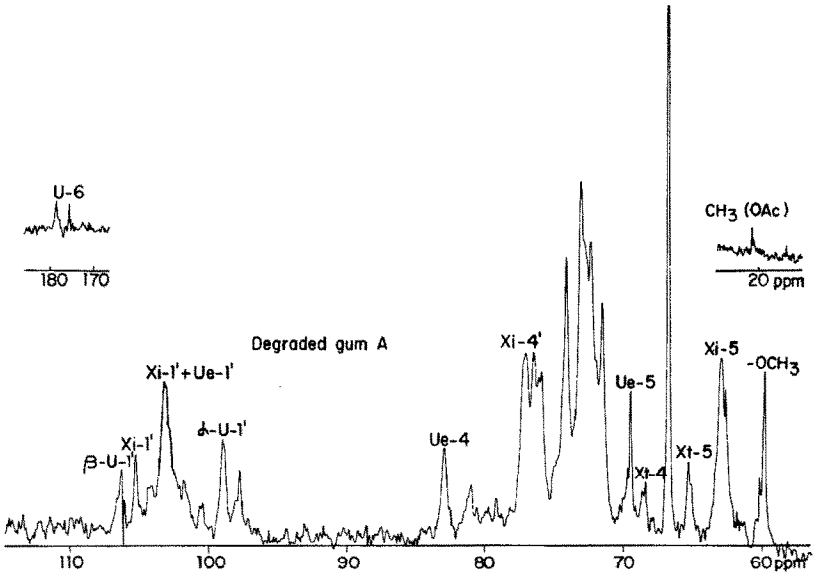

Fig. 3. ^{13}C NMR spectrum of degraded gum A recorded with a Bruker WP-80-DS spectrometer. 1,4-Dioxane (δ 66.67 ppm) was used as the internal standard.

103.79 ppm of the resonances for anomeric carbon atoms of β-L-arabinopyranose [20] and 2-*O*-substituted xylose residues by α-ether groups [18]. The resonances of the carbon atoms involved in the glycosidic linkages were attributed to 4-*O*- (75.35 ppm) [17] and 2,4-di-*O*- (79.22 ppm) [18] substituted xylose residues. This spectral evidence was supported by the isolation and characterization of neutral xylooligosaccharides and the aldobiouronic acid 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-β-D-xylose. The interpretation of the methylation analysis of the original gum [14] (Table 2), supported the spectral results. There is overlapping of some resonances assigned to β-D-xylose and β-D-glucuronic acid (Tables 3 and 4); thus the signal at 75.35 ppm, of high intensity, may be due to C-3 and C-5 of β-D-glucuronic acid, C-4 of 2-*O*-xylose residues linked to α-D-glucuronic acid, and of 4-*O*-xylose residues. The signal at 69.17 ppm is attributed to C-3 of β-L-arabinopyranose terminal residues [20] and C-5 of 4-*O*-methyl-α-D-glucuronic acid [18]. The resonances of β-L-arabinopyranose are very close to those of β-D-xylose residues. The signal at 66.67 ppm in the spectrum of the original gum, recorded with 1,4-dioxane as external standard, was unequivocally assigned to C-2 of (1 → 3)-β-L-arabinopyranose residues [23], as shown by methylation analysis [14] (Table 2). On the other hand the peaks of low intensity due to C-1 (108.80 ppm), C-4 (84.60 ppm), and linked C-3 (83.00 ppm) [23] of α-L-arabinofuranose residues may suggest the presence of these residues in the original gum.

Comparison of the spectra of original gum and degraded gum A (Figs. 2 and 3), show simultaneous decrease of the intensity of the peaks due to C-1 (103.79 ppm), C-2 (69.17 ppm), and C-5 (65.16 ppm) of terminal β-L-arabinopyranose residues,

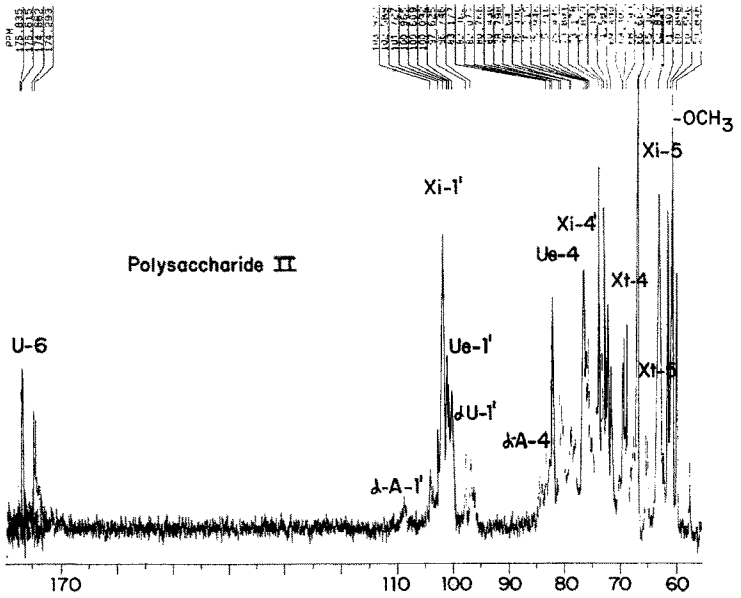

Fig. 4. ^{13}C NMR spectrum of polysaccharide II recorded with a Bruker AM-300. The same signals were observed in the spectrum of polysaccharide I. 1,4-Dioxane (δ 66.67 ppm) was used as the internal standard.

and the absence of the resonances attributed to α-L-arabinofuranose residues. This fact may be related to the removal of arabinose residues during the preparation of degraded gum A. The superposition of the resonances at 75.35 ppm in the spectrum of the original gum (Tables 3 and 4), is partially resolved in the spectrum of degraded gum A, namely, the signal due to C-4 of 2-*O*-xylose residues substituted by α-D-glucuronic acid (75.55 ppm) and that assigned to C-3 of β-D-glucuronic acid (76.12 ppm).

The spectra of polysaccharides I and II, as complex as that of the original gum, are very similar. They contain the resonances attributed to β-D-xylose, β-L-arabinopyranose, α-L-arabinofuranose, α-D-glucuronic acid, and 4-*O*-methyl α-D-glucuronic acid residues (Tables 3 and 4. Fig. 4). The spectrum of polysaccharide II (Fig. 4) does not show the signals that appear at extreme fields in the spectrum of the original gum (Fig. 2), and this may be interpreted as the partial removal of α-D-glucuronic acid residues bearing metal ions [19] and other sugars substituted by acetyl groups [21] vulnerable to the redox process. There are also resonances due to C-5 (61.4 ppm) and linked C-3 (80.07 ppm) of 3,4-di-*O*-xylose residues [24]. This spectral evidence may indicate the existence of those residues in the gum.

The signals of β-D-xylose were observed in all of the spectra studied (Figs. 2–4). These resonances were attributed to 4-*O*-, 2,4-di-*O*-, and terminal xylose residues (Table 3). There is also spectral evidence for 3,4-di-*O*-xylose residues. The resonances assigned to β-D-glucuronic acid were only observed in the spectra of the original gum and degraded gum A, whereas those corresponding to α-D-glucuronic

acid and its 4-methyl ether were observed in all the spectra studied. The absence of resonances for β-D-glucuronic acid in the spectra of polysaccharides I and II may indicate its presence as terminal residues in the original gum, and which were removed in the preparation of the polysaccharide I.

The spectral and chemical evidence indicates that the backbone of the molecular structure is basically a β-(1 → 4) xylan substituted at O-2 by 4-*O*-methyl-α-D-glucuronic acid and α-D-glucuronic acid residues. β-D-Glucuronic acid residues are probably terminal. Arabinose is present as the α-L-furanose and β-L-pyranose.

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