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Structural study of the polysaccharide isolated from *Samanea saman* gum

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Abstract

The polysaccharide isolated from the gum of *Samanea saman* (Jacq.) Merr. contains galactose, as main component, arabinose, rhamnose, glucuronic acid and 4-O-methyl-glucuronic acid. The study of a series of degraded products, obtained by mild acid hydrolysis and Smith degradation of the original polysaccharide, revealed interesting structural features of the polymer studied. Chemical analysis and ^{13}C-N.M.R. spectroscopy indicate a branched structure for the polysaccharide. β-(1→3) galactan is the backbone and the side chains are constituted by 6-O-linked galactose predominantly, terminal and 3-O-linked- α-L-arabinofuranose, α-D-glucuronic acid, β-D-glucuronic acid and its α-4-methyl analogue. The structure of this gum is similar in many aspects to those reported for *Acacia* gums.

Key words: ^{13}C-N.M.R. spectroscopy; gum exudate; Leguminoseae; *Samanea saman;* structural study.

Estudio estructural del polisacárido aislado de la goma de *Samanea saman*

Resumen

El polisacárido aislado de la goma de *Samanea saman* (Jacq) Merr. contiene galactosa como componente principal, arabinosa, ramnosa, ácido glucurónico y ácido 4-0-metil-α-glucurónico. El estudio de una serie de productos de degradación, obtenidos por hidrólisis ácida y degradación de Smith, permitió conocer los rasgos estructurales del polímero estudiado. Los resultados obtenidos por vía química y por espectrocopía de RMN de carbono-13 están de acuerdo con la existencia de una estructura ramificada. El esqueleto central es un -(1→3) galactán; las cadenas laterales están constituidas por residuos de galactosa 6-O-enlazados, arabinosa (α-L-arabinosa), ácido glucurónico (α y β) y ácido α-4-O-metil glucurónico. El polisacárido de *Samanea saman* tiene rasgos estructurales parecidos a los reportados para muchas gomas de *Acacia.*

Palabras clave: Espectroscopía; estudio estructural; exudado gomoso; Leguminoseae; R.M.N C-13; *Samanea saman.*

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Introduction

Many gums from Leguminosae, highly disseminated in Venezuela, have been investigated so far (1-5). *Samanea saman* (Jacq.) Merr., Mimosoideae, exudes gum easily. Analytical studies of the gum exudates from two *Pithecelobium* (*Samanea*) have been published recently (6). This work reports the structural features of the polysaccharide isolated from *S. saman* gum exudate.

Experimental

Origin, collection and purification of the gum sample

Gum from *S. saman* (Jacq.) Merr., known as "saman" in Venezuela, was collected by the authors in March-April, 1992, from trees growing in Maracaibo, Venezuela, South America. The identification of the voucher specimen N° 323 (plant collection of the Departamento de Biología, Facultad de Humanidades y Educación, La Universidad del Zulia, Venezuela) was confirmed by Lic. Carmen Clamens. The dissolution of the gum in cold water (3%), collected two weeks after the injury was made, was carried out in a day. The solution, light brown in colour, was passed through musslin and Whatman 41 and 42 papers, and dialyzed against running tap water for 48 h; after dialysis the gum was recovered by freeze-drying (47.70%).

General experimental methods

Standard methods of gum analysis were used (7,8). 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-0.1M HCl-1-butanol; (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 analysis of the methylglycosides by g.l.c. was carried out with a VARIAN 2700 instrument with a flame ionization detector with a N_2 flow rate of 40 mL/min. The glass column (168 x 0.57 cm) used was 10% by weight of polyethylene glycol adipate on Chromosorb WHD at 190°C. Retention times for the methyl ethers are quoted relative to that of methyl 2,3,4,6-tetra-O-methyl-O-β-D-glucopyranoside. ^{13}C-N.M.R. spectra were recorded on a BRUKER AM-300 spectrometer. Data points were accumulated overnight at 37°C and with complete proton decoupling. The samples (100-200 mg) were dissolved in D_2O (1 mL) and the spectra were calibrated by the addition of 1,4-dioxane. The procedures for partial and total hydrolyses, quantitative analysis of sugars, identification of aldobiouronic acids and methylations of the oligosaccharides and polysaccharides have been described (4,5,7).

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 (4,5,7). Degraded gum A (3.40 g) was obtained by mild acid hydrolysis from purified gum (12 g). Drastic periodate oxidation of degraded gum A (1.84 g) gave degraded gum B (55 mg). Preliminary small-scale experiments showed that 96 h were required for the preparation of degraded gum B.

Smith-degradation studies

Two sequential Smith-degradations (periodate oxidation, reduction and acid hydrolysis) were performed with pure gum as starting material (14 g) to obtain polysaccharide I (1.41 g). Polysaccharide I (665.3 mg) gave polysaccharide II (53.4 mg). The experimental conditions for these degradations have been described previously (4,5,7).

Results

Sugar composition and methylation analysis are shown in Tables 1 and 2. Ta-

bles 3-5 contain the interpretation of ^{13}C-N.M.R. spectra of the original polysaccharide and its degradation products.

Discussion

The polysaccharide from *S. saman* gum, contains galactose, arabinose, rhamnose, glucuronic acid and its 4-O-methyl analogue, Table 1. The major sugar component is galactose as has been reported for many Leguminosae gums (1,2,7). Column chromatography of neutral and acidic components, of the formic acid hydrolysate, corroborated the presence of the above neutral sugars and yielded three oligosaccharides, which were characterized, by their chromatographic behaviour, specific rotation, sugar and methylation analyses as 6-O-6-0-(β-D-glucopyranosyluronic acid)-β-D-galactopyranosyl-D-galactose, R_{gal} 0.13(a) (8), 4-O-(α-D-glucopyranosyluronic acid)-D-galactose, R_{gal} 0.35 (a), $[\alpha]_D$ +87° (9), and 4-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-galactose, R_{gal} 0.60(a), α_D + 10° (7). The last aldobiouronic acid was also isolated by partial hydrolysis of the original gum and further characterized as was described above. These oligosaccharides have been reported for others Leguminosae gums (5, 10, 11).

Methylation analysis of the gum studied, Table 2, showed the presence of terminal rhamnopyranose, terminal and 3-0-substituted arabinofuranose, 3-O-, 4-O-, 6-O- and 3,6-di-O- substituted galactose and terminal uronic acid residues, as constituents of the structure of the polymer.

Degraded gum A, obtained by mild acid hydrolysis of the original gum, led to the isolation of two bioses, which were characterized by their chromatography behaviour, specific rotation, sugar and methylation analyses as 3-O-(β-D-galactopyranosyl)-L-arabinose, and 4-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-galactose. This aldobiouronic acid was also isolated and characterized from the original gum.

Degraded gum B, obtained by drastic oxidation of degraded gum A (0.25 M) corresponds basically to a (1→3) galactan. Uronic acids (17%) are still present in the nucleus; steric hindrance might have prevent their complete removal by periodate oxidation. The relatively low yield (3%), of this polymer, is a good indicator of the existence of many sugar residues vulnerable to periodate oxidation in the original polymer.

Smith-degradation processes, carried out on the original polysaccharide as starting material, led to the preparation of poly-

Table 1
Analytical data of Sugar compositions[a] of Samanea saman polysaccharide and its degradation products

		Sugars[a], %			
Polymers	Yield %	Gal	Ara	Rha	U.A.
Original Polysaccharide	48	50	17	11	22
Degraded gum A	28	62	4	3	31
Degraded gum B	3	83	-	-	17
Polysaccharide I	10	72	11	4	13
Polysaccharide II	8	70	11	-	19

[a]Corrected for moisture. U.A.= uronic acids.

Table 2
Methylation analysis of Samanea saman polysaccharide.

0-methyl ethers	T (min)[a]	Type of linkage
2, 3, 4 -Me_3-L-Rha	0.46	Rhap (1—>
2, 3, 5-Me_3-L-Ara	0.68	L-Araf(1—>
2,5-Me_2-L-Ara	1.26 ; 2.20	—>3) L-Araf (1—>
2, 3 ,4, 6-Me_4-D-Gal	1.67	Galp (1—>
2, 3, 6-Me_3-D-Gal	(2.97) (3.91) (4.54)	—>4) Galp (1—>
2, 4, 6-Me_3-D-Gal	(3.91) (4.52)	—>3) Galp (1—>
2, 3, 4-Me_3-D-Gal	5.68 ; 6.01	—>6) Galp (1—>
2, 4-Me_2-D-Gal	12.22 , 13.70	—>3,6) Galp (1—>
2, 3, 4-Me_3-D-GlcA[b]	(2.42) (2.80)	GlcA (1—>

[a]Relative to methyl -2,3,4,6, -tetra-0-methyl-β-D-glucopyranoside. [b]As methyl ester glycoside. Figures in parenthesis indicate T values of components that were not completely resolved.

saccharides I and II. No oligossacharides were detected during the preparation of these polysaccharides. From the partial hydrolysis of polisaccharide II it was isolated 4-O-(4-O-methyl-α-D-glucopyranosyluronicacid)-D-galactose.This aldobiouronic acid was characterized using the same techniques described above. The relatively low yields of the above polysaccharides, Table 1, made necessary to repeat thrice the Smith-degradation process.

Signal assignments of the spectra was made on the basis of chemical results and previous works (4,5,7,12). The interpretation of the simpler spectra, those exhibited by degraded gum B and polysaccharide II, made it easier to study the more complex spectra.

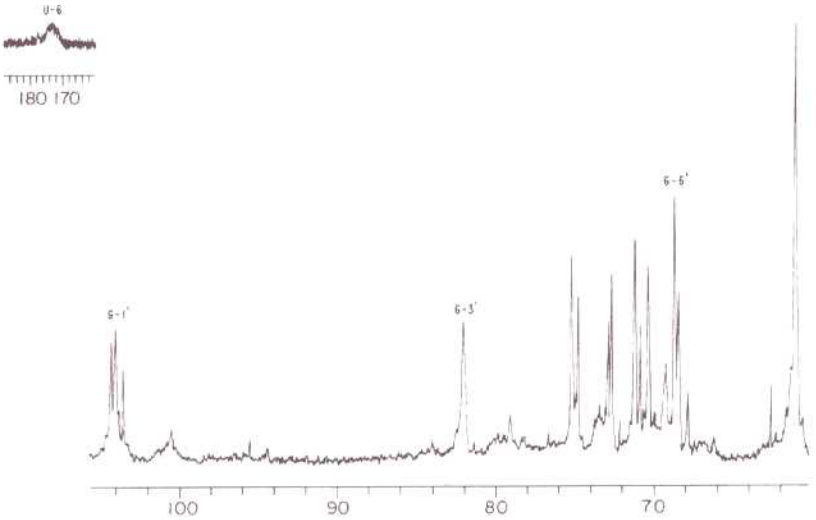
The ^{13}C-N.M.R. spectrum of degraded gum B, the backbone of the structure, shows the signals due to 3-O- and 6-O-substituted-galactose residues, Table 3. This is consistent with the presence of a β-(1→3) galactan and it is supported by the isolation of oligosaccharide 6-O-(β-D-glucopyranosyluronic acid)-6-O-(β-D-galactopyranosyl)-D-galactose obtained by formic acid hydrolysis. The anomeric region of this spectrum shows the resonances due to 4-O-methyl-α-D-glucuronic acid (100.00 p.p.m) (4,5), β-D-galactose (103.99 p.m.m.)(12) and β-D-glucuronic acid (104.33 p.p.m.) (4,5). The signals attributed to C-6 of uronic acids (179.09; 179.68 p.p.m.) (13) appear at lower field than those reported for other analogous polymers (4,5,7). The resonance of the methoxyl group (60.37 p.p.m.) (4,5) corroborated the existence of the 4-O-methyl-α-D-glucuronic acid as was demonstrated by chemical studies; this uronic acid has been reported in *Acacia* gums (7,11) and other analogous polymers (4,5,12). The presence of the uronic acids in the backbone of the structure of the gum studied, demonstrated by chemical and spectral evidences, suggests that those sugar acids were not vulnerable to periodate attack or the experimental conditions were not sufficient to remove them (4,5).

Degraded gum A, obtained by mild acid hydrolysis of the original gum, shows a ^{13}C-NMR spectrum, Figure 1, relatively more complex than that of the spectrum of degraded gum B, although there is a better resolution of the signals assigned to β-D-galactose and uronic acid residues, Tables 3

Table 3
Spectral data[a] of Carbon-13 NMR of β-D-galactose in the *S. saman* polysaccharide and its degradation products

Type of linkage	Polymer	C-1	C-2	C-3	C-4	C-5	C-6
→3(β-D-Gal(1→[b]		104.90	71.10	82.90	69.90	75.60	61.80
	d. g. B	104.16[c]	71.11	81.98	69.00	74.97	60.95
	d. g. A	103.58	71.28[c]	82.00	69.52[c]	75.17[c]	60.98[c]
	I	103.78	70.86	81.64[c]	69.39[c]	75.04[c]	60.97[c]
	II	103.73[c]	71.14	81.68[c]	69.59[c]	74.94[c]	61.01
→6)β-D-Gal (1→[b]		103.30	70.40	72.80	67.08	73.00	68.10
	d. g. B	103.98	70.14	72.60	66.60	72.60	68.59
	d. g. A	102.50[c]	70.12[c]	72.63[c]	67.95[c]	72.89	68.43[c]
	I	103.29	70.02	72.44[c]	67.55	72.57	68.38[c]
	II	103.49	70.30	72.73[c]	67.85	72.83	68.56[c]

[a]Values relative to the signal of 1,4-dioxane (δ 66.67 p.pm.). [b]Refs.7, 5. [c]Average values. d.g.B=degraded gum B; d.g.A=degraded gum A; I,II= polysaccharides I and II. The same signals were observed in the original polysaccharide.



and 4, as has been observed previously (5). The resonances that appeared in this spectrum suggest the existence of many environments of galactose and uronic acid residues within the polymer molecule of degraded gum A. There are the unequivocal resonances attributed to methoxyl group of 4-O-methyl-α-D- glucuronic acid (59.95 p.p.m.) (4,5) and C-6 of 3-O-substituted--galactose residues (60.78; 60.98; 61.20 p.p.m.) (5, 7). The anomeric region shows the signals due to α-D-glucuronic acid (98.33 p.p.m.) (4), 4-O-methyl-α-D-glucuronic acid (100.55 p.p.m.) (4,5), 6-O-galactose

Table 4
Carbon-13 NMR data[a] of uronic acids residues in *S. saman* polysaccharide 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→[b]		99.70	72.70	73.30	82.70	70.80		61.10
	d. g. B	100.00	72.06	73.22	81.98	70.14		60.37
	d. g. A	100.55	71.80[c]	73.42[c]	82.00	70.70		59.95
	I	100.34	71.89	-	82.03	70.27		60.86
	II	100.47	72.11	73.30[c]	82.01	70.72[c]		60.56
β-D-glcA(1→[b]		104.70	75.50	77.10	73.30	77.50[c]	177.50	
	d. g. B	103.33	75.00	76.67[c]	73.22	77.80	179.39[c]	
	d. g. A	104.11[c]	75.75[c]	76.25	73.42[c]	76.90[c]	175.50[c]	
	I	104.07[c]	74.94	76.36	-	76.36	-	
	II	104.25	75.14	76.61	73.55[c]	76.61	173.00	

[a]Values relative to the signal of 1,4-dioxane (δ- 66.67 p.p.m). [b]Ref. 4. [c]Average values. d. g.B= degraded gum B; d. g. A= degraded gum A; I, II = polysaccharide. The same signals were observed in the original polysaccharide.

(102.41; 102.61 p.p.m.) (5,11), 3-O-galactose (103.37; 103.46; 103.84 p.p.m.) (7,13) and β-D-glucuronic acid residues (104.05; 104.16 p.p.m.) (4,5). The resonances due to terminal and 3-O-substituted-arabinofuranose residues, were not observed in this spectrum, as was expected by the dearabinosylation of the original polymer in the hydrolytic process. The signal at lowest field in the spectrum (181.17 p.p.m.) may be due to salt formation in the α-D-glucuronic acid residues (4); the resonance at 98.33 p.p.m. is also attributed to this uronic acid. The ash composition of the gum revealed the presence of calcium (223100 p.p.m.), magnesium (34140 p.p.m.), sodium (25500 p.p.m.) and potassium (11050 p.p.m.) mainly; these values, comparable to those reported for other gums (14,15) support the assumption of salt formation in the α-D-glucuronic acid residues. In addition, there are two signals (175.24; 175.75 p.p.m.) which were attributed to C-6 of uronic acids.

The spectrum of the original gum, Figure 2, shows the resonances due to 3-O- and 6-O-β-D-galactose residues (5,7,12), Table 3, as was observed in the above spectra studied. The anomeric carbons of these residues and those corresponding to β-D-glucuronic acid appear overlapped (102.90 p.p.m.) but those attributed to C-1 of the 4-O-methyl-α-ether (100.63 p.p.m.) (4,5), terminal (109.43 p.p.m.) and 3-O-α-L-arabino-furanose residues (107.34 p.p.m.) (5,7) are well resolved, Tables 4 and 5. The signals attributed to C-6 of uronic acids, described previously, in the spectra of degraded gums A and B, were also observed in this spectrum and those that appear at high field, were unequivocally assigned to the methyl group of rhamnose (16.50 p.p.m.) (5,7,12) and acetyl groups (19.94 p.p.m.) (4,11).

The spectrum of polysaccharide I, Figure 3, less complex than those exhibited for the original gum, contains the signals corre-

Table 5
Carbon-13 NMR spectral data[a] of α-L-arabinofuranose residues in *S. saman* polysaccharide and its degradation products

Type of linkage	Polymer	C-1	C-2	C-3	C-4	C-5
α-L-Ara (1→[b]		109.20	81.80	77.50	84.90	62.40
	o.g.	109.43	81.28	76.50	83.90	61.20
	I	109.07	81.05	76.36	83.68	62.32
	II	109.07	81.40	76.61	83.95	62.24
→3) α-L-Ara (1→[b]		108.20	80.70	83.20	83.60	62.00
	o.g.	107.34	80.19	83.90	83.90	61.20
	I	107.34	80.06	83.68	83.68	61.07
	II	108.00	80.89	83.95	83.95	61.62

[a]Values relative to the signal of 1,4-dioxane (δ 66.67 p.p.m.). [b]Ref. 5. o.g.= original gum; I, II= polysaccharides I and II.

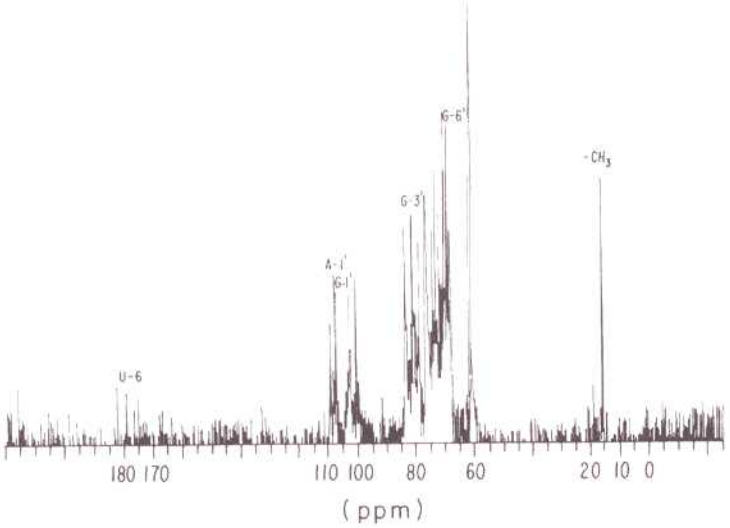


Figure 2. Carbon-13 NMR spectrum of *S. saman* (0-200 p.p.m.) gum. A-1'= C-1 linked α-L- arabinofuranose.

sponding to C-6 of 3-O-galactose residues in two environments (60.86; 61.07 p.p.m.). The resonance due to the methoxyl group of 4-O-methyl-α-D-glucuronic acid may appear overlapped with one of those environments (60.86 p.p.m.). The hydroxylated secondary carbon atoms of 3-O-galactose residues resonate in many environments, this is, C-3 (81.13; 81.75, 82.03 p.p.m.), C-4 (69.05; 69.74 p.p.m.), C-5 (74.94; 75.0 p.p.m.). The multiplicity for C-6 of 6-O-galactose residues is also observed (68.30; 68.46 p.p.m.) (5). The resonances (107.22; 109.43 p.p.m.) of terminal and 3-O-α-L-arabinofuranose residues are still present in this spectrum, Table 5. It is interesting to note that

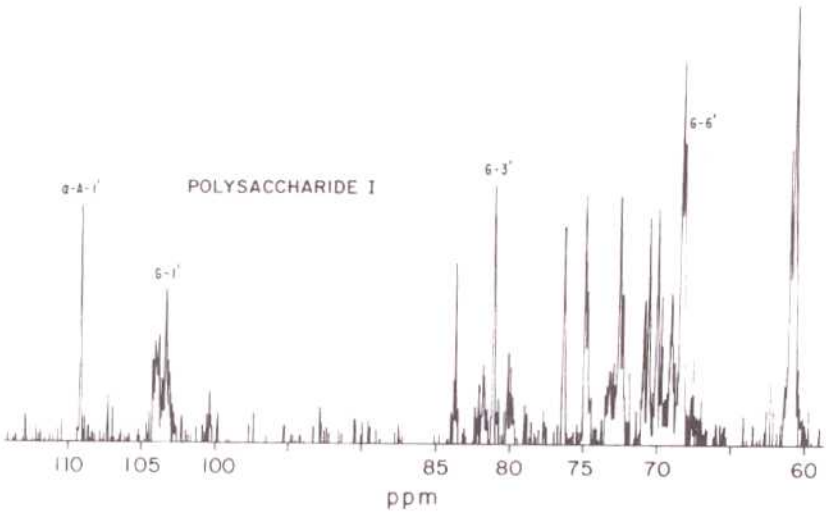


Figure 3. Carbon-13 NMR spectrum of polysaccharide I of *S. saman*.

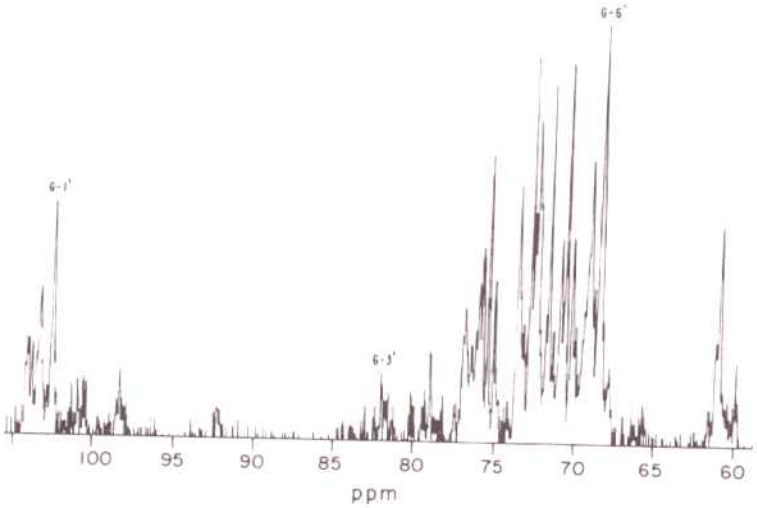


Figure 4. Carbon-13 NMR spectrum of polysaccharide II of *S. saman*.

the signal (100.34 p.p.m.) attributed to C-1 of 4-O-methyl-α-D-glucuronic acid has lower intensity than the equivalent signal in the spectrum of the original gum (100.63 p.p.m.) which may be related with the removal of these residues during the preparation of the polysaccharide I. The anomeric carbons of 6-O- (103.29 p.p.m.) (5) and 3-O-galactose residues (103.78 p.p.m.) (5,7,12) and β-D-glucuronic acid (103.96; 104.17 p.p.m.) (4), overlapped in the spectrum of the original gum, were resolved in this spectrum.

The spectrum of polysaccharide II, Figure 4, simpler than the previous one, contains the resonances unequivocally assigned to 3-O- and 6-O- galactose residues, Table 3. The signals due to arabinofuranose residues decreased its intensities. There is an unique signal (104.25 p.p.m.) for C-1 of β-D-glucuronic acid, in contrast with the two environments shown in the spectrum of polysaccharide I. On the other hand, the

anomeric region shows reducing terminal sugar residues (94.36; 95.49 p.p.m.) (7).

All the spectra discussed showed a signal at 79.03 p.p.m. which may be due to C-4 of galactose linked to 4-O-methyl-α-D-glucuronic acid (16) and/or C-3 of galactose linked to terminal α-L-arabinofuranose residues (17) which is in agreement with the isolation of 3-O-(β-D-galactopyranosyl)-L-arabinose and 4-O-(4-O-methyl-α-D-glucopyrano syluronic acid)-D-galactose during the preparation of degraded gum A.

Spectral evidence agrees with the chemical results and it indicates the existence of a branched structure for the polymer studied. The backbone of the polysaccharide is a β-(1—>3) galactan. Side chains are constituted by 6-0-substituted-galactose predominantly, terminal and 3-O-substituted arabinofuranose, and uronic acid residues. These last residues are constituted of α-D-glucuronic acid, β-D-glucuronic acid and its α-4-methyl analogue. The structure of *S. saman* polysaccharide reminds in many aspects those reported for *Acacia* gums. ^{13}C-N.m.r. spectra of the polymer studied showed interesting structural features.

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