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Spectral study of carbon-13 N.M.R. of three *Parkia* gums

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Abstract

Analytical data of gum from the seed pods of *Parkia pendula*, growing in Costa Rica, and the Nigerian gum exudates from *Parkia bicolor* and *Parkia biglobosa* have been published. Galactose, arabinose, glucuronic acid and its 4-0-methyl analogue are present in the three *Parkia* gums investigated. ^{13}C-NMR spectra of the polysaccharides, isolated from the *Parkia* gums corroborated the presence of the sugars mentioned above and the absence of rhamnose. The polysaccharides of *P. bicolor* and *P. biglobosa* showed very similar spectra. They contain the signals due to the sugars present in the polysaccharide structures. The spectrum of *P. pendula* original gum looks like the spectrum of and α - arabinan, but its dearabinosilation led a better resolution of the signals due to galactose and uronic acid residues. The spectral evidences found in this work are according with the sugar and methylation analyses.

Key words: ^{13}C-NMR spectra; *Parkia biglobosa; Parkia bicolor; Parkia pendula.*

Estudio espectral por RMN de carbono-13 de tres gomas de *Parkia*

Resumen

Se han publicado los datos analíticos de la goma de semilla de *Parkia pendula*, especie que crece en Costa Rica y de los exudados gomosos de *Parkia bicolor* y *Parkia biglobosa*, especies que crecen en Nigeria. Galactosa, arabinosa, ácido glucurónico y su 4-0 metil análogo estuvieron presentes en las gomas de *Parkia* investigadas. Los espectros de r.m.n. de Carbono-13 de los polisacáridos aislados de las gomas de *Parkia* corroboraron la presencia de los azúcares mencionados y la ausencia de ramnosa. Los espectros de los polisacáridos de *P. bicolor* y *P. biglobosa* fueron muy similares y muestran las señales debido a los azúcares presentes en las estructuras de los polisacáridos. El espectro de la goma original de *P. pendula* se parece al correspondiente a un α -L-arabinán, pero su dearabinosilación permite una mejor resolución de

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s señales debidas a galactosa y a los ácidos urónicos. Las evidencias espectrales, encontradas en este trabajo, están de acuerdo con los análisis de azúcares y de metilación.

Palabras clave: Espectros de ^{13}C-NMR; *Parkia biglobosa; Parkia bicolor; Parkia pendula.*

Introduction

Parkia (Leguminosae; Mimosoideae) is a pantropical genus with centres of distribution in South America, Africa and South-east Asia (1). Nigerian gum exudates from *Parkia bicolor* and *Parkia biglobosa*, and gum from the seed pods of *Parkia pendula* growing in Costa Rica have been analysed (1). This work deals with the ^{13}C-N.M.R. spectroscopy of the gums from the three *Parkia* sp. above mentioned.

Experimental

Origin and purification of gums

Seed pods from *Parkia pendula* (Willd.) Benth. were collected by Professor D.H. Janzen, Botany Department, University of Philadelphia, in Corcovado Natural Park, Puntarenas Province, Costa Rica, on March 20, 1977. The pods and seeds had a thick coating from which the gum was recovered by dissolution in water, followed by removal of seeds and pods by simple filtration. Dialysis and freeze drying yielded the polysaccharide.

Gum exudates from *Parkia bicolor* A. Chev. and from *Parkia biglobosa* (Jacq) R.Br. ex G. Don fil. were collected by Dr. H.C. Hopkins at the Forest Research Institute, Ibadan, on 10 February 1978. The gum exudates were dissolved in distilled water to give a 1% soln (w/w), filtered (muslin, then Whatman No. 1 and No. 42 papers), dialysed (vs tap H_2O for two days) and recovered by freeze-drying.

Analytical methods

The methods of analysis used have been described (2,3). The sugar composition was determined by the phenol-H_2SO_4 method (4). G.l.c. was carried out with a Pye 104 chromatograph fitted with a flame-ionization detector at nitrogen flow-rates of 25 mL/min. The column (200 x 0.3 cm) used contained 15% by weight of polyethylene glycol adipate on Universal B (phasesep). The temperature of analysis was 170°. Methyl ethers retention times are quoted relative to that of methyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside. Spectra were recorded with a Varian CFT-20 Carbon-13 Nuclear Magnetic Resonance spectrometer. Data points were accumulated overnight at a temperature of 36°C, a spin rate of 22 r.p.s. and with complete proton decoupling. The spectral width was 4000 Hz (200 ppm). Gum samples (100-200 mg) were dissolved in D_2O. Addition of 1,4-dioxane as internal standard to the samples permitted calibration of the spectra.

Methylation of the gums

The pure gums (300 mg) were methylated according to Haworth and Purdie (5,6) to give fully methylated derivatives. A portion of the product was methanolysed and examined by g.l.c.

Preparation of degraded gum A

The degraded gum A was prepared by reaction with 5mM H_2SO_4 as described previously (7).

Results

The spectra of the original gums from seed pods of *P. pendula* and from gum exudates of *P. bicolor* and *P. biglobosa* are shown in Figures 1 and 2. The spectrum of degraded gum A of *P. pendula* is shown in Figure 3. Chemical data of the studied polysaccharides are shown in Tables 1 and 2. The interpretation of their ^{13}C-N.M.R. spectra is presented in Tables 3, 4 and 5.

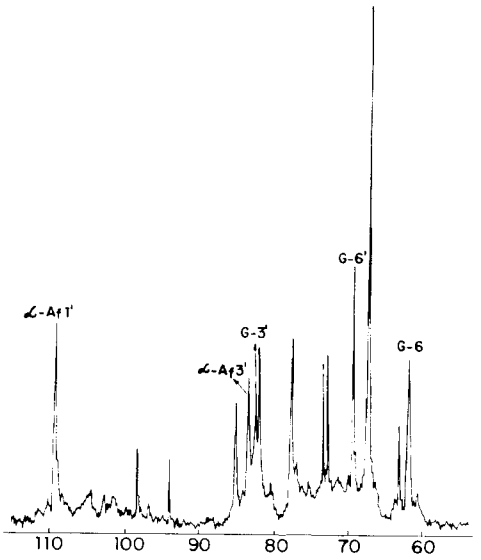

Figure 1. Carbon-13 N.m.r. spectrum of *P. pendula* gum. G = β -D- galactose.
A_f = α -L-arabinofuranose.
' = carbon involved in glycosidic linkage.

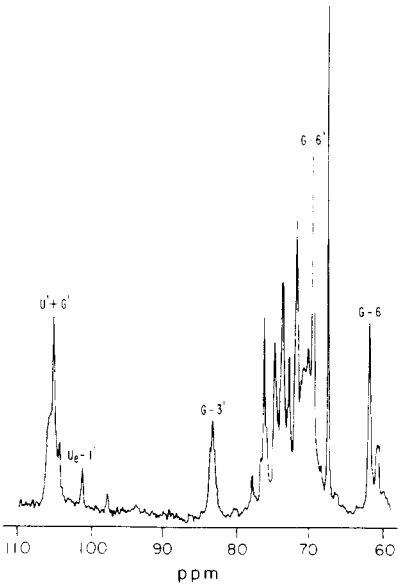

Figure 3. Carbon-13 N.m.r. spectrum of degraded gum A of *P. pendula* gum.
U' = C-1 of β -D-glucuronic acid.
G' = C-1 of β -D-galactose.
G = β -D-galactose
' = carbon involved in glycosidic linkage.

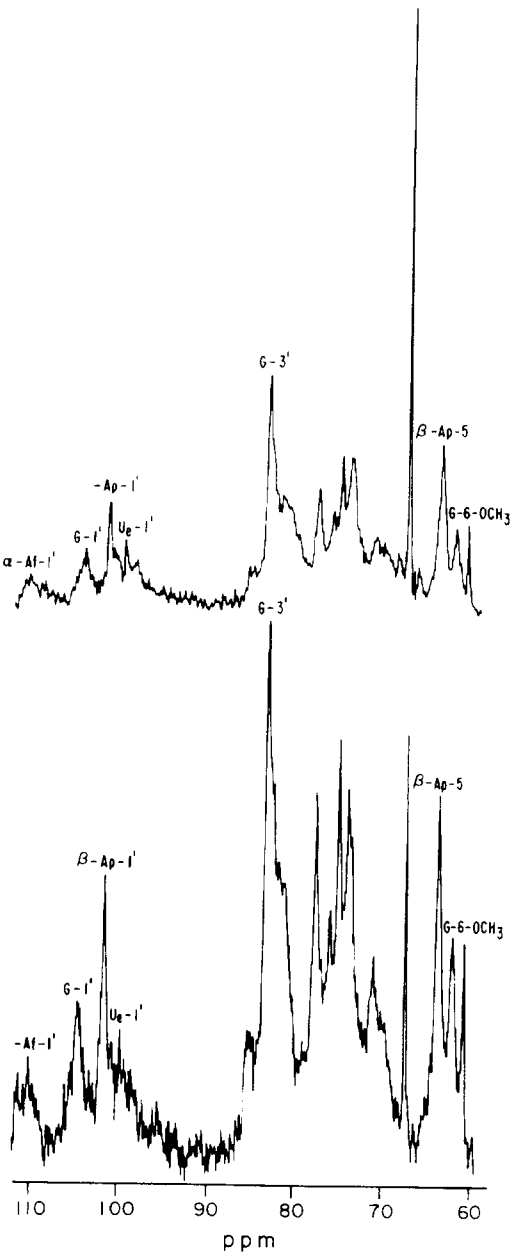

Figure 2. Carbon-13 N.m.r. spectra of P. biglobosa and P. bicolor gums.
G = β -D-galactose.
A_f = α -L-arabinofuranose.
A_p = β -L-arabinopyranose.
U_e = 4-O-methyl- α-D-glucuronic acid.
' = carbon involved in glycosidic linkage.

Table 1
Sugar composition[a,b] (%) of three *Parkia* gum exudates

	Species		
	P. pendula	*P. bicolor*	*P. biglobosa*
Galactose	30	74	73
Arabinose	62	9	
4-0-methyl glucuronic acid	4.3	7.5	6.4
glucuronic acid	3.7	9.5	11.6

[a] Corrected for moisture content. [b] Reference 1 Rhamnose was not evidenced.

Table 2
Methylation data for *Parkia pendula* gum

Methyl ethers	T (min)	Linkage	
2,3,5-Me -L-Ara		L-Ara (1—>	0.66
2,3,4-Me -L-Ara		L-Ara (1—>	0.87
2,5-Me-L-Ara		—> 3) -L-Ara (1—>	1.25; (
2,3,4,6,-Me -D-Gal		D-Gal (1—>	1.66
2,3,6-Me -D-Gal		—> 4) -D-Gal (1—>	2.45; (
2,4,6-Me -D-Gal		—> 3) -D-Gal (1—>	(3.50);
2,3,4-Me -D-Gal		—> 6) -D-Gal (1—>	5.70; 6
2,4-Me -D-Gal		→ 3,6) -D-Gal (1—>	12.31;
2,3,4-Me -D-GlcA		D-GlcA (1→	(2.22);

Relative to methyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside.
Reference 10. As methyl esther methyl glycoside. Figures in parenthesis indicate T values of components that were not completely resolved. The above sugars were observed in *P. bicolor* and *P. biglobosa* gums except 2,3,4-Me -D-Gal.

Discussion

The water soluble gums from *P. bicolor*, *P. biglobosa* and *P. pendula*, contain galactose, arabinose, glucuronic acid and its 4-O-methyl α -D-glucuronic acid, (Table 1). Rhamnose was not detected. Galactose is the main component present in *P. bicolor* and P. biglobosa gums but arabinose is the main sugar constituent in the gum of *P. pendula*.

The spectra of the original polysaccharides (Figure 1 and 2), performed in deuterium oxide, were well resolved and appear less complex than other analogous polymers (7,8). Signal assignment was based in chemical evidences (Tables 1 and 2), and by comparison with published results (7-10).

Table 3
Carbon-13 N.m.r. chemical shifts[a] of neutral sugar residues in *Parkia* gums

Type of linkage	Specie	C-1	C-2	C-3	C-4	C-5	C-6
—> 3) Gal (1—>[b]		105.0	71.2	83.0	69.3	75.6	61.8
	P. biglobosa	103.4 103.8	71.0	83.0	68.7	75.0 75.8	62.3
	P. bicolor	103.6	70.9	83.0	69.9	74.9	62.2
α -L-Ara (1—>[c]		109.2	81.8	77.5	84.9	62.4	
	P. biglobosa	109.6	-	77.4	-	62.3	
	P. bicolor	109.2	81.7	77.5	84.8	84.8	62.2
	P. pendula	108.3	81.7 82.2	77.4 77.6	84.8	84.8	62.0
—> 3) -L-Ara (1—>[c]		108.2	80.7	83.2	83.6	62.0	
	P. biglobosa	108.5	80.5	83.0	83.7	62.3	
	P. bicolor	108.6	80.9	83.0	83.0	62.2	
	P. pendula	108.3	80.2	83.2	83.2	62.0	

[a] The spectra were calibrated with 1,4-dioxane (δ 67.4 p.p.m.). [b] Reference 8. [c] Reference 10

The ^{13}C-N.m.r. spectrum of P. pendula gum (Figure 1), contains the resonances due to β -D-galactopyranose, α -L-arabinofuranose and β -L-arabinopyranose residues, (Table 3). The anomeric region shows the presence of at least three different types of linkages which were attributed to C-1 of terminal reducing sugars (93.43 and 97.63 p.p.m.) (7) and α -L-arabinofuranose residues (108.3 p.p.m.) (7). The arabinofuranose sugar may be present as terminal residues (8) and 3-O-linked, (Tables 2 and 3). There are evidences that support the presence of 3-0- (7,8) and 6-0- (10) galactose residues (82.21 and 69.34 p.p.m.). The spectral evidences agree with the methylation analysis, (Table 2). The signals due to uronic acid residues were not detected because of the relatively low content of these sugars in *P. pendula* gum, (Table 1). Dearabinosilation, during reaction to obtain degraded gum A, permitted a better resolution of the resonances of galactose and uronic acid residues as was shown in the spectrum of degraded gum A (Figure 3, Table V). This fact has been reported previously (7,10).

The spectra of *P. bicolor* and *P. biglobosa* gums (Figure 2), are very similar although the peak intensities in the spectrum of former are higher due to experimental conditions (transients numbers and the amount of sample used). These spectra show the resonances due to β -D-galactose, α -L-arabinofuranose, β-D-glucuronic acid and 4-O-methyl- α -D-glucuronic acid residues, (Tables 3 and 4). The anomeric region contains the peaks assigned unequivocally to C-1 of 4-O-methyl-α-D-glucuronic acid (99.00 p.p.m.) (9), terminal β -L-arabinopyranose (11) (100.81 p.p.m.), β -D-galactose (103.49 p.p.m.) (7,8) and of α -L-arabinofuranose (109.44 p.p.m.) (7,10) residues. The resonances at 103.49 and 82.98 p.p.m. agree with the presence of 3-O-linked galactose residues (7,8,10), in the structure of the gums of *P. bicolor* and *P. biglobosa*. The presence of terminal β -L-

Table 4
Carbon-13 N.m.r. chemical shifts[a] of the uronic acid residues of *Parkia* gums

Type of linkage	Specie	C-1	C-2	C-3	C-4	C-5	C-6	4-OMe
4-OMe-α-D-Glc (1—>[b]		99.7	72.2	73.0	82.7	70.8	-	61.1
	P. biglobosa	99.0	-	-	83.0	71.0	-	60.7
	P. bicolor	99.0	-	-	83.0	70.9	-	60.8
β-D-GlcA (1—>[b]		104.0	75.5	77.1	73.3	77.5	177.5	
	P. biglobosa	103.8	75.0	76.0	-	77.4		
	P. bicolor	103.6	75.8	77.5	-	77.5		

[a]The spectra were calibrated with 1,4-dioxane (δ 67.4 p.p.m.). [b]Reference 9. These signals were not observed in the spectrum of *P. pendula* gum.

Table 5
Carbon-13 N.m.r. chemical shifts[a] of sugar residues in degraded gum A of *Parkia pendula*

Type of linkage	C-1	C-2	C-3	C-4	C-5	C-6	4-OMe
—> 3) Gal (1—>	104.2	70.7	83.0	69.5	74.5	61.9	
b	105.0	71.2	83.0	69.3	75.6	61.8	
—> 6) Gal (1—>	103.5	70.1	72.6	67.4	72.6	69.5	
c	103.3	70.4	72.5	67.8	73.0	68.1	
4-OMe- D-GlcA (1—>	100.4	71.6	73.5	82.9	70.7		60.8
d	99.7	72.2	73.0	82.7	70.8		61.1
-D-GlcA (1—>	104.2	75.6	76.0	73.5	76.4		
d	103.8	75.5	77.1	73.3	77.5	177.5	

[a] The spectra were calibrated with 1,4-dioxane (δ 67.4 p.p.m.). [b] Reference 8. [c] Reference 10. [d] Reference 9.

arabinopyranose was corroborated by the assignment of the signal at 63.88 ppm due to C-5. The resonances assigned to the uronic acid residues (Table 4), suggest that β -D-glucuronic acid and its 4-O-methyl- α-ether (9) are present in the structure of these gums, as was demonstrated by chemical analysis, (Table 1). A very intense peak, at 82.96 ppm, observed in the spectrum of *P. bicolor* is, probably, the result of overlapping of the resonances due to C-3 linked galactose residues (7,8), C-3 of linked α -L-arabinofuranose residues (10) and C-4 of the 4-O-methyl-α-D-glucuronic acid (9).

The 13C-N.m.r. spectra of *P. bicolor* and *P. biglobosa* show the resonances due to 3-O-galactose residues, terminal and 3-0- linked α-arabinofuranose and uronic acid residues. The spectrum of P. pendula gum looks as that corresponding to an α-L-arabinan (12). Although there are evidences in favor of the presence of 3-O- and 6-O- galactose residues (7,8,10). A better resolution of these signals

and those due to the uronic acids was observed in the spectrum of degraded gum A of this polymer (Figure 3, Table 5).

The absence of a signal due to the methyl group of rhamnose, in all the spectra studied, confirmed the chemical data shown in Table 1.

The spectral evidences, based in chemical analyses and previous results (7-10) indicated that the interpretation of a well-resolved spectrum of these heteropolysaccharides may show many interesting structural features.

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