

# Simple $^{13}\text{C}$ -NMR Methods for Quantitative Determinations of Polyflavonoid Tannin Characteristics

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## SYNOPSIS

A simple  $^{13}\text{C}$ -NMR method for the quantitative determination of polyflavonoid tannin characteristics was developed. The system is effective for use on concentrated (25–50%) solutions of natural and modified tannins. It allows the determination of the average degree of polymerization ( $\overline{DP}_n$ ) of the polyflavonoid, resorcinol vs. phloroglucinol proportion of the A-ring and catechol vs. pyrogallol proportion of the B-ring. The results obtained are consistent with existing data determined by other techniques. The method was also tried with tannin extract that was modified to form thermosetting adhesive intermediates, and with tannin modified by sulfonation, a common commercial modification for these materials. The results were again consistent with what was expected. The method affords the possibility to follow by a simple technique the variations in  $\overline{DP}_n$  and  $\overline{M}_n$  (number-average molecular weight) induced by chemical modifications of polyflavonoid tannin extracts and thus to correlate them with relevant structural modifications affecting these parameters. The method is not capable of distinguishing the relative proportions of the four important flavonoid units present in commercial polymeric tannin extract. It can only distinguish the relative proportions of (i) (procyanidins + prodelphinidins) vs. (profisetinidins + prorobinetinidins) and (ii) (prorobinetinidins + prodelphinidins) vs. (profisetinidins + procyanidins). © 1995 John Wiley & Sons, Inc.

## INTRODUCTION

Polyflavonoid tannins have been used for adhesives for more than two decades,<sup>1</sup> but systems to quantitatively determine their characteristics in simple and rapid manner have not developed at the same pace. Among the more interesting of such materials' characteristics that are useful for their adhesives application are: average degree of polymerisation ( $\overline{DP}_n$ ) of the natural tannin, which is often determined in regard to their viscosity in the concentrated solutions in which they are used for adhesives<sup>1,2</sup>; relative proportion of phloroglucinol vs. resorcinol type A-rings, which determines their reactivity toward formaldehyde and hence their rate and extent of cross-linking; and relative proportion of pyrogallol vs. catechol B-rings, which influences heterocycle pyran ring opening determining which type or rear-

rangements occur in the structure of the polyflavonoid under acid and alkaline conditions.<sup>3</sup> These characteristics represent useful information not only on the natural tannin, but also on chemically modified tannins prepared as adhesive intermediates.<sup>3</sup>

Recently a  $^{13}\text{C}$ -NMR cross polarization, magic angle spinning (CP-MAS) method for solid tannins using a series of quite complex pulse sequences aimed at determining the relative proportions of prodelphinidin (phloroglucinol A-ring + pyrogallol B-rings) to procyanidins (phloroglucinol A-rings + catechol B-rings) was presented.<sup>4</sup> It is a good method but it suffers from some drawbacks, namely: it is somewhat complicated to use; it is not adapted for tannin solutions but is only applicable to solid samples; and it only addresses a small part of the characteristics of interest (outlined above). Also recently, extensive  $^{13}\text{C}$ -NMR studies on tannin and tannin adhesive intermediates in solution were conducted to confirm qualitatively, but directly on the tannin, what is the extent and type of rearrangements in the tannin structure that determine per-

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**Table I** Integrated Intensities of Relevant Bands of  $^{13}\text{C}$ -NMR

	A-Ring 156–158 ppm	B-Ring 146–148 ppm	C1'		C4–C8 Link ~110 ppm	Free C6 96–98 ppm	Free C8 95–96 ppm
			130–132 ppm	132–135 ppm			
Natural tannin extracts							
Mimosa	9.433	23.794	7.432	6.792	9.169	2.397	4.538
Quebracho	7.542	13.209	7.874	0	3.772	1.322	0.661
Pine	11.498	11.672	4.491	0	7.906	4.190	0.599
Pecan nut	8.724	11.633	6.190	5.418	9.221	3.095	3.010
Gambier	12.809	12.649	5.958	0	2.745	6.681	1.801
Tannin adhesive intermediates							
Mimosa	6.982	13.550	7.220	5.552	8.256	1.708	1.575
Quebracho	5.304	9.066	7.485	0	2.697	0.670	0.678
Pine	6.583	6.929	10.15	0	10.551	4.083	2.041
Pecan nut	4.486	5.864	5.632	2.234	8.714	3.460	1.922
Mixed tannins, mimosa : pine							
75 : 25	10.904	20.396	8.014	4.469	10.971	4.106	3.652
50 : 50	12.020	17.783	10.371	3.506	13.842	4.057	4.751
25 : 75	19.975	25.733	17.807	2.267	21.132	8.425	4.817
Sulfited tannins							
Mimosa	9.792	21.346	6.544	5.227	8.993	7.721	2.361
Quebracho	12.094	19.534	9.358	0	5.035	1.823	0.991
Pine	16.604	16.770	10.872	0	13.783	9.003	5.162
Catechin monomer (model)	7.576	7.820	11.567	~0	~0	12.113	2.094

formance in regard to their adhesive application.<sup>2,3</sup> On the basis of these studies<sup>2,3</sup> a simple  $^{13}\text{C}$ -NMR method for rapidly determining the wanted tannin characteristics was developed. This article presents such a method for concentrated (25–50%) tannin solutions in water and for modified tannin adhesives intermediates. It is based on the recent finding that correlation of  $^{13}\text{C}$ -NMR peak intensities ratio with the physical characteristics of synthetic resins such as urea-formaldehyde (UF)<sup>5</sup> and phenol-formaldehyde (PF)<sup>6</sup> is possible.

## EXPERIMENTAL

Forty percent water solutions of mimosa (*Acacia mearnsii*) bark extract, quebracho (*Schinopsis balansae*) wood extract, pine (*Pinus radiata*) bark extract, pecan (*Carya illinoensis*) nut pith extract, and gambier (*Uncaria gambir*) shoots and leaves extract were prepared and analyzed by  $^{13}\text{C}$ -NMR.  $^{13}\text{C}$ -NMR spectra were attained on a Bruker AC200 FT-NMR spectrometer, at a frequency of 50.3 MHz with the

sample at 35 Hz. Chemical shifts were calculated relative to  $(\text{CH}_3)_3\text{Si}(\text{CH}_2)_3\text{SO}_3\text{Na}$  for NMR control in  $\text{D}_2\text{O}$ . All spectra were run overnight. Acquisition time was of 1.08 s with 10,000 transients. All spectra were run with relaxation delay of 5 s and with a precision of 1 ppm. The spectra were run without nOe enhancement, spectral width was of 15,000 Hz, and digital resolution 0.825. Typical spin-lattice relaxation time constants have been reported as 0.06 s for protonated carbons and 1.7 s for nonprotonated carbons for solutions of *P. radiata* tannin<sup>4,7,8</sup> and these were used. The intensities reported and used are integrated areas and are shown in Table I. The samples were prepared by diluting 40% mass/mass aqueous solutions of tannin extracts with  $\text{D}_2\text{O}$  in 1 : 4 mass proportion. The chemical/heat treatments for the modified tannin extracts to form adhesive intermediates consisted of 3% acetic anhydride treatment followed by an NaOH treatment at 90°C as previously reported.<sup>2,3</sup>

The experiments were also repeated with tannin extract solutions of equal concentration but that had been modified by reaction with sodium sulfite. Three

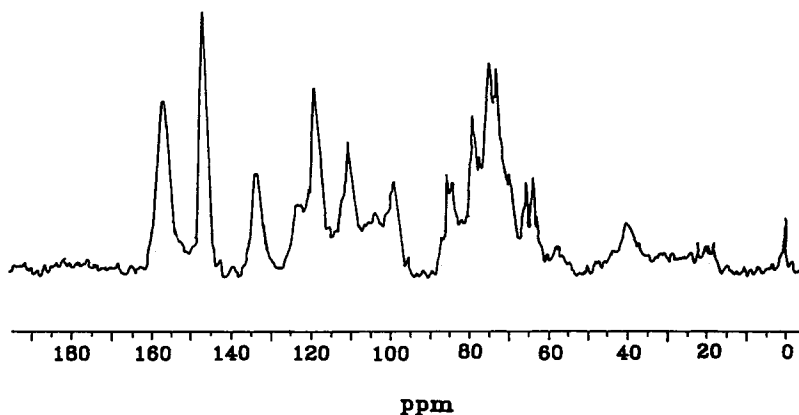


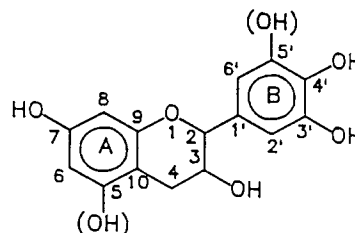
Figure 1  $^{13}\text{C}$ -NMR spectrum of pine tannin extract.

sulfited tannins were tested: commercial sulfited quebracho extract (8–10% sulfitation); commercial sulfited pine extract (8% sulfitation) in which the product had already been industrially sulfited; and laboratory sulfited mimosa extract prepared by 4 h refluxing of a solution of 100 parts mimosa tannin extract stirred in 200 parts water to which were added 15 parts sodium sulfite. This solution was then freeze-dried to obtain the modified tannin extract powder.

## DISCUSSION

The  $^{13}\text{C}$ -NMR bands of interest in the determination of the characteristics wanted are those at 156–158 ppm representing C5, C7, and C9 on the flavonoid A-ring, those at 146–148 ppm representing C3', C4',

and C5' on the B-ring, the unreacted "free" C6 band at 96–98 ppm, the unreacted "free" C8 band at 95–98 ppm, the C4–C8/C4–C6 interflavonoid linkage band at 110–111 ppm, and the C1' bands at 130–132 ppm (for catechol B-rings) and 132–135 ppm (for pyrogallol B-rings) (Figs. 1 and 2).



In Table I are reported: the area intensities of each of the bands for five different tannins used for adhesives (mimosa, quebracho, pine, pecan nut, and

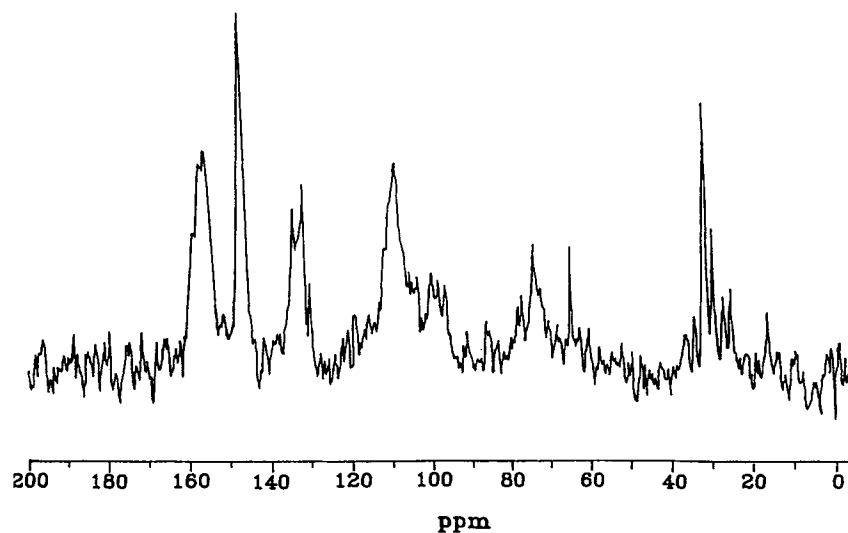


Figure 2  $^{13}\text{C}$ -NMR spectrum of pecan nut tannin extract.

gambier natural tannin extracts) of a pure flavonoid monomer (+)-catechin; of adhesive intermediates derived from tannin's chemical modifications already reported<sup>2,3</sup>; of three mixtures of the two more different tannins; and on sulfited tannins to check on the limits of the method presented.

In regard to  $\overline{DP}_n$  of the tannins, this is related to the relative proportion of C4-C8/C4-C6 interflavonoid linkages connecting one flavonoid with the others, in relation to the proportion of free C6 and free C8 sites. Taking the ratio of these band area intensities

$$y = \frac{\text{C4-C8/C4-C6 link}}{\text{free C8} + \text{free C6}}$$

(+)-catechin monomer  $\overline{DP}_n = 1$  for a ratio  $y = 0.093$  (Table II), while for mimosa tannin the known number-average molecule mass ( $\overline{M}_n$ ) is 1300-1350,<sup>9</sup> and taking the value reported by Riedl et al.<sup>10</sup> as 1340, hence the  $\overline{DP}_n = 4.90$  for a ratio  $y = 1.322$  (Table II). The relationship might of course not be exactly linear, but it is likely to be close to linearity. On this basis, the values presented in Table II lead to the following relationship for  $\overline{DP}_n$

$$\overline{DP}_n = 3.173y + 0.705.$$

The results of  $\overline{DP}_n$  for the other four tannins are approximately consistent with  $\overline{M}_n$  and  $\overline{M}_w$  (weight-average) values found experimentally, where these are available,<sup>9-13</sup> and are consistent with viscosity<sup>2,3</sup> and NMR<sup>2,3</sup> qualitative indications of the relative  $\overline{DP}_n$  for the five tannins. Thus, gambier is well known to have low  $\overline{DP}_n$  with up to 50% of the tannin extract being in monomeric (+)-catechin form.<sup>2</sup> Quebracho is known to be the most polymerized of the five tannins,<sup>2</sup> while pine and pecan nut are known to be of higher  $\overline{M}_n$  and  $\overline{DP}_n$  than mimosa but lower than quebracho.

In the determination of B-ring characteristics, the C1' bands are the most useful. A single peak indicates a pure catechol or pure pyrogallol B-rings. If both pyrogallol and catechol are present then two well-defined clear peaks appear. The downfield peak at higher ppm corresponds to pyrogallol.<sup>14</sup> Using the ratio

$$y = \frac{\text{catechol (130-132 ppm) peak}}{\text{catechol} + \text{pyrogallol (132-135 ppm) peak}}$$

Then from (+)-catechin monomer in which the B-ring is only composed of catechol  $y = 1.0$  (Table II) means 100% catechol, and from mimosa tannin in

which catechol is known to compose 20% of the B-rings<sup>15,16</sup> at a  $y = 0.523$  (Table II), it is possible to attain the following expression

$$\text{catechol percentage} = [1.677y - 0.677]100.$$

The results attained with this expression for the five tannins presented in Table II are consistent with what is known about their B-rings by other techniques.<sup>1,2,11,12,15,16</sup>

The relative proportion of hydroxy groups on A- and B-rings can be determined by using the A-rings C5, C7, C9 bands at 156-158 ppm (A) and the B-rings C3', C4', C5' bands at 146-148 ppm (B). Typical (not average) compositions of the A-rings in the five tannins are known for: mimosa,<sup>15,16</sup> catechin monomer, quebracho,<sup>14</sup> gambier,<sup>2</sup> pine,<sup>2,11</sup> and pecan<sup>2,12</sup> tannins. From the determination of B-ring fractions of catechol and pyrogallol already presented and using the ratio of band intensities A : B and dividing all by A, it becomes 1 : (B/A). Indicating the number of aromatic carbons connected to an —O—, or —OH, hence the number of OHs on the B-ring as  $b$  and with  $a$  the number of —OHs and —O— on the A-ring

number of —OHs on A ring ( $a$ )

$$= \frac{\text{number of —OHs on B-ring } (b)}{(B/A)}$$

where B and A are the <sup>13</sup>C-NMR bands area intensities. In this context the method is limited to give the preponderance of phloroglucinol vs. resorcinol A-ring character which is the main characteristic for use in adhesives, rather than the relative proportions of procyanidin, prodelphinidin, profisetinidin, and prorobinetinidin units. It is a step forward however over Newman's method<sup>4</sup> in which the proportions of profisetinidin and prorobinetinidin are not derived.

The mixtures of 75/25, 50/50, and 25/75 by mass of mimosa and pine tannin give results consistent with what was expected by the mixtures of the two tannins (results in Table II). It confirms the consistency of such a method in determining characteristics of flavonoid mixtures without relating this to what the original tannin might have been. The mixtures' results confirm that the assumption of linear relationships used above is correct.

Of interest are also the results obtained for chemically/heat modified tannin adhesives intermediates (Table II) that confirm some of the indications and qualitative results attained in previous studies.<sup>3</sup> Thus an increase in  $\overline{DP}_n$  following chemical

**Table II Peak Ratios and Results for B- and A-Ring Characteristics and Number-Average Degree of Polymerization ( $\overline{DP}_n$ )**

	B-Ring Ratio	% Catechol Ratio	B-Ring OH's	A-Ring OH's	Ratio	$\overline{DP}_n$	$\overline{M}_n$
Mimosa	0.523	20	2.80	1.11	1.322	4.90	1343-1406
Quebracho	1.000	100	2.00	1.14	1.902	6.74	1846
Pine	1.000	100	2.00	1.97	1.651	5.94	1722
Pecan nut	0.533	22	2.78	2.08	1.510	5.50	1683
Gambier	1.000	100	2.00	2.03	0.324	1.73	502
Mimosa adh. intermediate	0.565	27	2.73	1.40	2.505	8.65	2370
Quebracho adh. intermediate	1.000	100	2.00	1.17	2.001	7.05	1931
Pine adh. intermediate	1.000	100	2.00	1.90	1.723	6.17	1789
Pecan nut adh. intermediate	0.716	52	2.48	1.90	1.619	5.84	1787
Mimosa : pine mix							
75 : 25	0.642	40	2.60	1.39	1.414	5.19	
50 : 50	0.747	58	2.42	1.64	1.500	5.46	
25 : 75	0.887	81	2.19	1.70	1.596	5.77	
Sulfited							
Mimosa	0.556	25	2.75	1.26	0.892	3.54	970
Quebracho	1.000	100	2.00	1.24	1.789	6.38	1747
Pine	1.000	100	2.00	2.02	0.973	3.79	1098
Catechin	1.000	100	2.00	1.94	0.093	1.00	290

heat treatment for the profisetindin/prorobinetindin tannins (mimosa and quebracho) leads only to small or hardly any variation for pine tannins, and confirms the existence of some C4-C8/C4-C6 autocondensation for pecan nut tannin. The extensive C2-C8/C2-C6 pecan tannin autocondensation already reported<sup>2,3</sup> does not figure here because the C2-C8/C2-C6 found at 105-106 ppm was not taken into consideration in this study. The only ratios giving unacceptable results were the ones for modified mimosa and particularly modified pecan nut tannin in which the chemical modification yielded an apparent increase in the percentage of catechol type B-rings, which is absurd. What is not absurd however is the change of relative intensities of the C1' of pyrogallol and catechol B-rings. As the treatment includes acetic anhydride,<sup>2,3</sup> acetylation of the tannin in colloidal micelles,<sup>17</sup> as the initial step of an  $\alpha$ -set<sup>18</sup> attack (which develops subsequently through a Fries rearrangement<sup>19,20</sup>), would explain the decrease in intensity of the pyrogallol band with consequent apparent increase in relative percentage of catechol. This explanation however is unlikely, because the extent of apparent increase in catechol percentage is too high for pecan nut tannin to be explained by this route. It is this marked variation for the modified pecan tannin that gives an indication of what this represents: extensive opening of the heterocycle pyran ring in pyrogallol B-rings' fla-

vonoids ensures that the C1' signal at 132-135 ppm shifts for the open heterocycles, leading to a decrease in intensity for the normal form of the flavonoid. This leads to an increase in the apparent amount of catechol (which is not true). This effect is noticeable in only two tannins in the five studied in which pyran ring opening is facile.<sup>2,3</sup>

The other modification of the tannins looked at was sulfitation, sulfited mimosa, quebracho, and pine tannins. From the results shown in Table II it can be seen that the  $\overline{DP}_n$  of the sulfited tannins is somewhat lower than those for the natural tannins. This is an indication that such a treatment to some extent cleaves the interflavonoid, resulting in shorter polyflavonoid chains. This effect is greatest in the pine tannin, due to the well known lability of the interflavonoid link in procyanidins,<sup>21</sup> but sulfitation does not seem to affect the quebracho tannin to such a large extent. Mimosa tannin is proportionally affected more than quebracho but less than pine. This is unexpected as a interflavonoid link cleavage is rather difficult in this tannin.<sup>21</sup> These results are consistent with what would be expected based on viscosity and other qualitative observations previously determined.

As expected, the A-ring and B-ring characteristics of the sulfited tannins are similar to those of the natural tannins and the results obtained are within acceptable limits.

## CONCLUSION

Relative intensity ratios of selected  $^{13}\text{C}$ -NMR bands can be used to determine the relative proportion of phloroglucinol vs. resorcinol A-rings, and of pyrogallol vs. catechol B-rings, in natural tannin extracts in concentrated aqueous solutions. This is achieved by using standard  $^{13}\text{C}$ -NMR spectra without using any cross-polarization pulse sequence as proposed in other techniques.<sup>4</sup> Of interest in that  $\overline{\text{DP}}_n$ ,  $\overline{M}_n$ , and  $\overline{M}_w$  show good correlations with experimental results obtained with different techniques by other authors.<sup>9,10</sup> The method then affords the possibility of following by a single technique the variations in  $\overline{\text{DP}}_n$ ,  $\overline{M}_n$ , and  $\overline{M}_w$  induced by chemical modifications of polyflavonoid tannin extracts and thus to correlate those with the relevant structural modifications affecting these parameters. Although the method is very suitable for the determination of important parameters for the industrial use of polymeric tannin extracts, it is not capable of distinguishing the relative proportions of prodelphinidins to procyanidins to profisetinidins to prorobinetinidins units present in the polymer. It can only distinguish (procyanidins + prodelphinidins) vs. (profisetinidins + procyanidins).

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