

A Comparative C¹³ NMR Study of Polyflavonoid Tannin Extracts for Phenolic Polycondensates

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SYNOPSIS

Assignment of C¹³ NMR bands to important sites of polymeric flavonoid tannin extracts produced industrially for production of tannin-formaldehyde resins is reported. Five different tannin extracts were studied. The C¹³ NMR bands' position and relative intensities allowed us to qualitatively explain comparative differences in the five tannins' structural features and properties that are of importance for tannin adhesives applications. The five tannins could be divided into two classes (1) mimosa and quebracho tannins and (2) pecan nut, pine, and gambier tannins. Properties of relevance that varied considerably among the five tannins and that could be detected by ¹³C NMR were: the relative proportions of fisetinidin/robinetinidin units in relation to catechin/gallocatechin units; differences in number average degree of polymerization; the extent of open heterocycle forms present; branching; and relative proportion of pyrogallol versus catechol B-rings in the flavonoid repeating units. © 1993 John Wiley & Sons, Inc.

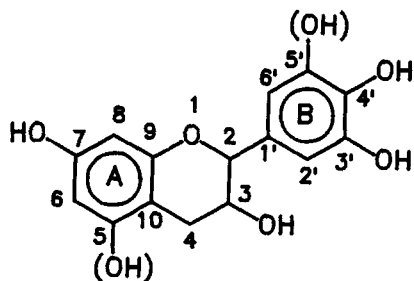
INTRODUCTION

Thermosetting polyflavonoid tannin-formaldehyde resins have performed well for the last 20 years in industrial applications as phenolic exterior adhesives for wood.¹ Industrial polyflavonoid tannin extracts are mostly composed of flavan-3-ols repeating units, and of smaller fractions of polysaccharides and simple sugars.¹ Polyflavonoids in such tannin extracts present phloroglucinol or resorcinol A-rings and catechol or pyrogallol B-rings (Scheme 1).

The repeating units are linked to each other C4-C6 or C4-C8, the former predominating in tannins mostly composed of fisetinidin (resorcinol A-ring; catechol B-ring) and robinetinidin (resorcinol A-ring; pyrogallo B-ring) repeating units. The C4-C8 interflavonoid linkage instead predominates in tannins composed of catechin (phloroglucinol A-ring; catechol B-ring) and gallocatechin (phloroglucinol A-ring, pyrogallol B-ring) repeating units. When the polymeric tannins are composed of fisetinidin/ro-

binetinidin units the polymers are respectively called profisetinidin/prorobinetidin; when they are composed of catechin/gallocatechin the polymers are called procyanidin/prodelphinidin, respectively.² The free C6 and C8 sites on the A-ring are the sites reactive with formaldehyde to form adhesives, under the usual conditions under which these materials are used. Some participation in the reaction with formaldehyde by the B-ring can occur when such a ring is pyrogallic in nature. Although the reaction of formaldehyde with the tannin extract occurs at reactive sites on the phenolic nuclei of the tannins, polysaccharides and simple sugars also can create conditions that influence the mechanism and route taken by the extract/formaldehyde reaction. This C¹³ NMR study is aimed at indicating the differences between the C¹³ NMR signals recorded and how they correlate with the characteristics of the tannin extracts and tannin structures that are of importance for the formulation of tannin-based adhesives and resins. The study is extended to five types of commercial tannin extracts: black wattle or mimosa (*Acacia mearnsii* formerly *mollissima* de Wildt) bark extract; quebracho (*Schinopsis balansae*, variety *chaqueno*) wood extract; pine (*Pinus radiata*) bark extract; pecan (*Carya illinoensis*) nut pith extract;

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Scheme 1

and gambier tannin (*Acacia catechú* and *Uncaria gambir*) leaf and shoot extract.

EXPERIMENTAL

C^{13} NMR

Solutions C^{13} NMR spectra were obtained on a Bruker AC200 FT NMR spectrometer, at a frequency of 50.3 MHz and with the sample spectra at 25 Hz. The samples were prepared by diluting 40% mass/

mass aqueous solutions of tannin extracts with D_2O in 1 : 4 mass proportion, except for monomeric catechin for which deuterated methanol was used as solvent. Distortionless enhancement by polarization transfer (DEPT), C^{13} NMR spectra were also obtained to add confirmatory evidence of correct assignment for $-CH$, $-CH_2-$, $-CH_3$, and quaternary carbons shifts.

The C^{13} NMR band intensities were reported to a 100% band intensity for the $C3'$, $C4'$, 145–146 ppm band, for parity of comparison. Results are shown in Table I and Figures 1–6.

Gel Times and Viscosity

Gel times of the reaction of tannin extracts with formaldehyde are an indication of the reactivity of the tannin sites and hence of some of the likely structural characteristics of the tannin repeating units important for the preparation of adhesives and resins.³ The gel time dependence on pH at 94°C was determined over the entire pH range shown in Table II for the five industrial tannin extracts under ex-

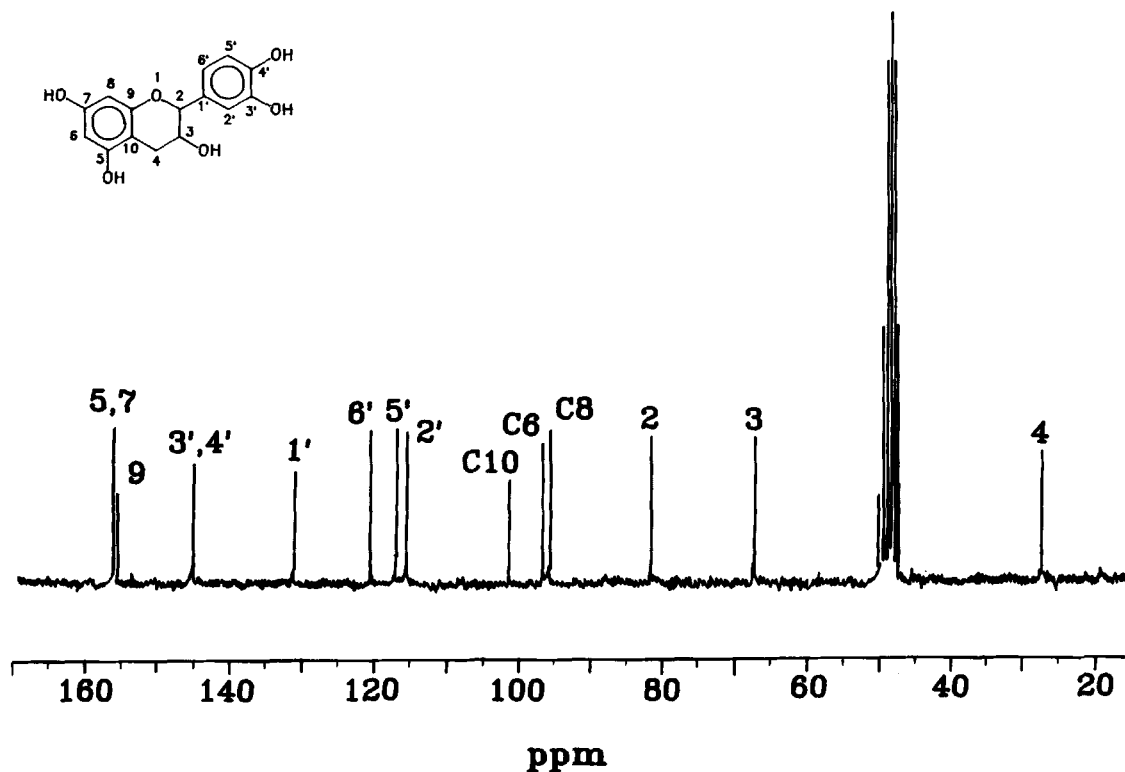


Figure 1 C^{13} NMR of pure monomeric catechin with carbon atoms assignments.

amination. All the gel times were determined for tannin extract solutions 40% by mass in water. Gelation is defined as the point at which the polycondensates formed by the reaction of tannin extract and formaldehyde ceases to be a viscous liquid and becomes a soft, elastic, rubbery solid. Ten grams of liquid tannin extract in a 40% water solution is weighed in a glass test tube. Paraformaldehyde 0.3 g (96% Degussa N fine powder) is added. A wire spring is placed in the test tube and the tannin extract solution gently mixed at ambient temperature for a few seconds. The test tube is then placed in a boiling water bath (temperature determined by altitude of location: our boiling point = 94°C), the wire spring is normally moved rapidly up and down in the tube, and the time taken to reach gel point measured using a stopwatch. The test is done in duplicate. The gel time measurement is used to relate to the speed of gelling of the extract/paraformaldehyde mixture under actual application conditions. Results are shown in Table III.

Viscosity measurements were carried out on 40% aqueous solutions of the tannin extracts conditioned at 25°C, using a Brookfield LVF viscometer. Results are shown in Table II.

DISCUSSION

The trends in relative C¹³ NMR band intensities, characteristic of different carbons in polyflavonoids, allow the identification of differences that might be significant for tannin-formaldehyde adhesives of the five tannins extracts under examination. The properties of comparative interest are number of potential reactive sites with formaldehyde, number average degree of polymerization of the tannin, amount of phloroglucinol A-rings present, the type and level of interflavonoid linkages, characteristics of the flavonoid B-rings and the extent of opening of the heterocycle.

The relative intensities of the free (unreacted) C6 and C8 sites on A-rings (Table I, Figs. 1-6) at 96-98 and 95-96 ppm, respectively, are a set of very sensitive bands indicating directly the reactivity and indirectly the degree of polymerization of the tannin. In this regard the five tannin extracts can be divided into two classes. First mimosa and quebracho that present much lower intensity of these two C¹³ NMR bands (Figs. 2, 3, Table I). Second, the set composed of pine, pecan nut, and gambier in which these two bands have much greater intensities. Within each of these two sets clear differences between the tan-

Table I Comparative C¹³ NMR Band Assignment and Relative Band Intensities (%) for Pure Catechin and Five Different Types of Polyflavonoid Tannin Extracts Suitable for Adhesive Preparation

Assignment	C5, C7	C9	C3', C4'	C1	C6'	C5', C2'	Phloroglucinol			Catechin			
							C5, C2'	C6	C8	C2	C3	Acid	C4
ppm	156-157	155	145-146	131	120-121	115-117	110	96-98	95-96	81-82	67-68	31-32	27-28
Pure catechin	97, 100	58	72, 76	73	97	98, 96	—	88	97	92	93	—	80
Mimosa bark	53	30	100	44	25	26	51	21	31	—	—	—	21
Quebracho wood	63	33	100	66	56	99	40, 52	20	20	—	—	—	32
Pine bark	71	71	100	49	39	76	60	47	23	—	—	—	—
Pecan nut pith	66	70	100	62	30	33	66	40	29	—	—	79	37
Gambier	100	50	100	55	69	106, 95	27	60	28	—	—	61	61

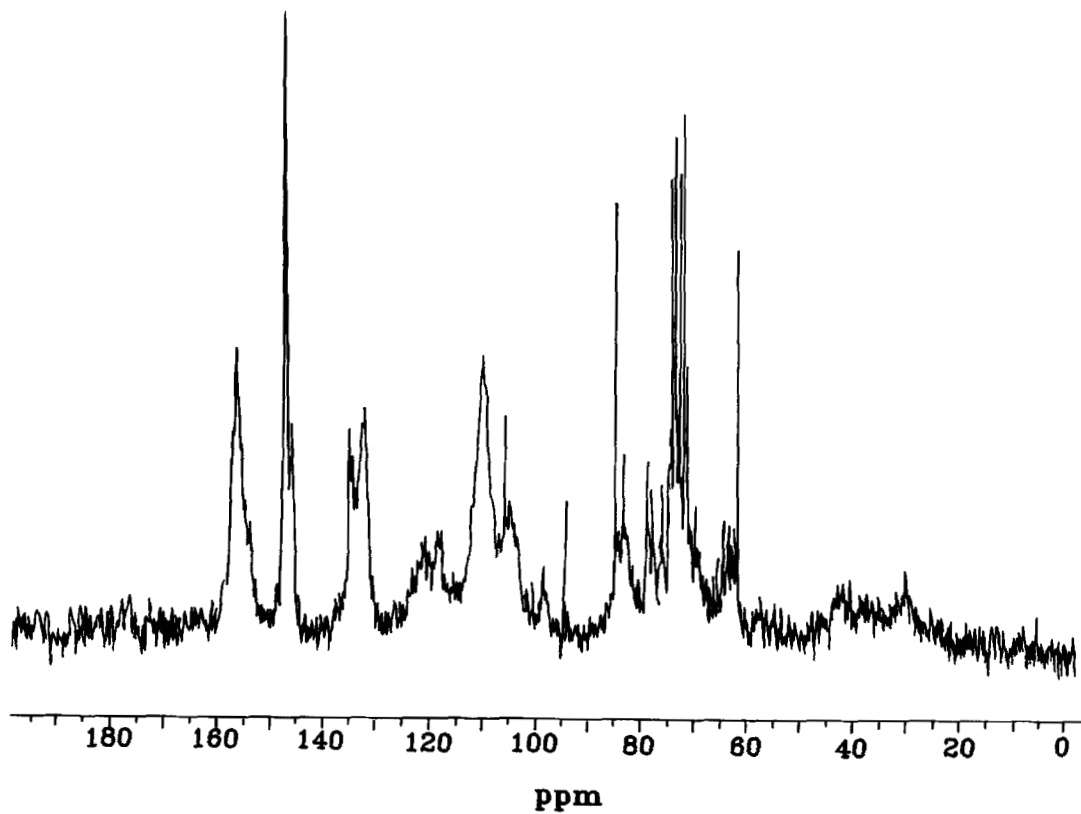


Figure 2 ¹³C NMR of industrial mimosa bark tannin extract.

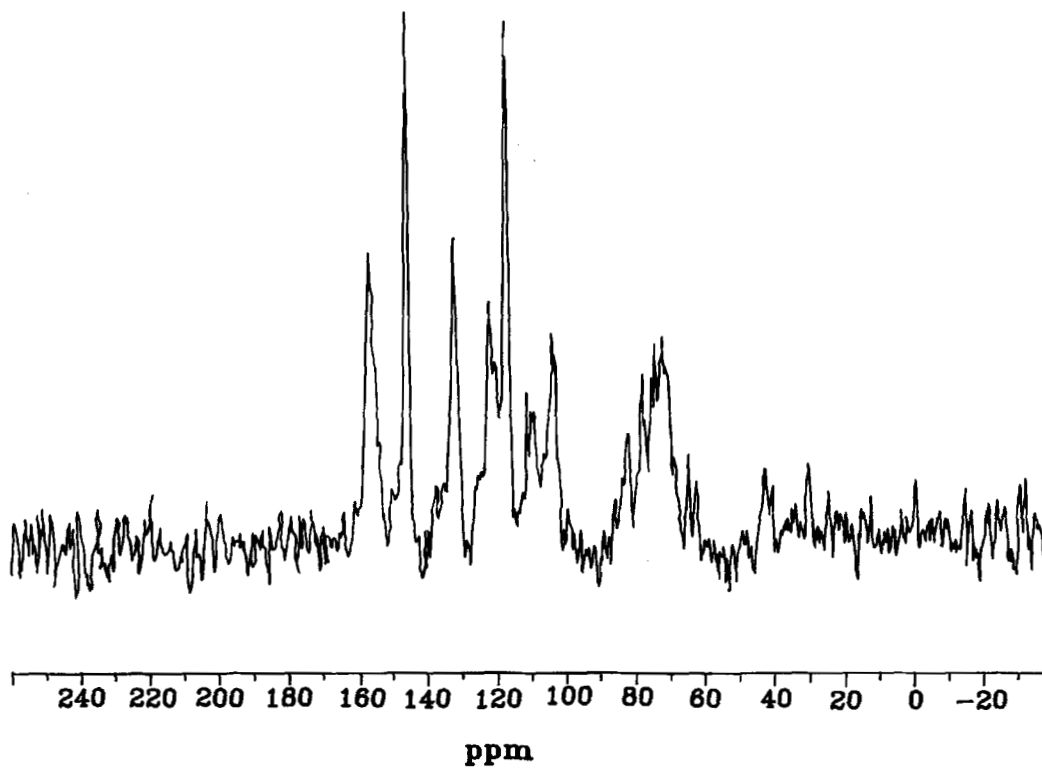


Figure 3 ¹³C NMR of industrial quebracho wood tannin extract.

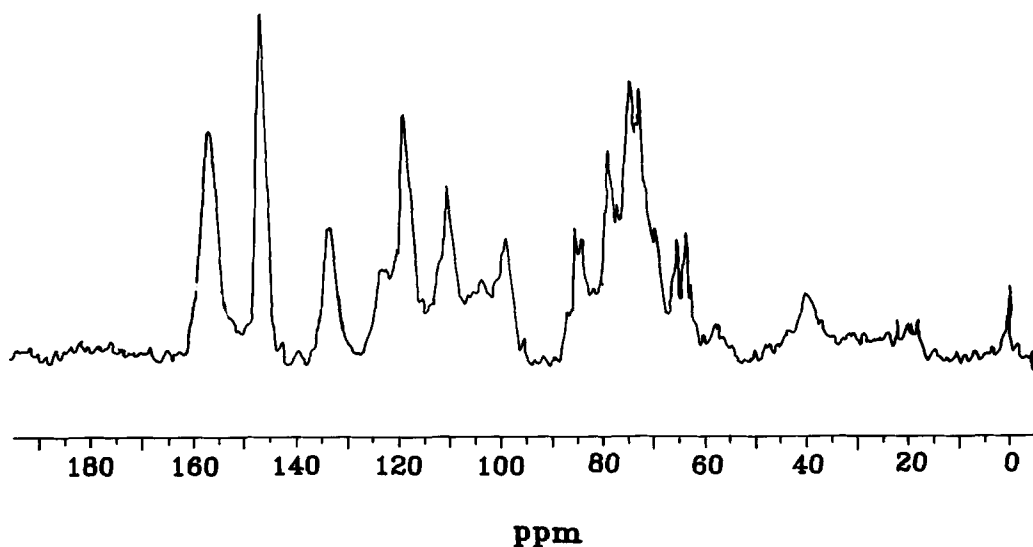


Figure 4 C^{13} NMR of industrial pine bark tannin extract.

nins can be seen. Quebracho for instance presents a total free C6 + C8 bands intensity much lower than mimosa indicating a likely higher degree of polymerization of the former (Table I, Figs. 2, 3). Furthermore it presents a much lower intensity of the free C8 band inferring a higher proportion of C4-

C8 interflavonoid linkages than in mimosa, in which the proportion of C4-C8 linkages is known to be relatively low.⁴ Thus, while mimosa units are predominantly C4-C6 linked with no more than 13-15% C4-C8 linkages, quebracho, although still presenting a predominant amount of C4-C6 linkages

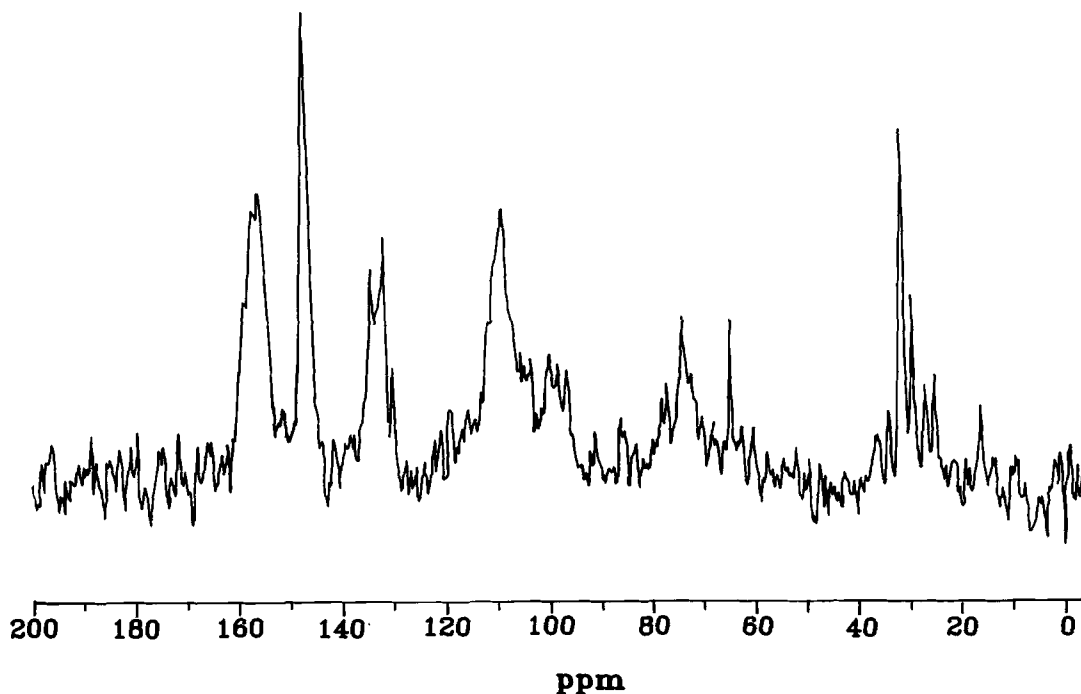


Figure 5 C^{13} NMR of industrial pecan nut pith tannin extract; note the extensive $-CH_2-$ from catechinic acid around 30-40 ppm ($-CH_2-$ in DEPT C^{13} NMR spectrum).

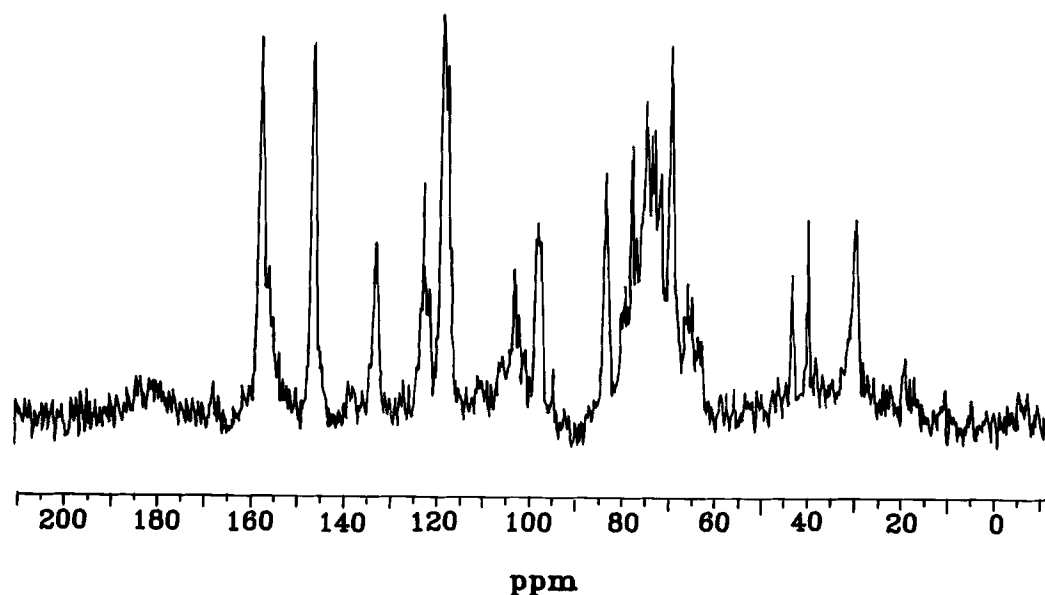


Figure 6 C^{13} NMR of industrial gambier shoots tannin extract; note the extensive $-CH_2-$ from catechinic acid around 30–40 ppm confirmed by DEPT spectrum.

shows clearly by inference a much higher proportion of C4–C8 linkages (about 20%) than mimosa. These facts are already ascertained by other techniques.⁵ The higher degree of polymerization and higher proportion of C4–C8 linkages infer that quebracho is a very “branched” tannin.

In the second set of tannins, the high proportion of free C6 clearly confirms the predominance of the C4–C8 interflavonoid linkage in these procyanidin- and prodelphinidin-based tannins, with pine and pecan nut showing a very similar degree of polymerization. The high value of the free C6, C8 band intensities indicates that gambier tannin has instead a much lower degree of polymerization (DP). Pure catechin, which is completely monomeric, presents the highest values of these two bands (Fig. 1). The low DP_n of gambier tannin leads to the highest proportion of free C8, given by the free C8 band relative intensity (Table I). Small differences are also notable between pecan nut and pine tannin: the former has a slightly higher intensity of the free C8 band and much lower intensity of the free C6 band, hence a higher amount of free C8 sites, inferring a noticeably higher proportion of C4–C6 linkages than in pine tannin (Figs. 5, 6).

Confirming evidence of the above can be gathered from the conspicuous but less sensitive band at 110 ppm indicating C8 and C6 sites participating in C4–C8 and C4–C6 interflavonoid linkages, in procyanidine/prodelphinidin type tannins. The low relative intensity of this band confirms for instance the very

low DP_n of gambier tannin (Fig. 6, Table I); its high intensity confirms that pecan nut tannin is the one of highest DP_n fairly closely followed by pine tannin. Again the low relative intensity of this band indicates that quebracho and mimosa tannins appear to be much less polymerized at least through phloroglucinol A-ring sites (they contain much lower amounts of phloroglucinol A-rings, thus much lower amounts of procyanidin or prodelphinidin units) with quebracho having slightly higher DP, or higher proportion of procyanidin/prodelphinidin units, than mimosa. The free C4 band (at 27–28 ppm) intensity is also in line with all of the above. The rel-

Table II Comparative Viscosities of Different Tannin Solutions at Different Concentrations at 25°C

	Percent Extract Solids Content, by Mass		
	50%	47%	40%
Mimosa bark tannin extract	1.8	—	0.25
Quebracho wood tannin extract	14.2	7.7	0.57
Pine bark tannin extract	—	4.4	0.51
Pecan nut pith tannin extract	57.0	—	1.8
Gambier tannin extract	3.0	1.7	0.32

Values expressed in Pa s.

Table III Comparative Gel Times at 94°C of Tannin Extract-Formaldehyde, of 40% Aqueous Solution of Tannin Extracts

pH	Pecan Nut (s)	Pine (s)	Gambier (s)	Quebracho (s)	Mimosa (s)
4.8	51	86 (natural pH)	217 (natural pH)	—	516
5.0	49	—	194	—	—
5.38	42 (natural pH)	—	—	—	—
5.5	41	74	146	534	180
6.0	37	60	113	338	165
6.5	33	48	89	214	—
7.0	28	39	72	136	102
7.5	25	32	58	86	82
8.0	22	26	48	54	59
r^a	0.98	0.95	0.99	0.99	0.96

^a Coefficient of correlation r of nonlinear correlation between pH and gel time.

ative relation of their degree of polymerization is confirmed by the relative proportion of the pine extract typical (not average) viscosities presented in Table II. Some differences in the process of industrial extraction occurs: for "natural" quebracho 5% metabisulphite and 97–100°C are used for extraction, 5% at 70°C is used in pecan, 2.5% and 70°C in pine, no metabisulphite and 97–100°C for mimosa. Use of metabisulphite for instance will tend to extract some higher molecular mass tannins to a certain extent increasing molecular mass by autocondensation,^{6,7} while the higher temperatures tend to slightly increase the amount of carbohydrates extracted. Such differences in extraction procedure have been shown to influence the final viscosity of the extract; these do not explain differences as extensive as those shown in Table II. These must be attributed in high proportion then to the structure and degree of polymerization of the tannin in each extract. The viscosities reported in Table II are consistent with the qualitative relative order of the average DP of the pine extracts that can be deduced by comparing the relative intensities of the relevant C^{13} NMR bands discussed above and reported in Table I. The correlation of relative C^{13} NMR band intensities and of typical viscosities in Tables I and II is clearly nonlinear and does not follow a simple nonlinear relationship.

Linked with the above are the intensities of the $C5$ and $C7$ bands (aromatic $\geq C-OH$) at 156–157 ppm, generally appearing as a single broad band (Table I, Figs. 1–6). This is a reasonably sensitive band in regard to intensity. It also allows a qualitative indication of the proportion of phloroglucinol

A-rings, thus of procyanidin/prodelphinidin units, in each tannin extract. Again confirming the other evidence, pure catechin and gambier tannin register the highest band intensity and have comparable values (Table I) as clearly seen comparing Figures 1 and 6 with the other figures indicating that gambier is completely catechinic in nature and again confirming its very low average DP_n . Again two sets of tannins are evident: mimosa and quebracho and the other three tannins. Mimosa and quebracho polyflavonoids presenting $C5$ not linked to $-OH$ groups, are in relative proportions as observed by other techniques.⁴ Thus, to their mainly profisetinidin/prorobinetinidin nature are superimposed procyanidin/prodelphinidin units in 13–15% proportion for mimosa and 18–20% for quebracho. If one considers then gambier tannin as composed of completely catechinic structures, pine tannin from the relative intensity of this band should present approximately > 10 profisetinidin/prorobinetinidin units (with resorcinol A-rings) (compare Figures 6 and 4, Table I). In pecan nut tannins the proportion of procyanidin-type units appears to be lower and of profisetinidin-type units slightly higher than in pine tannins (a fact also supported by pecan lower free $C6$ band) (Fig. 5, Table I).

C^{13} NMR analysis also predicts the reactivity of formaldehyde with tannins to be higher with higher proportion of units with phloroglucinol A-rings presenting free reactive sites. This is well proved experimentally.¹ However, it is interesting to see within each of the two sets of tannins why there are differences in their gel times. Pine and pecan nut tannins give gel times in line with their relative av-

erage DP_n under identical reaction conditions (Table III). Gambier, although mostly catechinic, gives much slower gel times, the faster reaction rate ascribed to the higher phloroglucinol A-rings content and higher proportion of free C6 and C8 reactive sites being strongly counterbalanced by the slowing down of the reaction due to the much greater number of steps needed to gelation as a consequence of the low DP_n . For quebracho and mimosa gel times are somewhat comparable (Table III), a clear indication that in quebracho, as already established for mimosa,¹ the phloroglucinol A-rings appear to be mostly in angular tannin configuration, and are not likely to contribute much to the rate of reaction. The higher quebracho average DP_n indicates that in viscous solution, as used in wood adhesives application, the higher viscosity and lowered molecular mobility have a stronger effect, reducing rather than increasing reactivity (Table II). The higher apparent average DP_n of quebracho tannin might also be due to the small amount of Na metabisulphite (not used in mimosa) used in its water extraction medium, allowing extraction of higher molecular weight tannin fractions.

The C^{13} NMR spectra also give valuable insights into the characteristics of the flavonoid units' B-rings in the pine tannins. Thus, the bands at 120–121 ppm (C6', Table I) and at 115–117 ppm (C5' and C2', Table I) appear to indicate by both the high values of the sum of their intensities and of the intensity of the C5', C2' band that quebracho and gambier are much lower in pyrogallol B-rings (Figs. 3 and 6). By the intensity of the same band (Figs. 2 and 5), mimosa and pecan nut tannins present relatively much higher proportion of pyrogallol B-rings, a fact determined also by other techniques.^{2,8} Pine tannin (Fig. 4) appears in this respect to be an intermediate case. This gives an important insight on the reactivity of the tannin. The B-ring reaction with formaldehyde is favored in tannin flavonoid units in which such a ring is pyrogallol. Because pyrogallol is faster reacting with formaldehyde than catechol, as well as for the occurrence of side reactions⁸ in units presenting a phloroglucinol A-ring/pyrogallol B-ring (prodelphinidin tannins), this combination renders such a combination particularly reactive.⁸ This is the case of pecan nut tannin, as their gel time (Table III) and high strength of unmodified glue lines³ also appears to confirm.

The interpretation of the C^{13} NMR band intensities for C6', and C5', C2' is however complicated by the C4–C6 and C4–C8 interflavonoid linkages in which the C6 and C8 involved belong to a fisetinidin or robinetinidin unit (a unit with resorcinol A-ring).

These also transmit in the same region.⁹ Thus, this type of C4–C6 can be calculated to transmit at 121–123 ppm although the band is of low intensity, and this type of C4–C8 is superimposed to the C5', C2' band and appears to be of more noticeable intensity (Figs. 1–6). However, this confuses the situation mostly in quebracho where relatively noticeable proportions of C4–C8 interflavonoid linkages coexist with a predominance of resorcinol-type A-rings.

Two other regions are of interest: the first being the C9 band at approximately 155 ppm (Table I, Figs. 1–6). The higher the intensity of this band, the higher the proportion of flavonoid unit heterocycles maintained close. Thus, pine and pecan nut tannins with the highest intensity of this band show very little or no opening of their units' heterocycles. This because metabisulphite extraction preferentially cleaves the interflavonoid link in procyanidin tannins.⁶ Mimosa and quebracho (Figs. 2, 3) show greater proportion (still small) of cleavage and opening of their units' heterocycles because in pro-fisetinidin/prorobinetinidin tannins this reaction is favored⁷ over interflavonoid cleavage, and their relative C9 bands are much lower. Gambier tannin (Fig. 6) having very low average DP_n present much less interflavonoid linkages to be cleaved, hence the heterocycle is more likely to open, a fact supported also by the higher intensity of the C9 band in pure catechin. Because in tannin extraction heterocycle opening is not extensive, this band appears to be very sensitive to detection of heterocycle opening and to molecular rearrangements as those induced in adhesives intermediates preparation.

A C^{13} NMR region of interest is also the one at 60–90 ppm, in which signals occur from carbons of polymeric and monomeric carbohydrates that are always present in industrial tannin extracts. These mask the C2 and C3 bands that are instead clearly discernible in pure catechin (Fig. 1). The carbohydrates region is also of importance to understand some of the properties obtained during preparation of tannin adhesives intermediates.¹

CONCLUSIONS

C^{13} NMR can indicate directly on concentrated solutions of the tannin extract structural characteristics and properties of importance in the use of these materials as phenolic adhesives. All five tannin extracts examined showed that they are suitable for the preparation of tannin–formaldehyde polycondensates, although important differences among them exist. The five tannins can be divided into two

classes: (1) mimosa and quebracho and (2) pecan nut, pine, and gambier tannins, the latter class having much higher reactivity toward formaldehyde due to the phloroglucinol nature of the A-ring of the predominant type of flavonoid repeating unit. The more important differences and characteristics detected were:

Pecan Nut Tannin Extract. Higher proportion than expected of C4–C6 interflavonoid linkages. Still predominantly a procyanidin/prodelphinidin-type tannin, but of much more mixed character than pine tannin. Higher proportion of pyrogallol-type B-rings indicating relatively high proportion of prodelphinidins. It has the highest degree of polymerization of all the five tannins examined. Little or no heterocycle opening. Proper polymer branching present.

Pine Tannin Extract. Predominantly C4–C8 linked mostly procyanidin-type tannin. Highly polymerized but less than pecan nut tannin. Low proportion of both C4–C6 and fisetinidin/robinetidin-type units. Low or no branching. Relative proportion of pyrogallol and catechol B-rings is intermediate between the two tannin classes.

Gambier Tannin. Very low degree of polymerization with high proportion of monomeric flavonoids. Totally or almost totally catechinic/epicatechinic. Low proportion of pyrogallol B-rings and high proportion of catechol B-rings. Higher proportion of open heterocycle form than in the other two procyanidin-type tannins.

Mimosa Tannin Extract. Lower degree of polymerization than pine, pecan, and quebracho tannins. Lower number of C4–C8 interflavonoid linkages and lower number catechin/gallocatechin units than in quebracho. Very predominantly a profisetinidin/prorobinetidin-type tannin. Higher proportion of pyrogallol B-rings in relation to all the other tannins,

except pecan nut tannin. Low proportion of branching and lower than quebracho tannin.

Quebracho Tannin Extract. Higher degree of polymerization than mimosa, but lower than pecan nut tannin. Higher C4–C8 and higher proportion of catechin/epicatechin-type units than mimosa. Clearly a branched tannin: highest level of branching other than pecan nut tannin. Lower in pyrogallol B-rings and higher in catechol B-rings. Greater proportion of heterocycle cleavage forms than pine and pecan nut tannins. Still predominantly a profisetinidin/prorobinetidin tannin.

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