An ESR Study of the Silica-Induced Autocondensation of Polyflavonoid Tannins

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SYNOPSIS

Radical mechanisms of silica-induced polyflavonoid tannin autocondensation and hardening through alkaline pyran ring cleavage are shown to be important, especially at different levels according to the reaction conditions and type of tannin. For prodelphinidin tannins, radical mechanisms are shown to be more important than are ionic ones even under mild alkaline reaction conditions. For procyanidin tannins, radical mechanisms appear to be of little or no importance under mild alkaline reaction conditions, but acquire more importance at more alkaline pHs, a result confirmed also with a (+)-catechin monomer as a model compound. Under the latter conditions, the radical mechanism of pyran ring cleavage inverts the relative importance of the interflavonoid bond cleavage and pyran ring opening for procyanidins. Relative intensities of phenoxide radical and radical anion ESR signals indicate that pyran ring opening and autocondensation are rapid under alkaline conditions.
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INTRODUCTION

Thermosetting polyflavonoid tannin–formaldehyde resins have performed well in industrial applications as phenolic exterior adhesives for wood for the last 20 years.[1] Industrial polyflavonoid tannin extracts are composed mostly of flavan-3-ols repeating units and smaller fractions of polysaccharides and simple sugars.[1] Polyflavonoid in such tannin extracts presents phloroglucinol or resorcinol A-rings and catechol or pyrogallol B-rings:
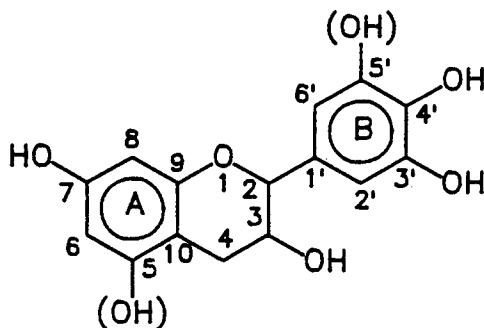
The repeating units are linked to each other as C4 — C6 or C4 — C8, the former predominating in tannins composed mostly of fisetinidin (resorcinol A-ring, catechol B-ring) and robinetinidin (resorcinol A-ring, pyrogallol B-ring). The C4 — C8 interflavonoid linkage instead greatly predominates in tannins 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/robinetinidin units, the polymers are, respectively, called profisetinidin/prorobinetinidin; when they are composed of catechin/gallocatechin, the polymers are called procyanidin/prodelphinidin, respectively.[2] The free C6 and/or C8 sites on the A-ring are the sites reactive with formaldehyde, due to their strong nucleophilicity, to form adhesives under the conditions of use of these materials.[1]

Recently, the autocondensation to hardened resins, without the use of any aldehyde, of polyflavonoid tannins induced by weak Lewis acids, in particular, silica and silicic acid, was described.[3,4] The mechanism of autocondensation was found to be based on the Lewis acid acceptance of electrons from the ether oxygen of the flavonoid unit pyran ring, with con-

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current and consequent facilitation of a base-induced heterocycle opening at the O1 — C2 bond.[3] The subsequent autocondensation of the reactive site formed (C2) with the A-ring of a flavonoid unit in another polymer chain leads to resin hardening.[3]

The base-induced pyran ring opening of flavonoids is well known.[5] The mechanism generally proposed[5] proceeds through ionization of the 4'-hydroxy group and B-ring quinone methide intermediates causing, e.g., epimerization[6-8] of the monomer model catechin to epicatechin and also rearrangement[9-14] of the former to catechinic acid.

More recently, however, it has been found that opening of the pyran ring of the monomeric model (+)-catechin at alkaline pH may proceed through a one-electron, radical mechanism[15] induced by the air singlet oxygen, rather than by a two-electron ionic mechanism.

The Lewis acid-induced autocondensation of tannins[3,4,16-19] indicated that an ionic mechanism appears to be present, but that a radical mechanism cannot be excluded. The present investigation was aimed at ascertaining directly on the polymeric tannins if a radical mechanism (i) is of importance in the silica-induced autocondensation of polyflavonoid tannins and if (ii) it occurs to a different extent in the silica-induced autocondensation of different types of flavonoid tannins.

EXPERIMENTAL

Tannin Extract Solutions

Tannin extract water solutions of 30% concentration were prepared by dissolving spray-dried powders of three commercial tannin extracts: pecan (*Carya illinoensis*) nut pith tannin extract (from the U.S.A.), pine (*Pinus radiata*) bark tannin extract (from Chile), and soluble sulfited quebracho (variety chaqueno, *Schinopsis balansae*) wood tannin extract (from Argentina) and mimosa (*Acacia mollissima*) bark extract (from South Africa). Solutions at pH 5, 9, and 12 were prepared. Solutions of (+)-catechin monomer were used as the control.

ESR

ESR spectra of the above solutions as such and with 10% fine SiO$_2$ powder were done. ESR spectra were recorded at 298 K on a Bruker ER 200 D spectrometer (X-band) equipped with a TE$_{102}$ sample cavity. The fine structure of the phenoxide radicals was

studied in the range 3465–3495 Gauss with a field modulation intensity of 0.8 and scan times of 500 s. The spectra under flux of nitrogen did only produce weak radical signals. The spectra in air, due to the requirement of the presence of singlet oxygen for the radical mechanism of pyran ring cleavage of flavonoids[15] to occur, are the ones reported. The tannin phenoxide radical signal was observed at 3482.4 Gauss and all had g = 2.003. Spectra were centered on the signal of DPPH as the standard[20] at 3483 gauss.

DISCUSSION

The ESR spectra at pH 9–10, without silica, of pecan nut tannin (a predominantly prodelphinidin-type tannin), of pine tannin (a procyanidin-type tannin), and of mimosa and sulfited soluble quebracho tannin (a profisetinidin/prorobinetinidin-type tannin) solutions show some interesting differences. In all of them, a clear, sharp phenoxide radical signal appears [Fig. 1(a)–(c)] in the identical position of the phenoxide signal of the monomer model (+)-catechin. For pecan nut and pine tannin, furthermore, an additional structure of six broad signals, distributed three by three symmetrically to the sharp phenoxide radical signal, also appear [Fig. 1(a) and (b)]. These six broad signals are not present in the sulfited quebracho tannin [Fig. 1(c)]. The presence of phenoxide signals would be expected if a radical
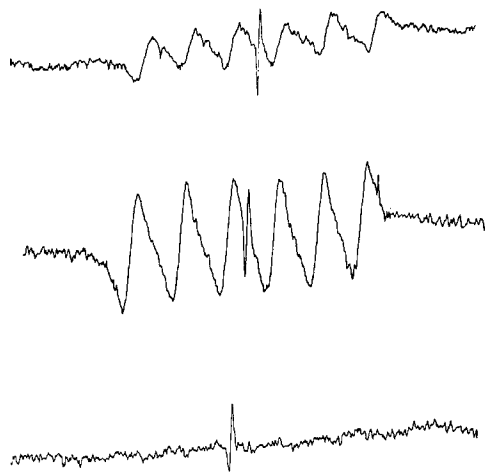

Figure 1 ESR spectra of polyflavonoid radical anions at pH 5 of (a) pecan nut tannin extract, (b) pine bark tannin extract, and (c) sulfited soluble quebracho wood tannin extract. Note the sharp phenoxide signal and the six symmetrical manganese bands for (a) and (b).

mechanism of the base-induced pyran cleavage is operative.[15] The six broad signals pattern is ascribed[20-22] and was checked to be due to the presence of Mn^{2+}. Electron spin resonance (ESR) is particularly sensitive to the presence of Mn^{2+} and this explains the intensity of the signal even if Mn^{2+} is present just in traces. The Mn^{2+} signals disappear at more alkaline pH due to Mn precipitation. Mn^{2+} is essential to the metabolism of many plants, although why it should be present particularly in two of the tannins is not known. In further ESR studies of tannins, Mn^{2+} might then be useful as an internal standard as regards the relative intensity of the phenoxide signal.

The sharp phenoxide signal is of considerably more interest. The presence of a phenoxide signal would be expected if a radical mechanism of the base-induced pyran cleavage reaction is operative.[15] The sharp phenoxide signal has been shown by model compound studies to belong to flavonoid radical anions formed in an alkaline environment.[12-15] At their natural pH, it is first of all of interest to compare the relative intensities of this signal for different tannins. Thus, pine tannin presents a signal that, at equal solution concentration, is double than that of pecan nut tannin and that of quebracho tannin. This might indicate that a more stable phenoxide radical is produced in procyanidins than in other tannins; it might also indicate, at the same time, that it is more difficult to increase or decrease radical concentration in procyanidins rather than in other tannins. This is consistent with the relative difficulty of procyanidin pyran ring opening in relation to other rearrangements.

The ESR spectra of the three tannins and of the monomer model (+)-catechin obtained 5 min after addition of finely powdered silica at pH 9 show considerable differences between the three tannins. In pecan nut tannin, the addition of silica has caused a strong surge of the phenoxide radical anion signal [Fig. 2(a)], while no changes have occurred for pine and quebracho tannins [Fig. 2(b) and (c)]. Thus, addition of silica at mildly alkaline pHs clearly indicate a marked increase in radical anions and in heterocycle pyran ring cleavage of prodelphinidins flavonoids. This again demonstrates that silica has then catalyzed a heterocycle ring opening, as already shown by other techniques.[3] It also means that at pH 8-9, in pecan nut tannin and in prodelphinidins, a radical mechanism of silica-induced autocondensation has considerable importance. It is not possible to deduce from the data available what the relative proportions of radical and ionic mechanisms could
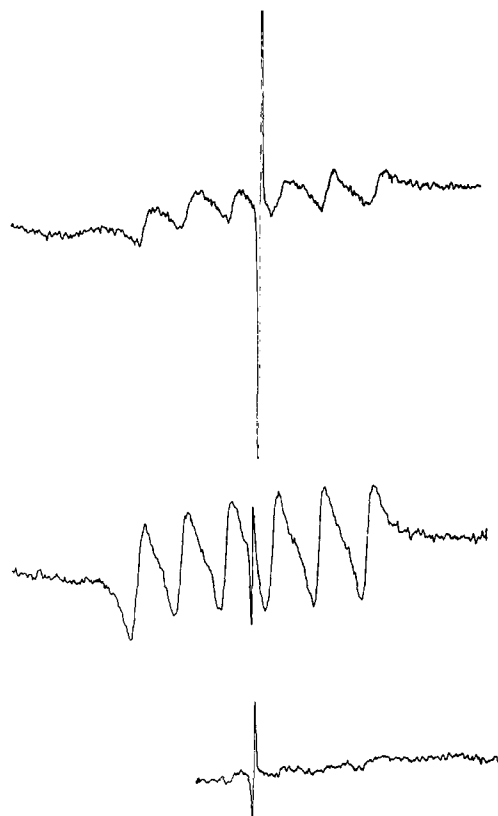

Figure 2 ESR spectra of polyflavonoid radical anions at pH 9 of (a) pecan nut tannin extract (note the marked surge in the sharp phenoxide signal in relation to the six symmetrical manganese signals), (b) pine bark tannin extract, and (c) sulfited soluble quebracho extract.

be, but the intense surge in the phenoxide signal is an indication that a radical mechanism under neutral and mild alkaline conditions is important for pecan nut tannin and prodelphinidins.

There is clearly no trace of a surge in radical anions for both pine and quebracho tannins. Since pine hardens by silica addition even at pHs as low as 8, this confirms previous findings that for these tannins an ionic mechanism is at least operative at pHs of 9 and lower.[3] In this regard, it is interesting to observe what occurs at more alkaline pHs. At pH 12, without any addition of silica, the ESR signal intensity for pine tannin is identical to that observed at lower pH [Fig. 1(b)]. However, on addition of SiO_2 to pine tannin at pH 12, the intense surge of the flavonoid signal, hence, in the proportion of the radical anion and of pyran ring opening, already observed at lower pH for pecan nut tannin, also occurs. This already starts to give some clear indications on what appears to occur. It is well known that in procyanidins, in an alkaline environment, interfla-

vonoid bond cleavage is facile (by an ionic mechanism) and pyran ring opening is a more difficult, less favorite reaction.[23] Thus, silica-catalyzed pyran ring cleavage by a radical mechanism appears to become the favorite reaction in procyanidin-type tannins such as pine. This confirms the already observed[3] inversion of the relative importance of pyran ring cleavage vs. interflavonoid bond cleavage in procyanidins, when silica is present. It also confirms the already presented[3] concept that the rate of catalysis (i) is pH dependent and (ii) it is faster for prodelphinidins, starting to show the effect at lower pH, than for procyanidins where it starts at higher pHs.

Of interest also is to observe the change in the fine structure of the phenoxide radical anion signal for the three tannins and for the monomer model (+)-catechin. The fine signal structure of pecan nut and quebracho tannins, in both of which a preponderant amount of pyrogallol B-rings is known to exist, resembles very closely at neutral pH the fine structure of pyrogallol phenoxides.[20,24,25] Thus, two triplets are present in the ESR spectra of pyragollol, pecan nut tannin, and quebracho tannin. These triplets represent then the radical anion signal of the flavonoid B-rings. Other signals, namely, two
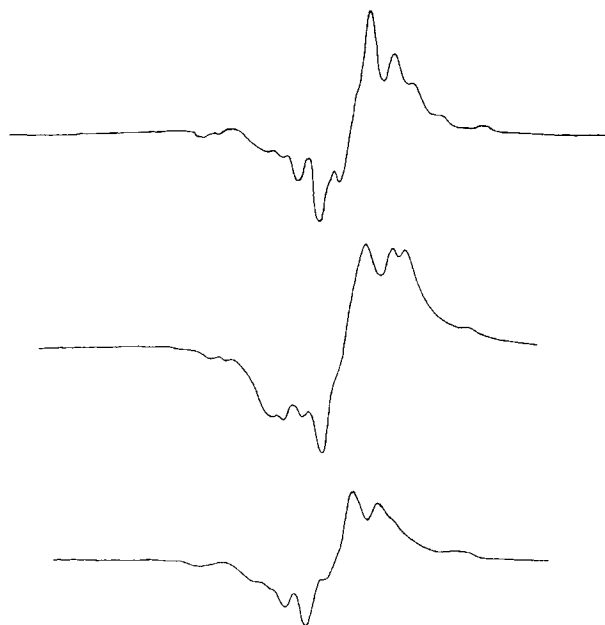

Figure 3 Fine structure of the sharp ESR phenoxide signal of the radical anions of a prodelphinidin tannin (pecan nut tannin extract solution) at pH 12: (a) by itself; (b) 5 min after addition of 10% fine silica powder; (c) 15 min after 10% silica addition. Note variation in the different signal intensities.
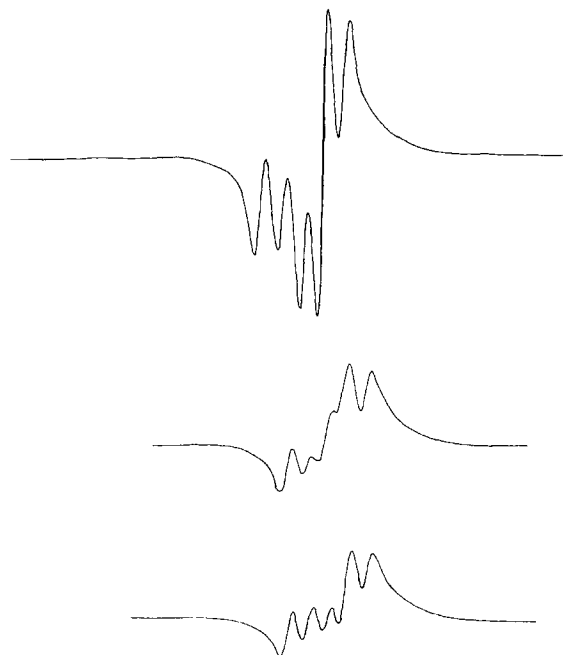

Figure 4 Fine structure of the sharp ESR phenoxide signal of the radical anions of a procyanidin tannin (pine bark tannin extract solution) at pH 12: (a) by itself; (b) 5 min after addition of 10% fine silica powder; (c) 15 min after 10% silica addition. Note variation in the different signal intensities and compare with Figure 6(a) and (b).

doublets in appearance [Figs. 3(a), 4(a), and 5(a)] are also visible for the tannins. In the case of pine tannin, the fine structure of the phenoxide signal appears to be more complex and very similar to that of the monomer model (+)-catechin [Fig. 6(a)]. In the latter, it is clear that two sets of overlapping triplets occur, the structure resolving itself in the two intense doublets nearest to the center of the ESR spectra, again representing the B-ring radical anion, and in the low-intensity doublet furthest away from the center of the spectra. Part of the triplets' overlap is shown, particularly in the catechin monomer case by a fifth low-intensity signal between the two doublets in the spectra. Pine tannin shows the same pattern of signals of the catechin monomer, but the polymeric nature of the tannin and consequent broadening of the bands renders it impossible to see this fifth peak.

On addition of silica, the relative intensities of the signals shift considerably. The high-intensity triplet (doublet in pine and catechin) decreases considerably in intensity while the signals further away from the center of the spectra increase considerably in intensity. This is particularly evident in the catechin monomer and in pine tannin where

the further away signals and the near signals invert in intensity [Fig. 4(b), 4(c), and 6(b)]. In pecan [Fig. 3(b) and (c)], mimosa [Fig. 5(a) and (b)], and quebracho [Fig. 5(c)] tannins, the same trend is evident but not to the point of presenting an inversion in intensity of the two sets of signals. The radical opening of the pyran ring of a flavonoid is known to go through the following mechanism[15]:
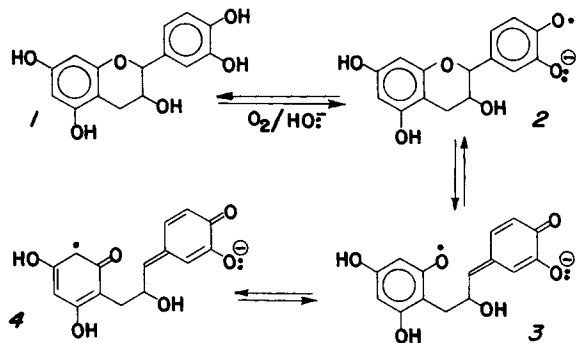
As the spectra near-center signals have been shown to belong to the radical anion in which the phenoxide radical is on the B-ring, this set of signals are then indicative of the relative abundance of species 2 in the system. The far spectra signals which clearly belong to another type of radical anion can then be assigned to species 3. Under neutral, mildly acid, and mildly alkaline conditions, the $2 \rightleftarrows 3$ equilibrium is considerably and always more shifted in favor of species 2. Increase of the pH to a more alkaline environment, while for some tannins increasing the total phenoxide signal intensity (pecan nut) and not for other tannins (pine and quebracho), leaves unaltered the relative intensities of the peaks which compose the phenoxide signal fine structure. Thus, just an increase in pH does not change the relative proportions at equilibrium of species 2 and 3. The marked inversion (pine and catechin) in fine structure signals on addition of SiO_2 indicates that addition of silica has caused a considerable increase in the relative proportion of radical anion 3 at the expense of radical anion 2. This clearly confirms that addition of silica induces a marked increase in the flavonoid pyran ring opening. How the variation in the relative proportions of species 2 and 3 occurs is open to debate. It might occur in two ways: (i) the equilibrium $2 \rightleftarrows 3$ is markedly and rapidly shifted to the right or (ii) the additional opening of the pyran ring does not pass through species 2 when silica is present. Both mechanisms might coexist. However, the absence of radical 4 observed by ESR and the absence of rear-
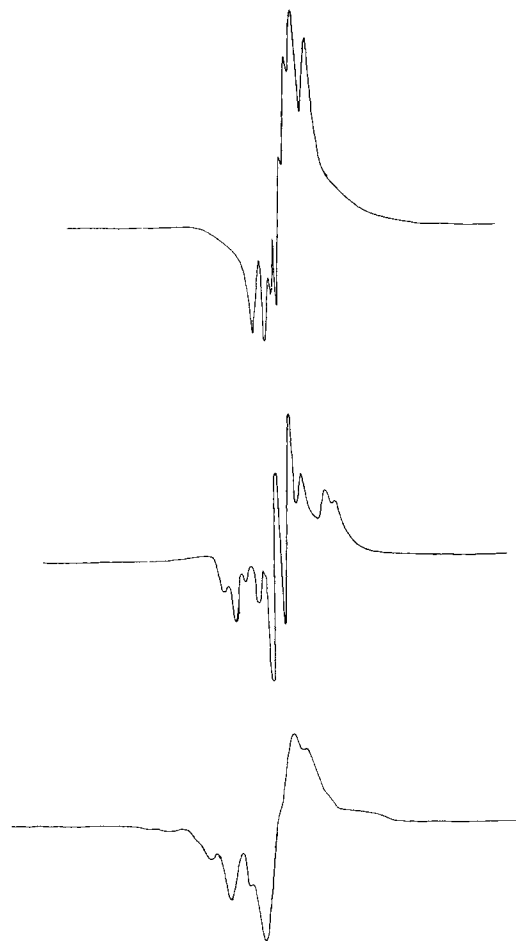
Figure 5 Fine structure of the sharp ESR phenoxide signal of the radical anions of profisetinidin/prorobine-tinidin tannins (mimosa and sulfited quebracho tannin extracts) at pH 12: (a) mimosa tannin by itself; (b) mimosa tannin 10 min after addition of 10% fine silica powder; (c) sulfited quebracho tannin by itself.

rangements to catechinic acid and phlobatannins, already confirmed by ^{13}C-NMR for SiO_2 hardened tannins,[3] indicate that the second mechanism above is superimposed over the normal radical mechanism of the base-induced pyran ring opening. It indicates that SiO_2-induced pyran ring cleavage and opening proceeds mainly through a different mechanism: a direct attack of the SiO_2 on the O1 or C2 or both of the flavonoid unit. This mechanism is superimposed to the normal base-induced radical reaction which clearly still occurs and which also further favors the SiO_2 attack. That radical 4 does not form or forms only to a minimal amount when silica is present is also confirmed by the very low proportion and often absence of any direct C — Si bonds as confirmed by ^{29}Si-NMR.[3] The result also confirms that while in
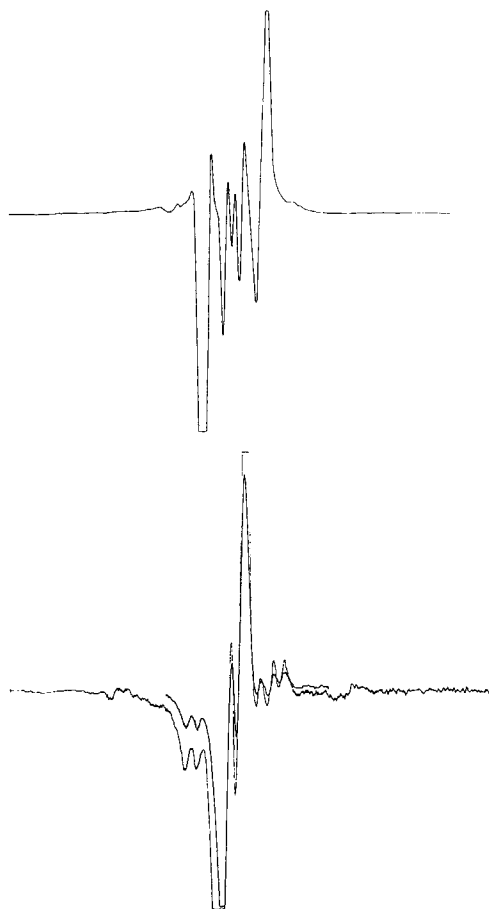
Figure 6 Fine structure of the sharp ESR phenoxide signal of the radical anions of the monomer model compound (+)-catechin at pH 12: (a) by itself; (b) 5 min after addition of 10% fine silica powder. Note variation in the different signal intensities and compare with the effect in procyanidin tannins (Fig. 4).

can nut tannins can still be resolved in their characteristic signal structure.

Quebracho and mimosa tannins appear to behave mostly as pecan nut tannin, confirming their high proportion of pyrogallol B-rings. Their rate of hardening is clearly slower[3] due to both the lower reactivity of their A-ring and the intensity of the radical mechanism appearing to be lower. This is again an indication that an ionic mechanism of autocondensation also appears to be at play—as already deduced by other techniques,[3] the lower nucleophilicity of the profisetinidin/prorobinetinidin tannins' A-rings having a hand in this—and that in heavily sulfited soluble quebracho,[3,26-28] a greater proportion of pyran rings are already open by the sulfitation process and fixed in such open configuration by insertion of a sulfonic group at C2.[26] Much less autocondensation is then possible for the latter tannin, and this at much higher pH, as already confirmed by sulfited soluble quebracho being by far the slowest gelling tannin by silica-induced autocondensation.

It is also worthwhile to again address the presence of Mn^{2+} in two of the tannins examined. It is well known that divalent metallic ions accelerate phenolic polycondensations by well-defined mechanisms.[29-33] Mn^{2+} is one of the metal ions showing this effect: Its presence in the two faster reacting tannins might also contribute to the second step of silica-induced tannin autocondensation (the autocondensation proper[3]), namely, the attack of the

procyanidins the inversion of the favorite reaction ensues when silica is present, in prodelphinidins, pyran ring opening is always, even in the absence of silica, the favorite reaction.

The above explains the existence of different trigger pHs and different rates of silica-induced hardening observed for pine and pecan nut tannins and, hence, for procyanidins and prodelphinidins, which have been observed experimentally.[3] It is also of interest to note that (a) the intensity of the phenoxide radical anion signal decreases considerably over a period of 2 h at ambient temperature (the case of pine tannin is illustrated in Figure 7, indicating that the crosslinking reaction is rapid, as observed experimentally,[3] and that (b) after 2 h reaction, the phenoxide radical signals of pine and pe-
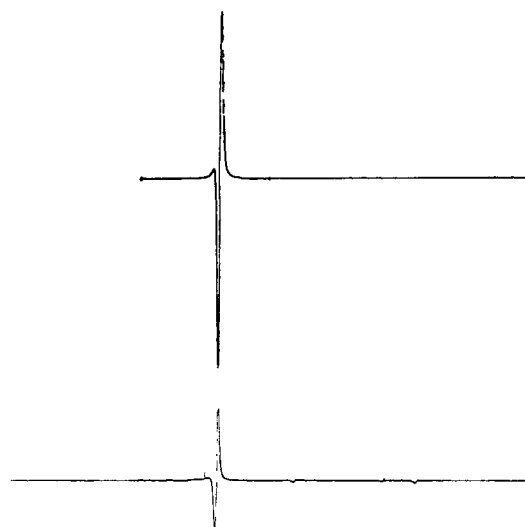

Figure 7 Variation of the relative intensity of the sharp phenoxide signal of a procyanidin tannin (pine bark tannin extract solution) at pH 12 after addition of 10% fine silica powder (a) after 15 min and (b) after 2 h from addition.

reactive C2 formed by pyran ring opening on a fla-
vonoid unit belonging to another tannin chain. This
again would confirm the presence of an ionic mech-
anism superimposed on the radical one described.

CONCLUSIONS

In conclusion, radical mechanisms are important,
especially at different levels according to conditions
and type of tannin, in the first step of the silica-
induced autocondensation and hardening of tannin
extracts solutions. In the case of prodelphinidins
such as pecan nut tannin, the radical mechanism
appears to be more important than is the ionic one
even under mild alkaline reaction conditions. For
procyanidins such as pine tannin and for the cate-
chin monomer model, the radical mechanism is of
little or no importance under mild alkaline condi-
tions while acquiring predominant importance at
more alkaline pHs. In procyanidins when the radical
mechanism of pyran ring opening becomes impor-
tant, it also ensures the inversion in the most fa-
vorite reaction from interflavonoid bond cleavage
(an ionic reaction[23]) to pyran ring opening followed
by interflavonoid autocondensation. In heavily sul-
fited tannins, such as soluble quebracho tannin ex-
tract, the pyran ring-opening mechanism has lower
importance, because, among others, a great propor-
tion of the pyran rings has already been opened and
fixed in such an open conformation by the reaction
of sulfitation: This leaves room for autocondensation
only under very alkaline conditions and at much
slower rates. In the case of profisetinidin/prorobi-
netinidin tannins, their facile ring opening and sta-
ble interflavonoid bond[34] also indicates that their
behavior will be more similar to prodelphinidins
than to procyanidins, autocondensation being
slightly less rapid due to the lower nucleophilicity
of their A-rings. An ionic mechanism is also clearly
superimposed to radical-based ones. Both shifting
of the equilibrium between two radical anion species
as well as the formation of one of these species by
direct silica attack indicates that in the first step of
silica-induced tannin autocondensation the main
radical mechanism by direct silica attack on the
heterocycle is still superimposed on the normal base-
induced radical anions' equilibrium. The existence
of silica-induced autocondensation might also reflect
on the autocondensation to a higher degree of po-
lymerization of tannins in the plant, in vivo, in spe-
cies where a high concentration of silica is known
to exist in the tree, such as mimosa and quebracho.

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