

Precipitation of Metal Ions by Plant Polyphenols: Optimal Conditions and Origin of Precipitation

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Precipitation of copper(II) and zinc(II) by low molecular weight phenols, penta-*O*-galloyl- β -D-glucose, and commercial tannins is studied at pH 5. The extent of copper precipitation, higher than that of zinc, depends on the initial concentration of both copper and phenolic compounds and on the control of acidification which results from complexation. The copper/phenol ratio in the precipitate, determined by elemental analysis, is independent of the initial concentration of catechol. However, it increases with chestnut tannin when the metal concentration is increased to a value corresponding to complexation with all *o*-dihydroxyphenyl chelating groups in the molecule. Stability of the precipitates obtained with copper(II) and various polyphenols in water, acetic acid, and ethylenediaminetetraacetic acid solutions is determined. Models for metal/polyphenol precipitation are proposed.

Keywords: Polyphenols; tannins; metal ions; copper; zinc; complexation; precipitation

INTRODUCTION

Proanthocyanidins (condensed tannins) and ellagitannins are among the most widespread polyphenol extractives in plants (Haslam, 1989). They often accumulate in large quantities in leaves, fruits, wood, or bark and contribute to the defense of the plant against herbivores, pathogens, and rots (Haslam, 1988; Scalbert, 1991). Previously, emphasis has been put on the study of their complexation properties with proteins to explain their protective properties.

Yet, most tannin molecules contain *o*-dihydroxyphenyl chelating functional groups and form stable complexes with many metal ions (Kennedy and Powell, 1985; Okuda et al., 1982; Slabbert, 1991; Powell and Rate, 1987). Recently, it has been suggested that complexation with metal ions required for microorganism growth such as iron(III) may be as important for plant defense against biotic aggressors as protein complexation (Scalbert, 1991; Mila and Scalbert, 1994). These properties are also of great importance in human nutrition as they contribute to the reduction of gastrointestinal absorption of some essential metal ions (Brune et al., 1989).

Numerous uses of tannins or tannin-containing materials in industry also rely on complexation of metal ions. These include anticorrosive primers for steel (Seavell, 1978) and non-ferrous metals (Sampat and Vora, 1975); modifiers of rheological properties of minerals and clays (Chang and Anderson, 1968) with applications in mineral flotation, ceramic, cement, and drilling industries; agents for metal ion recovery from waste waters (Randall et al., 1974); and fixation agents of metal micronutrients in foliar sprays (Durkee, 1965).

The blue-black color of iron(III)/tannin complexes has been at the origin of processes for dyeing leather (Meunier and Vaney, 1903), clothes, hair, etc.; tannins and iron have also been the main components of writing inks (Grimshaw, 1976) for several centuries.

Tannin/metal complexes often precipitate in water (Okuda et al., 1982). This property is best illustrated

by the washing fastness of tannin/iron dyes and inks, by the development of tannin–aluminum tannage (Sykes et al., 1980; Slabbert, 1981), or by the use of tannins in wood preservation treatments to fix fungicidal copper(II) ions (Laks et al., 1988).

In this paper, various parameters affecting the precipitation of copper(II) and zinc(II) by tannins and related low molecular weight phenols are studied with the objective of optimizing copper and zinc fixation in wood preservation treatments. The factors governing their insolubilization are discussed. Experiments were carried out at pH 5 for various reasons: most woods are naturally acid with a pH varying usually between 4 and 5 (Gray, 1958); in experiments with Al(III) and gallic acid, pH 5 was shown to be within the optimal range for precipitation (Öhman and Sjöberg, 1982); hydrolysis of copper is negligible at this pH (Steinhaus and Barsuhn, 1974) and autoxidation of phenols is limited.

EXPERIMENTAL PROCEDURES

Chemicals. Commercial tannins had the following origins: chestnut tannin, "N" Isoroy; quebracho tannin, Unitan ATO; pecan tannin, Px Inc. Other reagents were of reagent grade from either Fluka [tannic acid, gallic acid, (+)-catechin, catechol, *p*-hydroxybenzoic acid] or Merck (pyrogallol). Penta-*O*-galloyl- β -D-glucose was prepared by methanolysis of tannic acid (Merck) (Haslam et al., 1961; Haddock et al., 1982). *Pinus pinaster* proanthocyanidins were prepared by MeOH/water extraction of bark and chromatography of the aqueous soluble phase on Sephadex LH 20, a classical method for tannin purification (Hagerman and Butler, 1980). The good purity of the sample was confirmed by the value obtained for the vanillin/HCl assay ($E1\% = 166$) (Czochanska et al., 1980).

Metal Ion Precipitation by Polyphenols. Unless explicitly mentioned, the conditions are as follows: CuCl_2 or ZnCl_2 (25 mM), tannin (10 g/L), and 0.05 M acetate buffer, pH 5 (total volume 2 mL) are left stirring at room temperature for 2 days. Metal ion precipitation by *o*-dihydroxyphenyl derivatives is known to be a slow process (Öhman and Sjöberg, 1982). The maximum of copper precipitation was reached within 2 h for chestnut tannin, tannic acid, and gallic acid and within 1 day for pecan tannin and 2 days for quebracho tannin. A 2-day stirring duration was finally chosen. The mixture is then centrifuged. An aliquot of the clear supernatant diluted with H_2O is analyzed for Cu or Zn by atomic absorption spectroscopy ($\lambda = 324.7$ or 213.9 nm, respectively). Phenols

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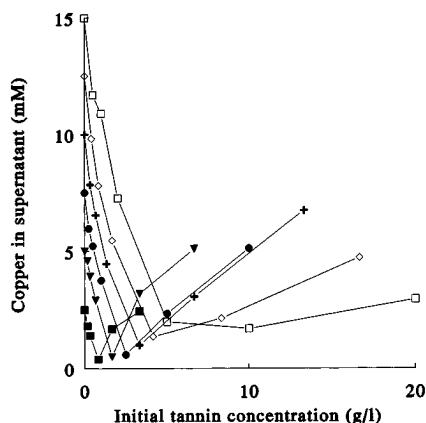


Figure 1. Copper precipitation by chestnut tannin with various initial copper concentrations.

in the supernatant were determined according to the Folin–Ciocalteu assay (Scalbert et al., 1989).

Elemental Analysis of the Precipitates. Carbon, oxygen, hydrogen, and copper were analyzed in the freeze-dried precipitates at the Service Central d'Analyse (CNRS, Vernaison). The copper/polyphenol ratio was calculated from the carbon and copper contents of the precipitates. The molecular weight of castalagin was used for calculation of molar ratios in chestnut tannin/copper precipitates. For *P. pinaster* bark extract, calculation of the molar ratios was based on the molecular weight of an epicatechin unit, the major unit in the proanthocyanidins (S. Matthews, I. Mila, and A. Scalbert, unpublished results).

Solubilization of Copper from the Precipitates. After precipitation in the conditions described above except for the polyphenol concentrations (chestnut, 15 g/L; tannic acid, 10 g/L; gallic acid, 5 g/L; catechol, 10 g/L), the supernatant was removed. Fresh solutions of either H₂O, acetic acid (AcOH), AcOH/H₂O 1:1, or aqueous ethylenediaminetetraacetic acid (EDTA) (2 mL) were added to the precipitates. The mixtures were ultrasonicated for 5 min and recentrifuged, and the supernatant was removed. This process was repeated three times altogether. Copper was determined in the three supernatants and the percentage of copper solubilized from the precipitate calculated.

RESULTS

Influence of Reagent and Buffer Concentrations on Copper Precipitation by Tannins. To compare polyphenols for their ability to precipitate copper, initial experiments were carried out to study the influence of certain parameters on metal precipitation.

Copper and Tannin Initial Concentrations. The extent of copper precipitation is dependent on both copper and tannin concentrations. For a given initial copper concentration, there is an optimal tannin concentration at which a maximum of precipitation is observed (Figure 1). When the initial copper concentration is increased, the amount of tannin required to reach the maximum of precipitation also increases.

For an initial copper concentration of 15 mM or less, chestnut tannin precipitated a constant amount of copper independently of the initial copper concentration (Figure 2). At higher copper concentrations, the capacity of tannins to precipitate copper was lower because of an insufficient buffer concentration to counteract acidification following complexation. In this case, the pH dropped from 5 to 3.5 upon complexation. The influence of pH control during complexation was further studied by varying the buffer concentration.

Buffer Concentration. In the absence of buffer, the pH before copper addition reflects the natural acidity

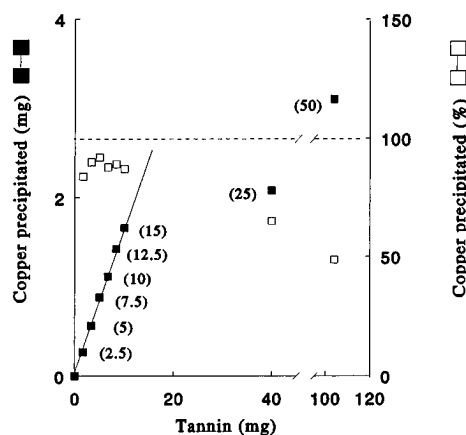


Figure 2. Yield of copper precipitation per weight unit of chestnut tannin and percentage of copper found in the precipitate. Figures in parentheses indicate the initial copper concentration (mM) used for the determination of each point. The tannin scale is expressed as the amount of tannin added to 2 mL of copper solution. All data presented correspond to experiments in which the initial copper/tannin ratio is optimal for copper precipitation.

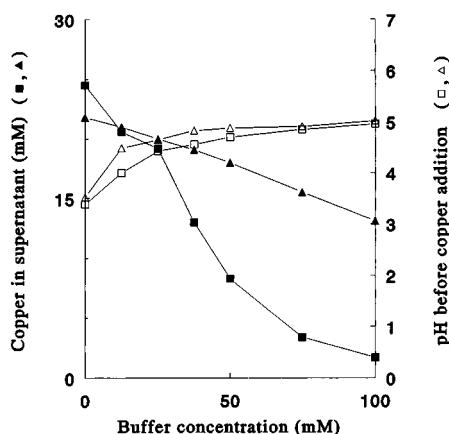


Figure 3. Effect of buffer concentration on initial pH before copper addition and on copper precipitation by chestnut tannin: (■, □), acetate buffer; (▲, △), pyridine buffer.

of the tannin extract. When acetate buffer is added and as its concentration increases, the initial pH of the tannin solution increases up to the buffer pH (Figure 3). Copper precipitation increases with an increase in buffer concentration. The same effect is observed when acetate buffer is replaced by pyridine buffer. This is not due to an increase in ionic strength as addition of NaCl (up to 0.1 M) had no influence on precipitation.

Some differences are observed between the two tested buffers. For a given buffer concentration, copper precipitation is higher with acetate buffer. This is not due to a kinetic effect as precipitation reached its maximum within less than 12 h for both buffers. The difference between the two buffers is more likely explained by the higher pK_a of pyridine (5.23) as compared to that of acetic acid (4.76), pyridine having a lower buffering capacity as the pH tends to decrease upon copper complexation. However, a possible influence of the buffer anion which may form weak complexes with copper cannot be excluded.

Precipitation of Copper by Commercial Tannins and Pure Polyphenols. Two hydrolyzable tannins, chestnut tannin and tannic acid, and two condensed tannins, pecan and quebracho tannins, were compared. For three of these tannins, a maximum of copper precipitation was observed at similar tannin concentra-

Table 1. Elemental Analysis of Copper/Polyphenol Precipitates

ligand (L)	precipitation		precipitate			
	initial L concn (g/L)	initial Cu/L molar ratio	elemental analysis (% dry wt)		Cu/L molar ratio	Cu/ <i>o</i> -dihydroxyphenyl group
			C	Cu		
catechol	1.00	2.8	39.6	19.4	0.6	0.6
	5.00	0.6	42.1	16.9	0.5	0.5
	10.00	0.3	45.2	13.9	0.4	0.4
(+)-catechin	1.25	5.8	44.5	7.7	0.5	0.5
gallic acid	2.50	1.9	29.0	31.0	1.4	1.4
penta- <i>O</i> -galloyl- β -D-glucose	1.25	18.8	28.5	15.0	3.9	0.8
chestnut tannin	2.50	9.3 ^a	30.5	18.0	4.6 ^a	0.9 ^a
	5.00	4.7 ^a	32.6	16.2	3.9 ^a	0.8 ^a
	15.00	1.6 ^a	38.2	9.1	1.8 ^a	0.4 ^a
pine tannin	4.0	1.8 ^b	43.3	5.1	0.9 ^b	0.9 ^b

^a L as castalagin (M = 934) for calculation. ^b L as procyanidin unit (M = 288) for calculation.

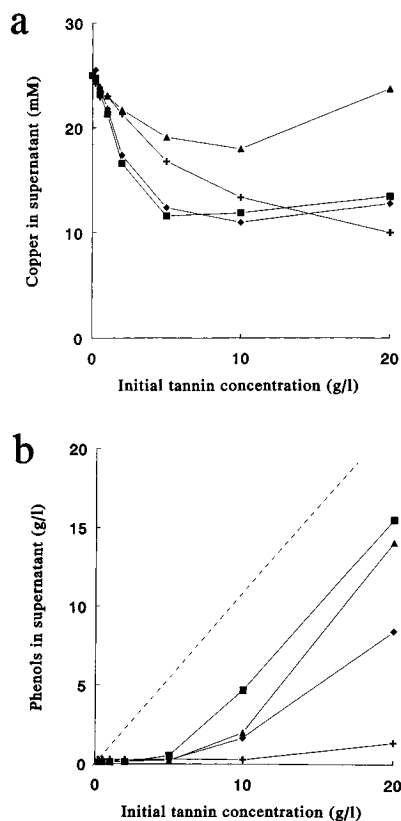


Figure 4. Copper/tannin precipitation with various commercial tannins [(a) copper in supernatant; (b) phenols in supernatant]: (◆) chestnut tannin; (■) tannic acid; (▲) quebracho tannin; (+), pecan tannin.

tion, and when an excess of tannin is added, an increasing part of copper (Figure 4a) and all of the excess tannin (Figure 4b) remain in solution. With pecan tannin, the maximum of precipitation was not reached at the highest tannin concentration tested and all of the tannin was precipitated.

The four commercial tannins also differ in the proportion of copper precipitated. The highest precipitation yield is obtained with pecan tannin as far as a sufficient quantity of tannin is used. Quebracho tannin gives the lowest yield of copper precipitation, and the two hydrolyzable tannins give intermediate results.

To determine the influence of polyphenol structure on copper precipitation, precipitation was studied using penta-*O*-galloyl- β -D-glucose (**1**) and various low molecular weight phenols. The best yield of copper precipita-

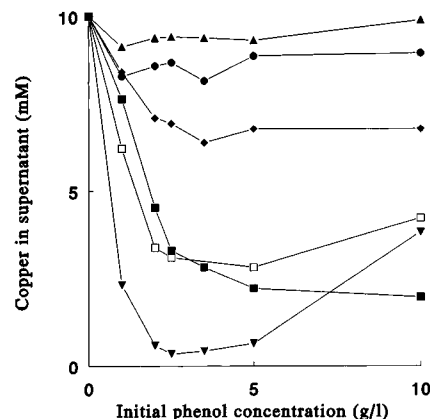


Figure 5. Copper precipitation by pure polyphenols: (▼) penta-*O*-galloyl- β -D-glucose; (■) pyrogallol; (□) gallic acid; (◆) catechol; (●), (+)-catechin; (▲) *p*-hydroxybenzoic acid.

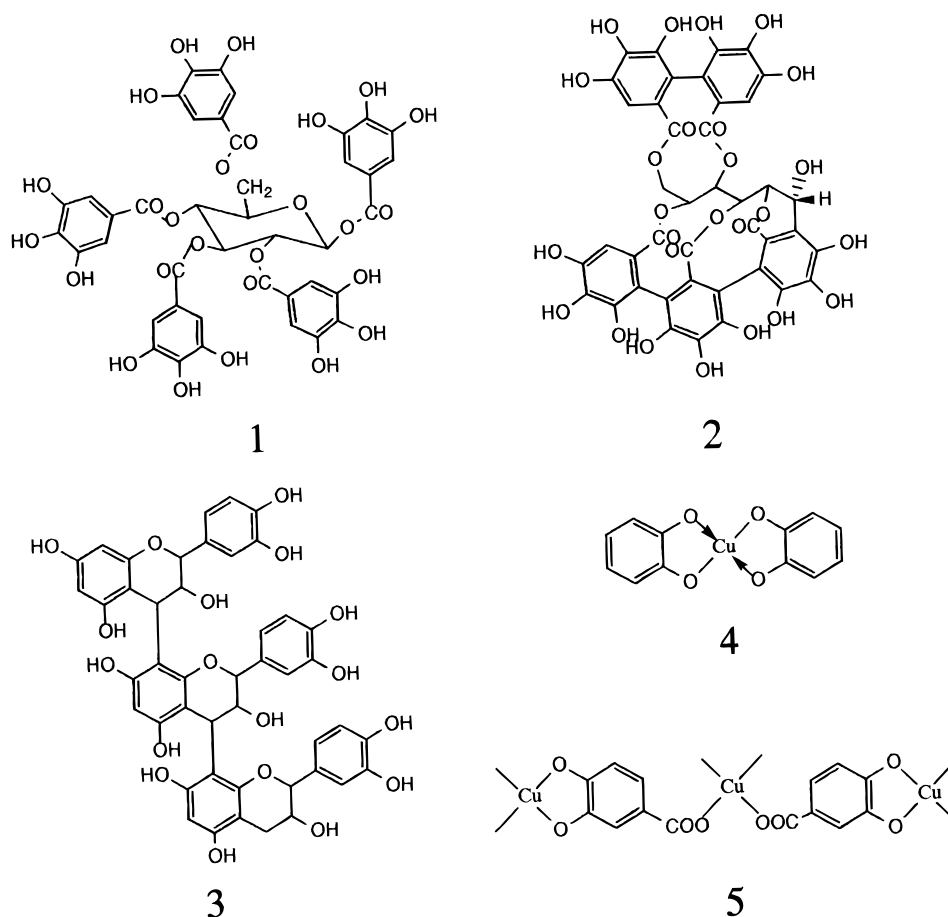
tion was observed with penta-*O*-galloyl- β -D-glucose (Figure 5). Pyrogallol and gallic acid also precipitate high amounts of copper, whereas catechol and (+)-catechin did not precipitate more than 35% copper. No precipitation was observed with *p*-hydroxybenzoic acid.

Precipitation of Zinc by Commercial Tannins and Pure Polyphenols. Precipitation of zinc by commercial tannins was examined using the same experimental conditions as for copper. No more than 20% zinc could be precipitated by either tannic acid or chestnut, quebracho, or pecan tannins with tannin concentrations ranging from 1 to 20 g/L. Using chestnut tannin, this yield of precipitation was only slightly increased to a value of 27% when the buffer concentration was increased to 0.2 M.

Pure polyphenols were also tested for zinc precipitation. No significant quantities of zinc could be precipitated by either penta-*O*-galloyl- β -D-glucose (**1**), pyrogallol, gallic acid, catechol, (+)-catechin, or *p*-hydroxybenzoic acid.

Stoichiometry of the Insoluble Copper/Polyphenol Complexes. Elemental analysis was carried out on copper-polyphenol precipitates. The ligand/copper ratio in the precipitate varies with the number of coordination groups (*o*-dihydroxyphenyl or carboxyl) within the ligand. Catechol and (+)-catechin with one *o*-dihydroxyphenyl group per molecule form insoluble complexes with two molecules of ligand and one copper ion (Table 1). Gallic acid with one extra carboxyl group forms complexes with two ligands and three copper ions. Penta-*O*-galloyl- β -D-glucose (**1**) with five *o*-dihydroxyphenyl groups forms insoluble complexes with four

Chart 1

**Table 2. Solubilization of Copper from Copper/Polyphenol Precipitates (Percent)**

	chestnut tannin	tannic acid	gallic acid	catechol
H ₂ O	17	9	5	12
AcOH	34	38	8	53
AcOH/H ₂ O 1:1	50	69	20	68

copper ions. Chestnut tannin, the main compound of which is castalagin (**2**) (Viriot et al., 1994) and closely related to penta-*O*-galloyl- β -D-glucose, gives a similar copper/polyphenol ratio in the precipitate as long as no excess of tannin is added. Procyanidins (**3**) form an insoluble complex having about one copper atom per monomeric unit. (Compound structures are given in Chart 1.)

Further precipitates were prepared with chestnut tannin and catechol with various initial ligand/copper ratios to assess the influence of this ratio on the composition of the precipitate. With chestnut tannin, the proportion of copper in the precipitate increases with the initial copper concentration (Table 1). With catechol, variation in the initial concentration of copper does not affect the composition of the precipitate.

Stability of the Polyphenol/Copper Complexes. Attempts to solubilize copper from tannin/copper precipitates with water, acetic acid, aqueous acetic acid, or EDTA were carried out with chestnut tannin and tannic acid. Less than 17% of copper in the precipitate is solubilized by water (Table 2). This percentage increases up to 69% when aqueous acetic acid is used. Even more copper can be solubilized with aqueous EDTA solutions. When the EDTA concentration was raised to 100 mM, 84% copper could be solubilized

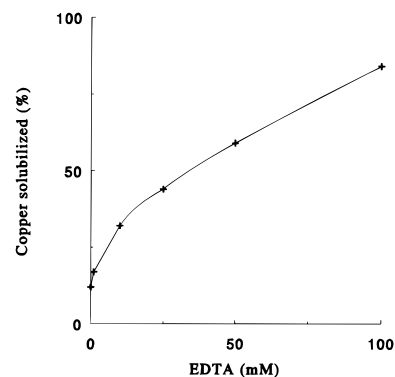


Figure 6. Dependence of ethylenediaminetetraacetic acid concentration on copper solubilization from copper/chestnut tannin precipitate.

(Figure 6). Similar results were obtained with catechol (Table 2). Gallic acid complexes were much more stable.

DISCUSSION

Stoichiometry and Origin of the Insolubility of the Complexes. Complexation of metal ions could influence solubility of both phenol and copper through (i) formation of an uncharged complex from the metal cation, (ii) reduction of the polarity of the tannin molecule, and (iii) formation of high molecular weight complexes. Two factors may contribute to the formation of high molecular weight complexes, either linear or branched:

(1) Metal ions such as copper(II) catalyze *o*-dihydroxybenzene oxidation by molecular oxygen at a pH of 4.5–5.5, at which uncatalyzed autoxidation does not occur

(Balla et al., 1992). The resulting quinones or semiquinones may polymerize and precipitate with copper ions.

(2) Polyfunctional tannins with several *o*-dihydroxyphenyl functional groups per molecule may also form high molecular weight complexes in the absence of oxidation reactions. Each tannin molecule may bind two or more metal ions, and each metal ion [copper(II) and zinc(II) have coordination numbers of 4] may form chelates with *o*-dihydroxyphenyl groups belonging to two different tannin molecules.

Determination of the proportion of copper (M^{2+}) and ligand (L) in the precipitate gives further insight to the origin of the insoluble complex formed (Table 1).

With catechol, the copper/catechol stoichiometry in the precipitate suggests the formation of a complex between two catechol molecules and one copper atom. It cannot be the bis(catecholato) complex $L_2MH_{-4}^{2-}$ (Aplincourt et al., 1987) as its negative charge would prevent its precipitation. Oxidation of catechol by molecular oxygen may lead to the formation of semiquinones which form neutral complexes with copper or zinc (4), insoluble in all common solvents and water (Röhrscheid et al., 1966; Abakumov et al., 1981). Polymerization of quinones or semiquinones could also explain the formation of a precipitate. Further support for the role of oxidation was given by the absence of any recognizable catechol in the acetic acid extract of catechol/copper precipitate.

With (+)-catechin, the same stoichiometry suggests the formation of a similar semiquinonic or quinonic complex. A study of copper(II) interactions at pH 4 by means of HPLC has shown the instantaneous formation of a catechin/copper complex which disappears with time (over 30 min) with the concomitant appearance of a catechin oxidation product only formed in the presence of copper (Weber, 1990).

Gallic acid differs from catechol by the presence of an additional carboxyl group which can also complex copper (Öhman and Sjöberg, 1981). The stoichiometry of the gallic acid/copper precipitate suggests the formation of the uncharged $M(LH_{-3})M(LH_{-3})M$ complex (5). Formation at pH around 4 of a similar insoluble complex with two 3,4-dihydroxybenzoic acid and three copper(II) ions has been reported (Gerrega et al., 1987). Chain binary complexes between gallic acid and Al(III) were also shown to form when gallic acid and Al(III) were mixed in equimolar concentrations and as long as the concentrations were high enough (Öhman and Sjöberg, 1982).

Penta-*O*-galloyl- β -D-glucose (1) has five *o*-dihydroxyphenyl chelating groups. The ratio Cu/L found in the precipitate (3.9) suggests the formation of either a high molecular weight complex of the type $M[(LH_{-10}M_3)M]_n^{(2n-2)-}$ (Figure 7a) or a nonpolymeric and uncharged complex $LH_{-8}M_4$ (Figure 7b). The present data do not allow differentiation between these two forms.

With commercial tannins, the number of metal atoms per *o*-dihydroxyphenyl group in tannin-copper precipitates (Table 1) varies within the same range as that reported previously for quince tannin: 0.4, 0.5, and 0.8 for Cu(II), Al(III), and Fe(III), respectively, at pH 6.8 (Kennedy and Powell, 1985) or 0.2 for Al(III) at pH 6 (Powell and Rate, 1987). However, the present study stresses the dependence of the initial metal/tannin ratio on the stoichiometry of the insoluble complex.

Chestnut tannin, a mixture of castalagin (2) and other related ellagitannins (Viriot et al., 1994), behaves very

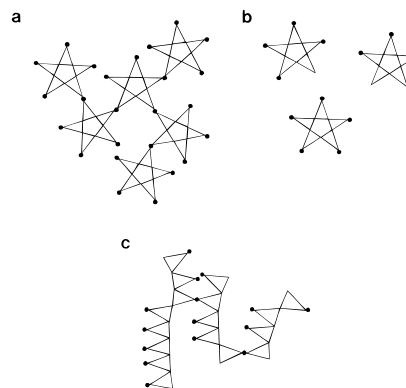


Figure 7. Proposed structure for polyphenol/metal ion precipitates: (●) metal; (△) *o*-dihydroxyphenyl group in a tannin molecule; (a, b) gallotannins and ellagitannins; (c) proanthocyanidins.

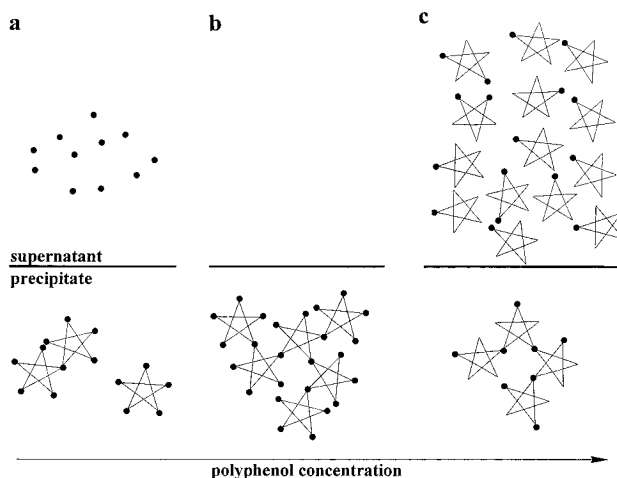


Figure 8. Schematic view of the influence of tannin concentration on metal ion precipitation: (●) metal; (five-arm star) tannin with five *o*-dihydroxyphenyl groups. Tannin concentration: (a), low; (b), intermediate; (c), high. Copper and tannin ratios are those reported in Table 1 for chestnut tannin.

much like gallotannins in terms of copper precipitation. The Cu/L ratio found in precipitate is similar to that measured with penta-*O*-galloyl- β -D-glucose. Very similar copper precipitation curves were obtained with chestnut tannin and tannic acid (a mixture of galloyl esters related to penta-*O*-galloyl- β -D-glucose) (Figure 4a). Similar structures for insoluble castalagin/copper complexes and penta-*O*-galloyl- β -D-glucose/copper complexes can be inferred. The structure of castalagin differs from penta-*O*-galloyl- β -D-glucose by three biphenyl bonds and the loss of six hydrogens. The presence of these biphenyl bonds imposes restrictions in conformational mobility which were shown to affect the ability to form complexes with macromolecules such as proteins (Spencer et al., 1988). However, the present results show that it does not affect the ability to precipitate metal ions, probably because the small metal ions reach the functional groups of polyphenols more easily than the interacting groups of proteins.

Copper precipitation (Figures 1 and 4) by commercial tannins and the composition of the precipitate (Table 1) are greatly influenced by the copper and tannin relative concentrations. If for a given copper initial concentration the chestnut tannin concentration is reduced below a value corresponding to the maximum of copper precipitation (Figure 8b), the yield of copper precipitation is reduced and the Cu/L ratio in the precipitate increases slightly to give a value

that corresponds to an average complex structure $(LH_{-10}M_4)M(LH_{-10}M_4)^{2+}$ (Figure 8a). If the chestnut tannin concentration is increased, yields of both copper and tannin precipitation are reduced and the Cu/L ratio in the precipitate is reduced to a value of 1.8, which could correspond to a polymer such as $M[(LH_{-6}M)M]_n^{(2n-2)-}$ (Figure 8c) or to a monomer $LH_{-4}M_2$. However, it is more doubtful that complexation of two *o*-dihydroxyphenyl groups among the five present in castalagin would be enough to induce its precipitation.

The Cu/L ratio (0.9) in precipitates formed with procyanidins L_n purified from *P. pinaster* bark suggests that all *o*-dihydroxyphenyl groups form chelates with copper. The structure of the chelate would thus be the uncharged $(LH_{-2}M)_n$. Some reticulation between two procyanidin chains is not excluded (Figure 7c).

Oxidative reactions catalyzed by copper similar to those discussed above for catechol may also contribute to the precipitation of tannin/copper complexes. However, in contrast to catechol for which precipitation relies on its oxidation (see above), precipitation of tannin/copper complexes can be explained in the absence of oxidation by the formation of uncharged complexes or of high molecular weight complexes, branched or not, charged or uncharged. It was observed that copper precipitation by either chestnut tannin or tannic acid was not affected by bubbling nitrogen in the reagent solutions and carrying out precipitation in stoppered SVL tubes (not shown). Lattice-building properties similar to those described here for tannins have been reported for other polydentate ligands such as carbonates, oxalates, or phosphates (Chaberek and Martell, 1959); such lattice precipitates usually dissolve in an excess of ligand, just as is observed here for commercial tannins (Figure 4), penta-*O*-galloyl- β -D-glucose, or the bifunctional gallic acid (Figure 5).

In any case, the proposed schemes for tannin/copper precipitation (Figures 7 and 8) would not be significantly altered by a partial oxidation of tannins and polyfunctionality will remain a key factor to explain the particularly efficient copper precipitation by tannins (compare penta-*O*-galloyl- β -D-glucose and low molecular weight phenols in Figure 7).

Some analogies between the model for tannin-metal ion precipitation presented here and that proposed by Haslam for complexation/precipitation of proteins by polyphenols (Spencer et al., 1988) are evident. Both schemes propose either the formation of high molecular weight complexes with possible reticulation or the reduction of the polarity of the tannin or protein molecules through complexation.

Zinc(II) was found to be much less easily precipitated by both tannins and low molecular weight phenols. This is in agreement with a previous study showing the absence of zinc precipitation by tannic acid (Okuda et al., 1982) and with the relative stabilities of zinc and copper chelates with various chelating agents (Chaberek and Martell, 1959). Both zinc(II) and copper(II) have coordination numbers of 4, and differences in the stability of zinc and copper may be explained by the different configurations of their complexes, respectively tetrahedral and planar.

Nature of Commercial Tannins and Copper Precipitation. As the results of elemental analysis (Table 1) have shown that about one copper atom is precipitated per *o*-dihydroxyphenyl group in the tannin molecule, the optimal Cu/L weight ratio should depend

on the number of *o*-dihydroxyphenyl groups per tannin molecular weight unit. These Cu/L values can be calculated as approximately 0.34 for hydrolyzable tannins (penta-*O*-galloyl- β -D-glucose, chestnut tannin, and tannic acid) and 0.22 for proanthocyanidins (pine bark and quebracho tannins). These values suggest that more proanthocyanidins will be required as compared to hydrolyzable tannins to precipitate similar amounts of copper. However, due to the broad nature of the precipitation maximum (Figure 6a), our data do not allow confirmation of this.

Pecan tannin differs from the other proanthocyanidins as much more tannin is required to reach the precipitation maximum (Figure 6a). It could be simply explained by the presence of large amounts of non-tannin impurities. This was indeed suggested by the relatively low phenol content of pecan tannin measured by the Folin-Ciocalteu assay (Scalbert et al., 1989) (490 mg/g of tannin) in comparison to those of tannic acid (676 mg/g), quebracho (643 mg/g), or chestnut tannin (608 mg/g).

The chemical structure of tannins and in particular the type of hydroxyl substitution could also explain differences in their ability to precipitate copper. Pecan proanthocyanidins differ from quebracho or pine tannins by the 3',4',5'-OH substitution pattern of B-rings (pyrogallol rings) instead of 3',4'-OH substitution (catechol rings) (McGraw et al., 1992). Experiments with low molecular weight phenols have shown that pyrogallol precipitates copper more efficiently than catechol (Figure 5). If one considers that oxidation plays a substantial role in copper precipitation by low molecular weight phenols (see above), the easier oxidation of pyrogallol as compared to catechol may explain this difference. More experiments with model molecules are, however, required to ascertain the effect of substitution type on copper precipitation.

Commercial tannins also differ in the proportion of copper precipitated at the maximum of precipitation. Commercial tannins could contain some non-tannin chelators which would form soluble chelates with higher affinities for copper than tannins. These chelators if present cannot be phenolic as all phenols are precipitated (Figure 4b) in conditions under which only 60% of the copper is precipitated (Figure 4a). These differences are more likely explained by some pH effects.

pH, Chelators, and Copper Precipitation by Tannins. Chelation of metal ions by *o*-dihydroxyphenyl derivatives is pH dependent as it appears in the following reaction:



A reduction of pH will displace the equilibrium toward the left and the extent of complexation and therefore of precipitation will be reduced. Three factors contribute to lower the pH: the natural acidity of the tannin extracts, the acidity of copper chloride, and the complexation reaction. Acidification can be counteracted by adding a buffer. Increasing its concentration improves copper precipitation, and a 100% copper precipitation can be reached as long as the buffer concentration is high enough (Figure 3). Quantitative copper precipitation was also achieved at pH 6.8 kept constant through addition of KOH (Kennedy, 1985).

Differences in the proportion of copper precipitated by different commercial tannins might thus be explained

by differences in the pH of the tannin samples or in their intrinsic buffering capacity. However, the pH values of the different tannins (quebracho, 5.1; pecan, 5.0; tannic acid, 2.8; and chestnut tannin, 3.4, for 10 g/L solutions) would suggest a higher yield of copper precipitation for quebracho and pecan condensed tannins, whereas the opposite is observed (Figure 4a). Some other factors related to the chemical structure of the tannins should explain differences between tannins.

pH effects influence not only copper precipitation but also the stability of copper/tannin complexes. Acetic acid displaces part of the copper from the insoluble complexes (Table 2). Strong chelators such as EDTA, with a higher affinity for copper than tannins, are even more efficient than acetic acid in displacing copper from the precipitate (Figure 6).

CONCLUSIONS

Polyfunctional tannins with several *o*-dihydroxyphenyl functional groups in their molecule are good chelators that can form precipitates with metal ions. The present results obtained with copper(II) as well as previous works with other metal ions (Okuda, 1982; Kennedy, 1985) suggest that two main factors affect coprecipitation of tannins and metal ions: (i) an optimal ratio of tannin and metal concentrations is required to obtain the highest yield of metal precipitation; (ii) pH control is essential to counteract acidification resulting from complexation. When these conditions are met, a nearly quantitative precipitation of tannin and metal ion can be obtained.

The amount of complexed metal ions in the precipitate is not constant but depends on the relative concentrations of tannin and metal ion. An increase in the proportion of metal will increase the proportion of metal in the precipitate up to the limit of one metal atom per *o*-dihydroxyphenyl group.

The physicochemical mechanisms governing insolubilization are poorly understood, and the present experiments do not indicate if formation of a tannin/metal polymeric network is essential or not. Deeper insight into the influence of polyphenol structure on precipitation should help to clarify this point.

The significance of tannin-metal precipitation in plant biology can be further addressed. Precipitation could contribute directly to deprive plant pathogens and rots from essential metal ion micronutrients. Otherwise, precipitation could displace the complexation equilibrium toward the complexed forms of metal ions, thus reducing their availability. The significance of metal ion precipitation by tannins, if it is confirmed, may help to explain why so many plant tissues accumulate tannins rather than low molecular weight polyphenols.

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