Solubility of Tannins and Preparation of Oil-Soluble Derivatives

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Abstract: Tannins are plant defense substances that exhibit a strong astringent taste and precipitate proteins, leading to the inhibition of protein functions; however, owing to their relatively low toxicity, tannins must be accumulated in high concentrations in cell vacuoles. Therefore, the solubility of tannins is crucial for their functions. In this review, the structure and reactions of tannins related to solubility; insolubilization of persimmon proanthocyanidins on fruit ripening; pigment formation from cinnamon procyanidins by reaction with cinnamaldehyde in case of wounding; and insolubilization of ellagitannins in chestnut wood is discussed. In addition, the development of functional polyphenols including oil-soluble tea catechins is introduced.

Key words: catechins, tannins, polyphenols, antioxidant, proanthocyanidins, cinnamaldehyde, essential oil

1 INTRODUCTION

Tannins are a group of plant polyphenols that can precipitate proteins and heavy metals in aqueous solutions. The precipitation of salivary proteins in oral cavities via interaction with tannins leads to an unpleasant astringent taste, and interactions of tannins with digestive enzymes decrease absorption of sugar and lipid from the digestive tract. Ironically, the decreased uptake of sugar and lipid from the digestive tract has been recognized as health benefits of tannins in developed countries for the prevention of diabetes and hyperlipidemia. Tannins suppress the proliferation of microorganisms and exhibit antioxidative activities similar to other polyphenols such as flavonol and hydroxycinnamate. Based on these physicochemical and biological properties, in addition to previously reported results in chemical ecology, it is generally accepted that tannins are plant defense substances. In addition, the wide distribution of tannins in the plant kingdom including ferns, gymnosperms, and angiosperms, suggests that plants began to biosynthesize tannins in the early stage of plant evolution. However, in the current stage of evolution, the toxicity of tannins is considerably weaker than those of sophisticated toxic alkaloids and terpenoids, and herbivores, including humans, can cope with the toxicity of tannins.

Many herbivorous mammals synthesize and secrete a group of proline-rich peptides in the saliva with a high affinity for tannins, and the peptides precipitate tannins in the oral cavity prior to interaction with digestive enzymes. Tannins are water-soluble compounds, and the tannin–protein interaction is mainly accounted for by hydrophobic association, hydrogen bonding, and π–π and CH–π interactions. The solubility of tannins in water depends on not only the hydrophobicity but also the flexibility of molecules; therefore, the modification of tannin chemical structure leads to the change in the physicochemical properties. In addition, the molecular weight of tannins is related to the tannin–protein interactions: tannins with larger molecular size usually shows stronger interactions with proteins. However, epigallocatechin-3-O-gallate strongly interacts with proteins despite being a relatively small molecule (MW 458, Fig. 1). This interaction is caused by the highly hydrophobic cavity formed by the B-ring, C-ring, and the galloyl ester at the C-3 hydroxy group of 4. The decrease of astringency via modification of tannin structures is important in food science and chemical ecology. Conventional food processing methods, including the production of black tea and microbial fermentation of green tea, probably aim to decrease the astringent tea catechins. The modification of tannin structures during fruit ripening is related to the dispersal method of plant seeds. In this review, examples of chemical mechanisms for the structural change in tannins and the application of these reaction mecha-
nisms to the development of functional derivatives of poly-
phenols including oil-soluble tea catechins are introduced.

2 PROANTHOCYANIDINS AND FLAVAN-3-OLS

2.1 Structure and properties

Proanthocyanidins or condensed tannins are abundant
in nature next to cellulose, hemicellulose, and lignin\textsuperscript{21},
which are widely distributed in ferns, such as bracken fern
and Osmunda fern; gymnosperms, such as pine and
conifer; and angiosperms, such as grape, apple, cocoa,
blueberry, and cranberry\textsuperscript{9}. Proanthocyanidins are flavan-
3-ol oligomers in which each monomer unit is connected
by C–C linkages between C-4 and C-8 (or C-6) (B-type link-
ages) (Fig. 1). The heating of proanthocyanidins under
strongly acidic conditions affords anthocyanidins, which
determine subclasses of proanthocyanidins. Proanthocy-
anidins yielding cyanidins are classified as procyanidins,
such as procyanidin B2 (2), while those yielding delphin-
idins are called prodelphinidins, such as prodelphinidin B2
(3). Typically, the B-rings of flavan-3-ol units comprise
catechol (3,4-dihydroxyl groups) or pyrogallol (3,4,5-tri-
dihydroxyl groups), and catechol-type proanthocyanidins are
considerably more common in nature\textsuperscript{9}. The oxidation
of B-rings affords A-type double linkages with additional C-2–
A-ring-O ether linkages\textsuperscript{21}. Interestingly, limited A-type

![Figure 1](image1.png)

**Fig. 1** Structure of epigallocatechin-3-O-gallate (1), procyanidin B2 (2), prodelphinidin B-2 (3), and cinnamtannin B1 (4).

![Figure 2](image2.png)

**Fig. 2** Fragmentation of proanthocyanidin polymers by nucleophilic substitution with tea catechins.

Flavan C-4 methine carbons of the extension (upper)
units are located between two phloroglucinol rings (A-
rings); thus, these carbons are extremely sensitive to nu-
cleophilic substitution. This chemical property can be ex-
plained for the structural determination of proanthocyanidins. The treatment of proanthocyanidins
with the excess of nucleophiles, such as phloroglucinol and
thiol compounds, under acidic conditions is useful for
identifying the monomeric building blocks\textsuperscript{24}. In addition,
the C-8 (or C-6) carbons of flavan-3-ols and proanthocyan-
idins can act as nucleophiles; therefore, in an acidic solu-
tion, the C-4–C-8 (or C-6) linkage of proanthocyanidins un-
dergoes cleavage, yielding a carbocation at C-4, and the
carbocation reacts with the C-8 (or C-6) of the coexisting
flavan-3-ol units, affording different procyanidin skeletons.
Finally, a complex mixture is obtained. This chemical prop-
erty was exploited for the conversion of polymeric proan-
thocyanidins into dimeric and trimeric proanthocyanidi-

da\textsuperscript{25}. The method is quite simple. First, either polymeric
proanthocyanidins of persimmon fruits or unripe persim-
mon fruits are heated with tea catechins in a citric-acid-
containing aqueous solution, affording a mixture mainly
comprising dimeric and trimeric proanthocyanidins (Fig.
2). Currently, oligomeric products obtained from proanthocya-
nidin polymers in the litchi pericarp are commercially
used as functional foods, Oligonol\textsuperscript{26}.

2.2 Insolubilization of persimmon proanthocyanidins

The strong astringent taste of unripe persimmon fruits corresponds to the polymeric proanthocyanidins comprising (\textminus-)epigallocatechin, (\textminus-)epicatechin, and their 3-O-galloyl esters\textsuperscript{37}. However, the taste disappears after the seeds acquire germination ability, and animals such as monkeys and crows eat the sweet flesh and disperse the seeds. This is a dispersal method for persimmon seeds. In Japan, the astringent taste of persimmon fruits is artificially removed by soaking in warm water, placing in a CO\textsubscript{2}-filled chamber, or packing in a polyethylene bag containing a small amount of ethanol. Under these anaerobic conditions, acetaldehyde is generated from ethanol or pyruvate in the flesh, which undergoes cross-linking and insolubilizes the proanthocyanadin molecules in the tannin cell (Fig. 3)\textsuperscript{28}. In case of persimmon fruits on trees, acetaldehyde is known to be supplied from mature seeds\textsuperscript{29}. The above-mentioned thiol degradation method is directly applied to persimmon fruits containing insolubilized proanthocyanidins, and thiol degradation products with C\textsubscript{2}-units corresponding to acetaldehyde from the flavan-3-ol A-ring are isolated\textsuperscript{20}. Gelation in an aqueous solution of tea catechin 1, which is a structural component of persimmon proanthocyanidins, by the addition of acetaldehyde revealed a spontaneous, non-enzymatic reaction for the insolubilization of proanthocyanidins.

2.3 Polymeric pigments produced from cinnamon procyanidins and cinnamaldehyde

After peeling off a fresh bark from a Japanese cinnamon branch, the fresh wood surface changes from pale-yellow to reddish-orange within 30 min. The chemical mechanism of pigmentation has been reported to be caused by the formation of anthocyanidin-like chromophores via the condensation of proanthocyanidins with cinnamaldehyde and subsequent autoxidation\textsuperscript{30}. In addition, the reaction also cause the oligomerization of procyanidins. In contrast to reactions with acetaldehyde in persimmon fruits, the C--C bond formation between the carbonyl group of \textalpha,\textbeta-unsaturated aldehyde and the A-ring is followed by the addition of the A-ring phenolic hydroxyl group to the double bond (Fig. 4). The physiological meaning of the reactions occurring on the wound surface is not clear; however, the polymerization of procyanidins is probably related to the defense system, because in some plants such as tea leaves the enzymatic oxidation of polyphenols accompanies the production of oligomers and polymers in response to wounding\textsuperscript{16, 17}. Furthermore, pigment formation by oxidation with oxygen molecules is possibly accompanied by the production of reactive oxygen species, such as a superoxide anion radical or hydrogen peroxide, which exhibits antibacterial activity\textsuperscript{31}. Model experiments using catechins and dimeric procyanidins have revealed that the polarity of the reaction products with cinnamaldehyde is considerably less than that of the starting material due to the longer retention time observed in HPLC analysis and a higher Rf

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3.png}
\caption{Insolubilization and thiol degradation of persimmon proanthocyanidins.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig4.png}
\caption{Oligomerization and pigmentation of procyanidins by condensation with cinnamaldehyde. Bold line: cinnamaldehyde unit, white arrow: anthocyanidin chromophore.}
\end{figure}
value in TLC. The water solubility of the polymeric pig-
ments on the wounded wood surface is thought to be con-
siderably less than that of the original procyanidins.

2.4 Oil-soluble tea catechin derivatives
Since the 1990s, several biological and epidemiological
studies have revealed various health benefits of plant poly-
phenols and tannins\textsuperscript{32}. Currently, the daily intake of anti-
oxidative compounds originating from food has been re-
ported to reduce oxidative stress in various organs of the
human body and prevent cancer, myocardial infarction,
and cardiovascular diseases. Tea is the most popular drink
consumed worldwide; thus, tea catechins are the most im-
portant dietary polyphenols for humans\textsuperscript{33}. However, cate-
chins are soluble in water and not soluble to food oil. In
reality, 1, which constitutes the major component of tea
catechins, inhibits the peroxidation of lipids in the lipid
bilayer caused by the water-soluble radical initiator; how-
ever, it does not inhibit the oxidation caused by the li-
pophilic radical initiator. Therefore, some studies have re-
ported the preparation of lipid-soluble derivatives of tea
catechins. Mixtures of fatty acid esters of the epigallocate-
chin-3-O-gallate B-ring and galloyl hydroxy groups (5) were
prepared, and their antibacterial, antifungal\textsuperscript{34}, antitumor\textsuperscript{35},
and antiviral activities were reported (Fig. 5)\textsuperscript{36, 37}. In the
derivatives, phenolic hydroxyl groups of the B-ring and
galloyl groups were inactivated by esterification; there-
fore, the aforementioned condensation of the proanthocyanidin
A-ring with aldehyde is applied to the synthesis of oil-solu-
ble tea catechin derivatives with the intact B-ring and
galloyl groups. The treatment of 1 with formaldehyde
affords polymeric gels, and the subsequent degradation of
the polymer with various alkyl thiols afforded hydrophobic
derivatives with thiethers at C-6 and C-8 of the A-ring (6)\textsuperscript{38}. The
derivatives exhibit a strong inhibition activity against the
lipid peroxidation of liposome caused by lipid-soluble
and water-soluble radical initiators. In addition, similar de-
rivatives have been prepared by reactions with various
alkyl aldehydes and methanethiol\textsuperscript{39}. Furthermore, the ob-
served reaction of the α,β-unsaturated aldehyde in cinn-
amon wood was applied to another group of hydrophobic
derivatives\textsuperscript{40}. The heating of tea catechins with natural es-
sential oils comprising partial structures of an unsatu-
rated aldehyde or an allyl alcohol affords highly hydrophobic de-
rivatives (8-11). These derivatives are soluble to triglyc-
rides and exhibit radical scavenging activities. In addition,
some of the derivatives such as 9–11 have been reported
to upregulate the activity of neprilysin, which is a major
enzyme responsible for the degradation of amyloid-β
peptide\textsuperscript{41}.

3 HYDROLYZABLE TANNINS
3.1 Structure and water solubility
Hydrolysable tannins are basically categorized into two
groups, i.e., gallotannins and ellagitannins (Fig. 6). A ma-
jority of gallotannins are galloyl esters of glucose, with a
typical example being 1,2,3,4,6-pentagalloyl-β-D-glucose
(12). Compound 12 is found in peony root (Paeonia sp.)
and bearberry leaves (Arctostaphylos uva-ursi). In addi-
tion, quinic acid, shikimic acid, and 1,5-anhydro-D-glucitol
have been reported as core polyalcohols of polygalloyl glu-
coses of Caesalpinia spinose, Castanopsis cuspidata,
and Acer ginnala, respectively. By itself, pentagalloyl glucose does not accumulate in plant tissues, and it is typically accumulated as a mixture of polygalloyl glucoses produced by the further galloylation of the circumferential phenolic hydroxyl groups of pentagalloyl glucose (Fig. 6). Pentagalloyl glucose is a highly flexible with a flat starfish-like shape. Despite the presence of 15 circumferential hydroxyl groups, the molecule center is highly hydrophobic. Therefore, pentagalloyl glucose exhibits strong interactions with the hydrophobic site of proteins, leading to the strong inhibition of the biological function of proteins. However, this property is probably not convenient for accumulation in plant cell vacuoles as high concentrations of this compound lead to gel formation by self-association.

Tannins are supposed defense substances against herbivores and are typically accumulated in high concentrations due to their lower toxicity compared to toxic alkaloids and terpenoids. In contrast, the water solubility of polygalloyl glucoses is greater than that of pentagalloyl glucose, and high concentrations of this compound are accumulated in gallnuts from Rhus semialata and Quercus infectoria, which are important sources of tannic acid. Ellagitannins are found in commercially important plants, such as oak, chestnut, walnut, raspberry, pomegranate, and Acer ginnala, respectively. By itself, pentagalloyl glucose does not accumulate in plant tissues, and it is typically accumulated as a mixture of polygalloyl glucoses produced by the further galloylation of the circumferential phenolic hydroxyl groups of pentagalloyl glucose. Pentagalloyl glucose is a highly flexible with a flat starfish-like shape. Despite the presence of 15 circumferential hydroxyl groups, the molecule center is highly hydrophobic. Therefore, pentagalloyl glucose exhibits strong interactions with the hydrophobic site of proteins, leading to the strong inhibition of the biological function of proteins. However, this property is probably not convenient for accumulation in plant cell vacuoles as high concentrations of this compound lead to gel formation by self-association.

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extractable ellagitannins in the inner heartwood is confirmed by the acid hydrolysis of wood debris after extraction, affording ellagic acid ($\text{H}_2$). Although the current evidence is not sufficient, insolubilization is probably caused by the formation of C–O or C–C bond at the C-1 benzylic carbon of tannins with plant cell-wall components (Fig. 8). Model experiments have indicated that vescalagin spontaneously forms ether linkages at the C-1 position with glycerol at room temperature and with glucose on heating$^{64}$. Furthermore, heating with sinapylaldehyde, a component of lignin, afforded a C–C bond-connected adduct$^{64}$. Previously, our group has reported that Japanese chestnut wood contains castacrenins, such as castacrenin B (19), as the characteristic metabolites detected only in the heartwood center$^{63}$, which is possibly generated by the degradation of insolubilized ellagitannins (Fig. 8). The immobilization of antimicrobial ellagitannins in the dead area of the wood is probably a strategy of these trees for defense against microorganisms.

4 CONCLUSION
Proanthocyanidins, tea catechins, and hydrolysable tannins are the so-called quantitative defense substance that must be accumulated in high concentrations in plant tissues due to low toxicity compared to qualitative defense substance such as toxic alkaloids. The bitter, astringent taste and protein function inhibition of these polyphenols are only effective when their concentration are greater than the concentrations that are disfavored by herbivores. To accumulate the polyphenols for effective defense, plants modify structures to increase the solubility, and when it is necessary, decrease the solubility and are insolubilized in the tissues. Still, sufficient evidence for the actual function of tannins and polyphenols in plants is not available, and the information obtained from the studies on the reactivity of tannins is applicable to the development of functional materials such as oil-soluble polyphenols.
Preparation of Oil-Soluble Catechins

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