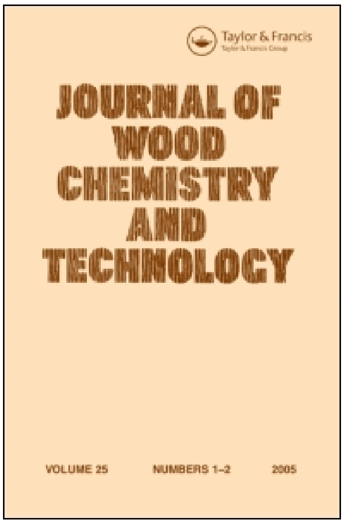

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Effects of the Hydroxylation Patterns and Degrees of Polymerization of Condensed Tannins on their Metal-Chelating Capacity

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EFFECTS OF THE HYDROXYLATION PATTERNS AND DEGREES OF POLYMERIZATION OF CONDENSED TANNINS ON THEIR METAL-CHELATING CAPACITY

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ABSTRACT

We studied the effects of the hydroxylation patterns and the degrees of polymerization of condensed tannins on their metal-chelating capacity. These effects were evaluated by the relative stability of aluminium complexes with proanthocyanidin. The relative stability of aluminium complexes was determined qualitatively in methanol solution by the competition with the metallochromic reagent (chrome azurol S). A comparison of these capacities indicates the following results: 1) phenolic hydroxy groups in tannins are essential sites for the chelation with metal. 2) the sites of tannins interacting with metal ions are mainly *o*-dihydroxyphenyl groups in B-ring. 3) increasing degrees of polymerization of tannins tends to increase the relative stability of condensed tannin-aluminium chelate.

INTRODUCTION

The condensed tannins (procyanidins) are polymeric phenolic compounds occurring widely in plants, especially in bark.[1]

Several kinds of natural condensed tannins with differently hydroxylated B-ring have been found. Many of them are known to be 3',4'-dihydroxylated or

3',4',5'-trihydroxylated B-rings. In addition, 4'-hydroxylated B-rings in admixture with the above B-rings have been also found in those of the extractives of oolong tea,[2] *Kandelia candel* bark,[3] *etc.* Condensed tannins with expressly high molecular weight exist in ferns[4] and the persimmon (*Diospyros kaki* L.) fruit.[5]

The condensed tannins precipitate from aqueous solution by albumin, gelatin and other proteins. Owing to this protein-precipitating capacity of tannins, in plant, they appear to have many important functions; they can interact with various proteins and enzymes to protect plants from the attack by microbes and inhibiting wood-decaying fungi.[6]

The condensed tannins are also strong complexing agents for metal ions, such as iron (III) and aluminium (III), in neutral solution; the related hydrolysable tannins have been used for the quantitative precipitation of trace metals from solution.[7] The stability and low solubility of their metal complexes implies that they may be involved in the mobilization and subsequent deposition of metal ions in soils.[8]

In the previous papers, we reported the synthesis and their protein-precipitating capacity of a series of condensed tannins with non-, mono-, di-, tri-hydroxylated B-rings, and showed that a synthetic condensed tannin with only a 4'-hydroxylated B-ring had almost the same protein-precipitating capacity as that of condensed tannin with 3',4'-dihydroxylated or 3',4',5'-trihydroxylated B-rings.[9] Relationships between protein-precipitating capacity and the degree of polymerization of condensed tannin have been also reported by Haslam[10] and Bate-Smith.[11]

However, relationship between metal-chelating capacity and the B-ring hydroxylation pattern or the degree of polymerization of condensed tannins have been scarcely studied. The significant sites of condensed tannin for metal complexation have been thought to be vicinal phenolic hydroxyl groups, such as 3',4'-dihydroxylated and 3',4',5'-trihydroxylated B-rings, but it has not been proved by the direct experiments. It is interesting to establish whether condensed tannins with only 4'-hydroxylated B-rings have metal-chelating capacity or not.

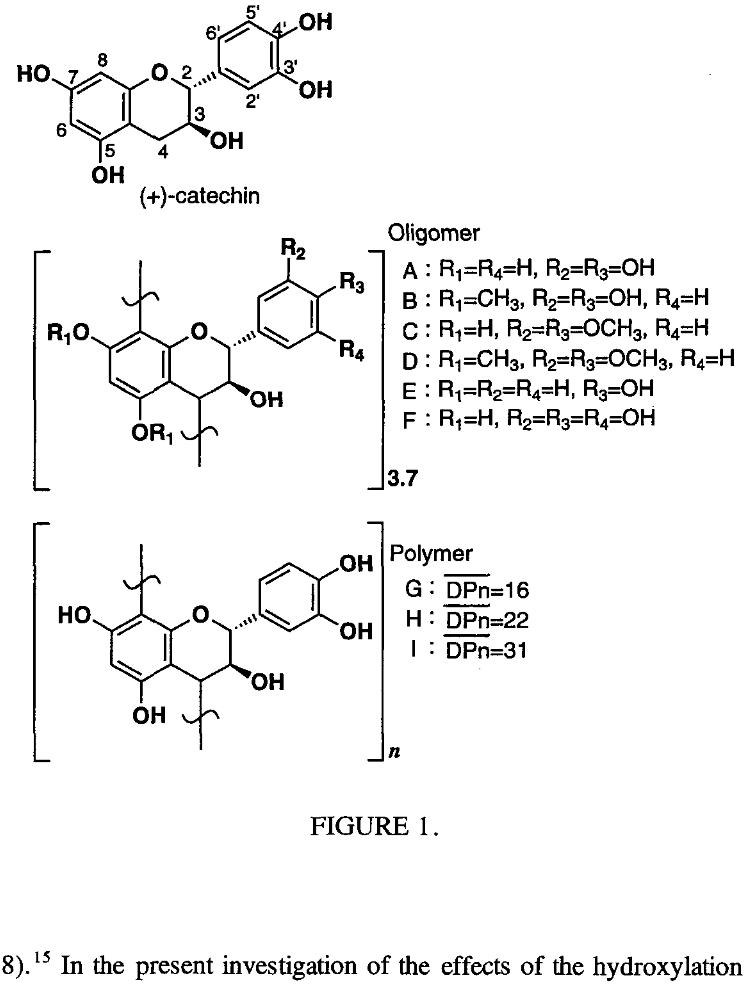
In this paper, we discuss the effects of the hydroxylation patterns of the B-rings and the degree of polymerization on condensed tannin-aluminium interaction in methanol solution.

RESULTS AND DISCUSSION

In the present investigation, the following samples were selected (Figure 1); catechin as a monomer, procyanidin oligomer A, regiospecifically methylated procyanidin oligomers B–D, propelargonidin oligomer E, prodelphinidin oligomer F and procyanidin polymers G–I. Oligomers A to F were synthesized by the condensation of their corresponding flavan-3,4-diols and subsequent debenzylation of the condensed products.[9, 12, 13] Polymers G ($\overline{\text{DPn}}$=16), H ($\overline{\text{DPn}}$=22) and I ($\overline{\text{DPn}}$=31) were synthesized by the condensation of benzylated flavan 3,4-carbonate and subsequent debenzylation of the condensed products.[14]

Determination of the relative stability of aluminium complex

Powell and Rate have qualitatively determined the relative stability of aluminium complex with tannin (citrate, oxalate *etc.*) by competition with the metallochromic reagent chrome azurol S in 0.005 M hexamine aqueous solution



Oligomer

A : $R_1=R_4=H$, $R_2=R_3=OH$
B : $R_1=CH_3$, $R_2=R_3=OH$, $R_4=H$
C : $R_1=H$, $R_2=R_3=OCH_3$, $R_4=H$
D : $R_1=CH_3$, $R_2=R_3=OCH_3$, $R_4=H$
E : $R_1=R_2=R_4=H$, $R_3=OH$
F : $R_1=H$, $R_2=R_3=R_4=OH$

Polymer

G : $\overline{DPn}=16$
H : $\overline{DPn}=22$
I : $\overline{DPn}=31$

FIGURE 1.

(pH 4.8).[15] In the present investigation of the effects of the hydroxylation pattern and the degree of polymerization on condensed tannin-aluminium interaction, the relative stability of aluminium complexes with catechin, oligomers A–F and polymers G–I were determined qualitatively by their competition method with chrome azurol S. The relative stability of aluminium complexes with tannins may be determined in methanol, because oligomers B–D and polymers G–I are slightly soluble in water, but soluble in methanol. Flavonoids have been reported to form the complex with aluminium ion in absolute methanol.[16]

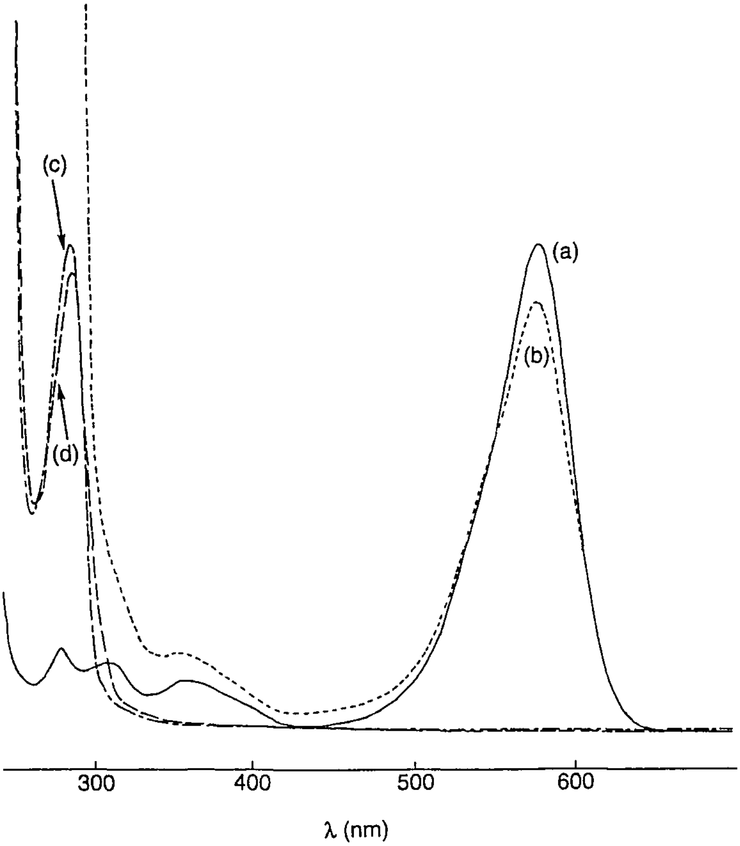


FIGURE 2. UV-VIS spectra: (a)aluminium-chrom azurol S complex, (b) aluminium-chrom azurol S complex containing condensed tannin, (c) condensed tannin, (d)aluminium-condensed tannin complex.

By our preliminary experiment, molar ratio plots based on the absorption of the aluminium–chrom azurol S complex at 578 nm indicated to form a 1:1 complex in methanol.

Figure 2 shows UV–visible spectra of aluminium–chrome azurol S complex having a maximum absorbance at 578nm [Figure 2(a)]. This maximum absorbance decreases by addition of condensed tannin [Figure 2(b)]. This decrease occurs on account of the inhibition of aluminium-chrome azurol S complexation by the condensed tannin added; the more stable complexes of ligands with aluminium show the smaller degrees of absorbance at 578nm.

On the other hand, condensed tannin [Figure 2(c)], or aluminium-condensed tannin complex [Figure 2(d)] does not show the absorbance at 578nm; the absorbance at 578nm is not influenced by the absorbances of aluminium, condensed tannin or aluminium–condensed tannin complex. Consequently, the relative stability of aluminium complexes with condensed tannins may be evaluated by the degree of decrease of the absorbance at 578nm.

In the determination of the relative absorbance, the concentrations of tannin derivatives are expressed as molarity of repeating units. Relative absorbances at 578nm for aluminium–chrom azurol S solutions containing competing tannin derivatives are presented in Figures 3–5.

Influence of the phenolic hydroxyl groups of condensed tannin

It has been believed that the sites of tannins interacting with metal ions is *o*-dihydroxyphenyl groups of the B-ring in condensed tannin molecules:[17] the hydroxyl groups of A-ring are not concerned in these interactions. The metal–chelating capacity of isolated phenolic hydroxyl group seems to be very low, but direct experimental proof using condensed tannins has not yet been obtained. Figure 3 shows the results of the metal–chelating capacity of three regiospecifically methylated procyanidin oligomers. The capacity of oligomer B was found to be

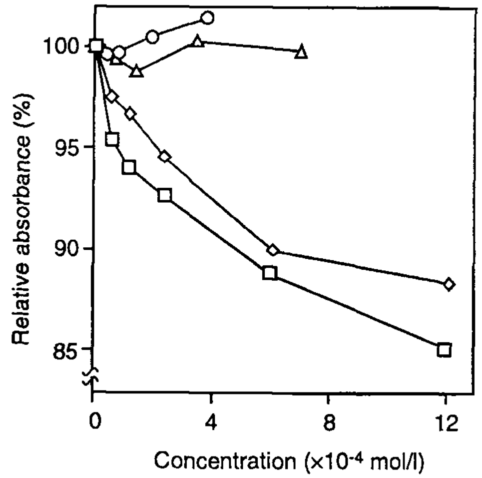


FIGURE 3. Relative absorbance at 578nm for aluminium-chrom azurol S solutions containing oligomer A-D:
Legend: ○: oligomer D (with methylated A- and B-ring)
△: oligomer C (with methylated B-ring)
□: oligomer A, ◇: oligomer B (with methylated A-ring)

nearly equal to that of oligomer A. However, that of oligomer C was very low, and oligomer D had little complexation ability.

Figure 4 shows the relationships between the hydroxylation pattern of the B-ring and the metal-chelating capacity. The hydroxylation patterns of B-ring are effective in the order of 3',4',5'-trihydroxylated > 3',4'-dihydroxylated > 4'-hydroxylated B-rings. 4'-hydroxylated B-ring has very low capacity. Thus, 4'-hydroxylated B-ring is considered to be of little importance for tannin–metal chelation as well as A-ring.

The comparison of the complexation ability of these oligomers indicates following results: 1) phenolic hydroxyl groups in tannins are essential sites for the

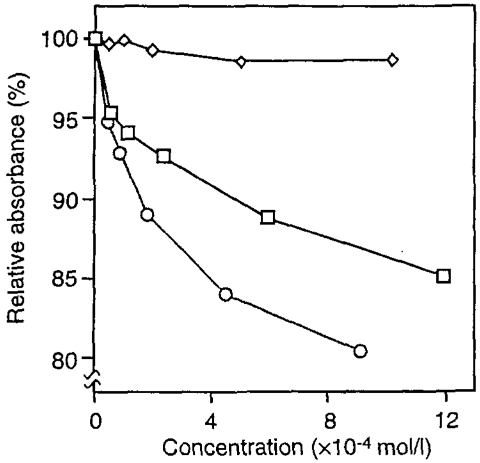


FIGURE 4. Relative absorbance at 578nm for aluminium-chrom azurol S solutions containing competing oligomer A, E and F.
Legend: ◇: oligomer E (with monohydroxylated B-ring)
□: oligomer A (with dihydroxylated B-ring)
○: oligomer F (with trihydroxylated B-ring)

chelation with metal. 2) the A-ring is of little importance for tannin–metal interactions. 3) the site of tannins interacting with metal ions is mainly *o*-dihydroxyphenyl groups of B-ring in condensed tannin molecules. The metal–chelating capacity of 3',4',5'-trihydroxylated B-ring is highest in three hydroxylation patterns; three vicinal phenolic hydroxyl groups may raise the metal–chelating capacity by more chelating sites with aluminium ion than *o*-dihydroxyphenyl group.

Previous paper reported that a condensed tannin with only a 4'-hydroxylated B-ring has almost the same protein-precipitating capacity as that of a condensed tannin with 3',4'-dihydroxylated or 3',4',5'-trihydroxylated B-ring.[8] This point is

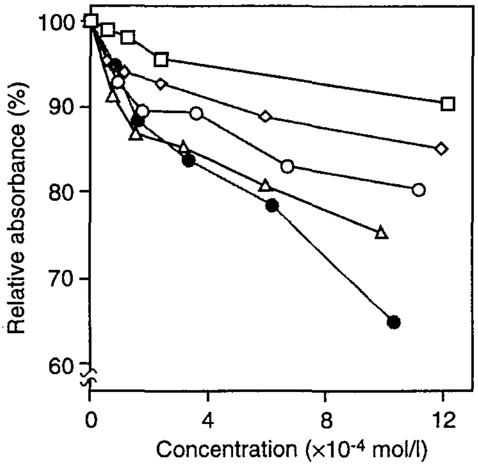


FIGURE 5. Relative absorbance at 578nm for aluminium-chrom azurol S solutions containing competing catechin, oligomer A, polymer G, H and I.

Legend: □: catechin, ◇: oligomer A ($\overline{DPn}$=3.7)
○: polymer G ($\overline{DPn}$=16), △: polymer H ($\overline{DPn}$=22)
●: polymer I ($\overline{DPn}$=31)

interesting difference between the complexation with metal ion and protein.

It is thought that the difference between the complexation with protein and metal ion is as follows. Complexation of polyphenols with proteins may be considered as a two-stage process; adsorption and agglutination. In the adsorption stage, the hydrogen bond between the phenyl group and the carbonyl group of peptide linkages is formed.[18] On the other hand, on the complexation with the metal ion, the coordinate bond between the oxygen of phenolic hydroxyl group and the metal ion is formed; coordinate bond is much more affected than a hydrogen bonding by hydroxylation patterns in condensed tannin on the metal complexation.

Influence of the degree of polymerization of condensed tannin

It is well known that procyanidin monomers and oligomers have the capacity to chelate with metal ions. However, the study on the metal-chelating capacity of condensed tannins with high molecular weight have been little reported hitherto, because it is difficult to obtain these condensed tannins from natural products. Recently, we established a synthesis of condensed tannins with high molecular weight.[14] Thus, the metal-chelating capacities of the tannin with high molecular weight were investigated by the use of polymer G–I synthesized.

Figure 5 shows the relationships between the degrees of polymerization of condensed tannins and the metal–chelating capacity. In concentration of the condensed tannins above about 4×10^{-4} M on solutions, increasing degrees of polymerization of tannins tend to increase the relative stability of condensed tannin–aluminium chelate. But, below that concentration of condensed tannins, a difference in the stability of condensed tannin–aluminium chelate was not appreciably detected.

Powell *et al.* described that the chelate effect of intramolecule of tannins is small as the tannin molecule is comparatively rigid.[15] But, it is expected that the stability of polyphenol–aluminium chelate is raised by the additional new chelating sites arising from the three dimensional structure of the high molecular weight condensed tannin; the intramolecular coordinate of tannins to metal ions contributes to the stability of polyphenol–metal chelate.

In previous paper, we reported that procyanidin monomers and dimers have no protein-precipitating capacities, but the capacity increases with increasing molecular weights of the condensed tannins.[19] The results of the complexation with protein was coincident to those of the complexation with metal ion. It is suggested that it is necessary for condensed tannins to have a proportion of polymeric chains to act with optimum efficiency, which may explain the general predominance of procyanidin polymers over lower oligomers in nature.

EXPERIMENTAL

General. — Oligomers A to F and polymers G–I were synthesized as reported previously.[9, 12, 13, 14] Molecular weight distributions of substituted polymers were analyzed by gel permeation chromatography (GPC) in tetrahydrofuran, using polystyrene standards. A Waters universal liquid chromatograph injector (Model U6K), a Waters solvent delivery system (Model 6000A), A Waters refractive index detector (Series R-400), a Waters absorbance detector (Model 440), and Shodex columns (KF802 and KF803) were used. The flow rate was 1.0 ml/min. A SHIMADZU UV-365 ultraviolet spectrometer was used for UV spectra.

Determination of the relative stability of aluminium complexes

Stock solution of aluminium–chrom azurol S chelate was made up in methanol with known concentration (4.5×10^{-4} M) from anhydrous aluminium chloride (Wako Pure Chemical Industries, Ltd.) and Chrome Azurol S (Nakarai Tesque Inc.). Stock solutions of condensed tannins were made up in methanol with known concentrations in the range of 2–3 mg/ml (or lower where necessary). From these a series of dilutions were made by taking volumes ranging from 0–0.5 ml and making up the volume to 0.5 ml with methanol in a vial. To these were added 3 ml of the stock solution of aluminium–chrom azurol S chelate. The final concentrations of procyanidin in the methanol solutions were in the approximate range $0.2–12\times10^{-4}$ M (as molarity of repeating units). The concentration of aluminium–chrom azurol S complex was 3.86×10^{-5}M. The contents of each vial were thoroughly mixed, and then left for 1 hour at room temperature. Solutions were kept out of exposure to light by cover of aluminium foil. These solutions were monitored at 578 nm in a 1 cm glass cell.

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