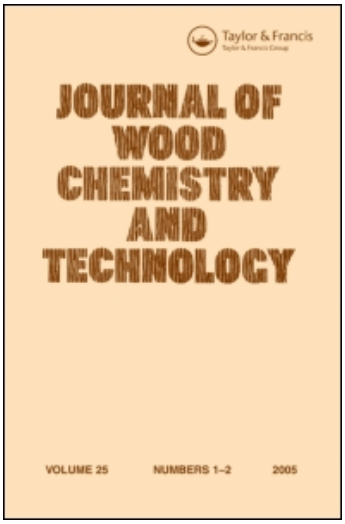

Journal of Wood Chemistry and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597282

Relationship Between the B-Ring Hydroxylation Pattern of Condensed Tannins and their Protein-Precipitating Capacity

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To cite this Article Kawamoto, Haruo , Nakatsubo, Fumiaki and Murakami, Koji(1990) 'Relationship Between the B-Ring Hydroxylation Pattern of Condensed Tannins and their Protein-Precipitating Capacity', Journal of Wood Chemistry and Technology, 10: 3, 401 — 409
To link to this Article: DOI: 10.1080/02773819008050248
URL: http://dx.doi.org/10.1080/02773819008050248

JOURNAL OF WOOD CHEMISTRY AND TECHNOLOGY, 10(3), 401-409 (1990)

RELATIONSHIP BETWEEN THE B-RING HYDROXYLATION PATTERN OF CONDENSED TANNINS AND THEIR PROTEIN-PRECIPITATING CAPACITY

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ABSTRACT

A series of condensed tannin derivatives with non-, mono-, di- and tri-hydroxylated B-rings were synthesized starting from phloroglucinol and benzaldehyde derivatives. The protein-precipitating capacity of these condensed tannin derivatives showed 1) 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-rings. 2) The complexing ability of phenolic hydroxyl groups in the B-ring are effective in the order of *p*- > *m*- > *o*-positions.

INTRODUCTION

Several kinds of natural condensed tannins with differently hydroxylated B-rings have been found and many of them are known to be 3',4'-dihydroxylated or 3',4',5'-trihydroxylated B-rings.[1] Recently, condensed tannins having 4'-hydroxylated B-rings in admixture with the above B-rings have been found in the extractives of oolong tea (commercial name: Shiraore)[2], *Kandelia candel* bark[3] and *Cassia fistula* leaf[4]. Since the significant sites of condensed tannin for protein complexation have been thought to be vicinal phenolic hydroxyl groups[5,6], such as 3',4'-dihydroxylated and 3',4',5'-trihydroxylated B-rings, it is

interesting to establish whether condensed tannins with only 4'-hydroxylated B-rings have protein-precipitating capacity or not. This question remains, because condensed tannins with only 4'-hydroxylated B-rings have not been found as natural products[1]. Such condensed tannins may be obtained only by chemical synthetic method reported previously[7].

We reported the synthesis and their protein-precipitating capacity of several regiospecifically methylated condensed tannins and concluded that both of the phenolic hydroxyl groups in the A- and B-rings play important roles in tannin-protein interaction and they may synergistically interact with protein[7].

In this paper, we describe the synthesis of a series of condensed tannins with non-, mono-, di- and tri-hydroxylated B-rings and discuss the relationship between the hydroxylation pattern of the B-ring and the protein-precipitating capacity.

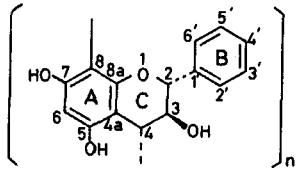
RESULTS AND DISCUSSION

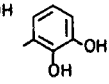
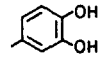
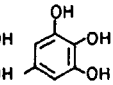
For the present investigation, a series of condensed tannin derivatives, oligomers A to G with several non- (oligomer A), mono- (oligomers B, C and D), di- (oligomers E and F) and tri- (oligomer G) hydroxylated B-rings, was selected (Table 1). Of these oligomers, oligomers D, F and G have been found as structural units in natural condensed tannins.

Oligomers A to G were synthesized by the condensation of their corresponding flavan-3,4-diols (with the protected phenolic hydroxyl groups by benzyl groups) and subsequent debenzylation of the condensed products[7]. The flavan-3,4-diols were synthesized via four reaction steps in 45.3-61.3% overall yields starting from phloroglucinol and benzaldehyde derivatives[8].

Each reaction step in the synthetic route proceeded smoothly except the final step, debenzylation; namely, the reactivity on the debenzylation was found to depend on the substitution pattern of the B-rings.

The benzyl groups of the benzylated oligomers E and F were quantitatively cleaved by using 10% Pd-C and H_2 in dioxane at



Oligomer	A	B	C	D	E	F	G
B-ring							

Stereochemistry is a relative configuration suggested by the reaction mechanism.

TABLE 1. A series of condensed tannin derivatives selected for the protein - precipitating test.

90°C for 3hr as previously reported[7]. However, longer reaction time (8 hours) was required for the debenzylation of the benzylated oligomers B, C, D and G, and more polar solvent system consisting of dioxane/ethanol (1/3, v/v) had to be used for the complete debenzylation of benzylated oligomer A.

The possibility of the side reactions during debenzylation, hydrogenolysis, was examined by the use of the corresponding monomers converted to oligomers B to G as an model compound. Under the same reaction conditions as those used for the preparation of their corresponding oligomers, the reductive cleavage of the C_4-hydroxyl group and the reductive ring-opening reaction of the C-ring occurred in 70 and 30% yields, respectively, as found in the previous experiments reported[7]. However, side reactions other than the reductive cleavage of the C_4-hydroxyl group may hardly proceed during debenzylation of oligomers, because the benzylated oligomers with higher molecular weights should have the lower reactivity than each monomer.

The protein-precipitating ability of these oligomers was evaluated by the formation of the precipitates with bovine serum albumin (BSA) in 0.2M acetate buffer (pH 4.5) at 20°C. The precipitated BSA was estimated by the ninhydrin method as reported previously[7].

Results of the BSA-precipitation tests of oligomers D, F and G are shown in Fig. 1. Interestingly, BSA-precipitating capacity of oligomers D and G are 83 and 87% of that of oligomer F, respectively, when 2.0, 3.0 and 4.0mg of BSA were used for 1.0mg of each oligomer. Thus, these results first indicate that the condensed tannin with only 4'-hydroxylated B-ring has almost the similar complexing ability as that with 3',4'-dihydroxylated or 3',4',5'-trihydroxylated B-rings. Porter and Woodruffe[9] reported that the condensed tannin extracted from Sterelitzia reginae leaf that contains 25% of 4'-hydroxylated B-ring also showed the similar complexing ability as other condensed tannins containing 3',4'-dihydroxylated or 3',4',5'-trihydroxylated B-rings. These results are now reasonably explained by the present results.

The relationships between the hydroxylation pattern of the B-ring and the protein-precipitating capacity are summarized in Fig. 2. The previous data obtained by the use of the regiospecifically methylated condensed tannins, oligomers H (with methylated hydroxyl groups in A-ring), oligomer I (with methylated hydroxyl groups in B-ring) and oligomer J (with methylated hydroxyl groups in both A- and B-rings) are also included in Fig. 2 for the comparison. The relative complexing ability (RCA-value) is a relative value normalized to the precipitating ability of oligomer F (RCA-value: 1.0) where 2.0, 3.0 and 4.0mg of BSA was used. The open circles are RCA-values of oligomers with free phenolic hydroxyl groups in the A-ring and the solid circles are those of oligomers with methylated hydroxyl groups in the A-ring. From the comparison of these RCA-values, the following relationships between the hydroxylation pattern of the B-ring and the protein-precipitating capacity were found.

1) Oligomers A, I and J with no phenolic hydroxyl groups in the

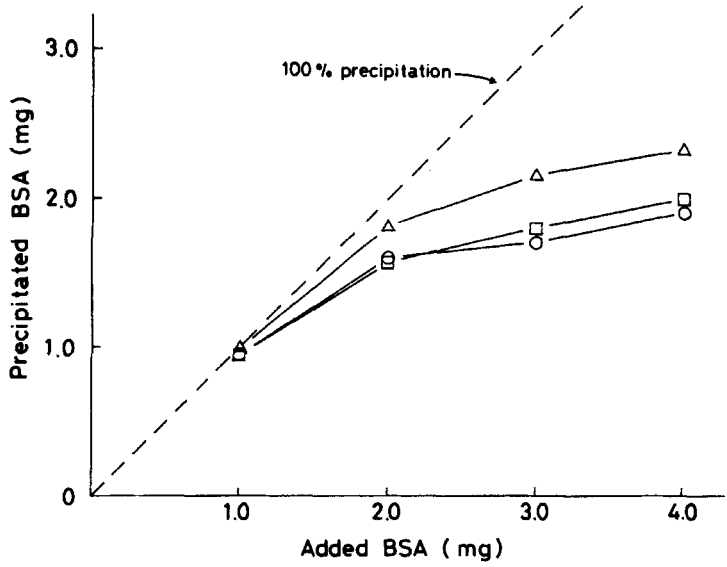


FIGURE 1. BSA - precipitating capacity of oligomers D, F and G with the B-ring hydroxylation patterns found in the unit structures of natural condensed tannins.
○: oligomer D, △: oligomer F, □: oligomer G.

B-ring show small RCA-values (0.22, 0.22 and 0.02, respectively). The phenolic hydroxyl groups in the B-ring are essential for complexing ability. Similar RCA-values of oligomers A and I indicate that there is no participation of the paired electrons around the oxygen atoms in methoxyls attached to the B-ring.

2) RCA-values of oligomers B, C and D that have only one phenolic hydroxyl group in the B-ring are 0.54, 0.72 and 0.83, respectively. The phenolic hydroxyl groups in the B-ring are effective in the order of *p*- > *m*- > *o*-positions on their complexing ability. These results indicate that less hindered phenolic hydroxyl group in the B-ring interacts with protein more effectively than hindered one.

3) Oligomer E, with two vicinal phenolic hydroxyl groups at the *o*- and *m*-positions in the B-ring, has a much smaller RCA-value

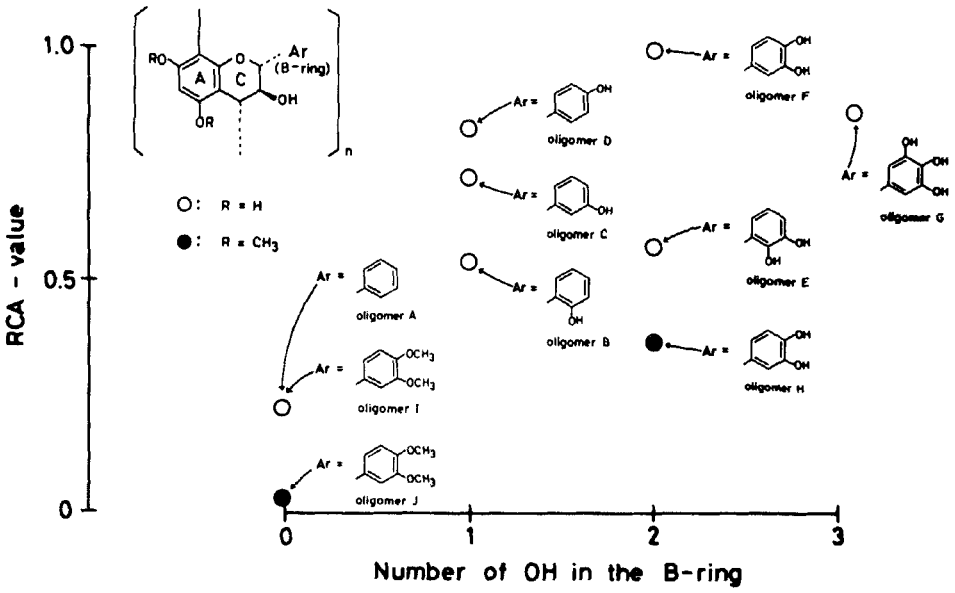


FIGURE 2. BSA - precipitating capacity of condensed tannin derivatives with the differently hydroxylated B-rings.

(0.58) than oligomer F (RCA-value: 1.00) with two phenolic hydroxyl groups at the m- and p-positions. These results are coincident with the above results; the most effective hydroxyl group is at the p-position.

The protein-precipitating capacity of oligomer F, the highest complexing ability in all oligomers tested is extremely reduced by the methylation of the hydroxyl groups in the A-ring as found in the RCA-value (0.36) of oligomer H.

4) Oligomer G with three phenolic hydroxyl groups in the B-ring has almost the same protein-precipitating capacity as oligomers D and F (RCA-values: 0.83, 1.00 and 0.87 for oligomer D, F and G, respectively). This indicates that the additional hydroxyl groups at the m-positions in the B-ring do not contribute much to the complexing ability of condensed tannins.

Thus, the acidic protons of the phenolic hydroxyl groups in tannin molecules play the most important role for the protein-precipitation. Furthermore, the most suitable positions (p > m > o) of the phenolic hydroxyl groups in tannin molecules is important for the effective protein-precipitating ability, but the number of the phenolic hydroxyl groups is not so important.

EXPERIMENTAL

Materials

Oligomer F was synthesized as reported previously[7]. Oligomers A to E and G were also synthesized by a similar method. Only the reaction conditions of the debenzylation reactions of benzylated oligomers were altered as mentioned in the previous section. The structures of the synthesized compounds were supported by the ^{1}H-NMR spectra (measured by JEOL FX-90Q FT NMR-(90MHz) spectrometer). The GPC data of benzylated oligomers A to E and G showed the similar number average degree of polymerization ($\overline{DPn}$=3.7[7]) as oligomer F. The melting points are uncorrected. A SHIMADZU UV-365 ultraviolet spectrometer was used for UV spectra.

Oligomer A

Monomer: Mp 183-184°C ; UV λ_{max}^{MeOH}nm (log ε): 259 (3.07), 265 (3.07), 269 (sh, 3.02). Anal. Calcd. for $C_{29}H_{26}O_5$: C, 76.6; H, 5.8. Found: C, 76.4, H, 5.7.

Benzylated oligomer A: Anal. Calcd. for $(C_{29}H_{24}O_4)_{3.7}OH\cdot H_2O$: C, 78.1; H, 5.6. Found: C, 78.1; H, 5.4, hereafter OH including in molecular formula means the hydroxyl group of the lowest terminal unit.

Oligomer A: Anal. Calcd. for $C_{15}H_{12}O_4\cdot 0.1H_2O$: C, 69.8; H, 4.8. Found: C, 69.7; H, 5.0.

oligomer B

Monomer: Mp 179-180°C ; UV λ_{max}^{MeOH}nm (log ε): 274 (3.55). Anal. Calcd. for $C_{36}H_{32}O_6$: C, 77.1; H, 5.8. Found: C, 77.1; H, 5.7.

Benzylated oligomer B: Anal. Calcd. for $(C_{36}H_{30}O_5)_{3.7}OH \cdot 3.7H_2O$: C, 78.1; H, 5.6. Found: C, 78.1; H, 5.4.

Oligomer B: Anal. Calcd. for $C_{15}H_{12}O_5 \cdot 0.3H_2O$: C, 64.9;, H, 4.6. Found: C, 65.2; H, 4.9.

Oligomer C

Monomer: Mp 168–170°C; UV λ_{max}^{MeOH}nm (log ε): 274 (3.30), 280 (sh, 3.22). Anal. Calcd. for $C_{36}H_{32}O_6$: C, 77.1; H, 5.8. Found: C, 76.9, H, 5.8.

Benzylated oligomer C: Anal. Calcd. for $(C_{36}H_{30}O_5)_{3.7}OH \cdot 1.5H_2O$: C, 78.0, H, 5.7. Found: C, 78.0; H, 5.5.

Oligomer C: Anal. Calcd. for $C_{15}H_{12}O_5 \cdot 0.5H_2O$: C, 64.0; H, 4.7. Found: C, 64.2; H, 5.4.

Oligomer D

Monomer: Mp 166–167°C; UV λ_{max}^{MeOH}nm (log ε): 260 (sh, 3.28), 266 (sh, 3.34), 270 (3.36), 274 (sh, 3.34), 281 (sh, 3.21). Anal. Calcd. for $C_{36}H_{32}O_6$: C, 77.1; H, 5.8. Found: C, 77.0; H, 5.7.

Benzylated oligomer D: Anal. Calcd. for $(C_{36}H_{30}O_5)_{3.7}OH \cdot 1.8H_2O$: C, 77.8; H, 5.7. Found: C, 77.8;, H, 5.4.

Oligomer D: Anal. Calcd. for $C_{15}H_{12}O_5 \cdot 0.7H_2O$: C, 63.0; H, 4.7. Found: C, 63.1; H, 5.4.

Oligomer E

Monomer: Mp 182–183°C; UV λ_{max}^{MeOH}nm (log ε): 260 (sh, 3.30), 2.66 (sh, 3.39), 271 (sh, 3.44), 274 (3.45). Anal. Calcd. for $C_{43}H_{38}O_7$: C, 77.4; H, 5.8. Found: C, 77.3; H, 5.8.

Benzylated oligomer E: Anal. Calcd. for $(C_{43}H_{36}O_6)_{3.7}OH \cdot 3.9H_2O$: C, 77.3; H,5.7. Found: 77.3; H, 5.3.

Oligomer E: Anal. Calcd. for $C_{15}H_{12}O_6 \cdot 0.6H_2O$: C, 60.2; H, 4.5. Found: C, 60.7; H, 5.2.

Oligomer G

Monomer: Mp 179–180°C; UV λ_{max}^{MeOH}nm (log ε): 259 (3.49), 265 (3.47), 270 (3.45). Anal. Calcd. for $C_{50}H_{44}O_8$: C, 77.7; H, 5.8. Found: C, 77.8; H, 5.8.

Benzylated oligomer G: Anal. Calcd. for $(C_{50}H_{42}O_7)_{3.7}OH \cdot 2.5H_2O$: C, 77.8; H, 5.7. Found: C, 77.8; H, 5.4.

Oligomer G: Anal. Calcd. for $C_{15}H_{12}O_7 \cdot 0.3H_2O$: C, 58.2; H, 4.1. Found: C, 58.4; H, 4.6.

Determination of the protein-precipitating capacity

The protein-precipitating capacity of each oligomer was evaluated by the method described in the previous paper[7].

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