Seasonal Changes of Flavanols in the Inner Barks of *Cryptomeria japonica* D. Don

Masahiro SAMEJIMA* and Tomotaka YOSHIMOTO*

1 Introduction

Flavanols are phenolic extractives widely distributed in higher plants, especially in woody plants[1]. Flavanols in plant tissues are usually mixtures of monomeric and various oligomeric forms. Oligomeric flavanols are composed of flavanol units chained with C_4—C_8 or C_4—C_6 linkages and this linkages are decomposed by acid treatment to give anthocyanidins. Therefore, oligomeric flavanols are generally called proanthocyanidins[2].

In Coniferae, much amounts of flavanols are universally distributed in foliage and bark[3),4)]. Moreover, the tissue clutured cells also produce flavanols in high yields[5),6)]. However, the structural characteristics of flavanols, such as the mean polymerization degree of flavanol unit, the composition of stereoisomers and hydroxylation patterns of flavanol units, are significantly different among species and tissues[4),6),7)]. Therefore, the fine structures of flavanols are thought to be characterized by both of phylogenetic and ontogenetic factors. Although little is known about the structural changes of flavanols in the same tissues in the same species, the authors could not observe any significant changes among various parts of the inner bark of *Cryptomeria japonica* as shown in our previous report[8)].

Recently, the authors have investigated on seasonal changes of the content and the structural characteristics of flavanols in the inner barks of *Cryptomeria japonica*. In the present paper, the results obtained are described.

2 Experimental

2.1 Sampling and extraction of the inner barks

The inner barks of *Cryptomeria japonica* D. Don were collected every four weeks from 20th May, 1980 to 19th May, 1981 in Tanashi experiment field of the Tokyo University Forest. The age of tree analyzed was five years old. Three samples of tree were felled and tested at each time. The barks were stripped from the freshly felled stem between 30cm and 90cm height from the ground. The outer barks were removed and discarded. The remaining inner barks were immediately cut into small chips, weighed, and then soaked in

* Department of Forest Products, Faculty of Agriculture, University of Tokyo.
東京大学農学部林産学科

one litter of methanol. The chips were extracted at room temperature for 24 hrs. After then, the chips were filtrated and the methanol extracts were obtained. The remaining chips were further soaked in another one litter of methanol and then homogenized by a Polytron in a ice bath for 15 min.. The mixture stood at room temperature for ca. 24 hrs. and then the methanol extracts were collected. The homogenized inner barks were further extracted by one litter of methanol three times. All of the methanol extracts were collected and concentrated and made up to 50 ml. The solution of the extracts obtained was used as a stock solution for the following analyses.

In parallel with this procedure, a part of the fresh inner barks were oven-dried at 105°C for 8 hrs. and determined the moisture content.

2. 2 Determination of the flavanol content

Total flavanol content of the fresh weight and the oven dried weight of the inner barks were determined according to the vanillin-HCI method previously described[4),9)].

2. 3 Structural analyses of flavanols

The mean molecular weight of the total flavanols were measured by gel permeation chromatography and the composition of the stereoisomers of monomeric and dimeric flavanols were determined by the HPLC analysis described in our previous report[10)].

3 Results

3. 1 Seasonal changes of total flavanol content

The seasonal changes of the total flavanol content by the fresh weight of the inner barks are shown in Fig. 1. The content at the first sampling day (20th May, 1980) was 3. 9%. From late spring to late summer (20th May to 9th September), the content rapidly decreased and the content measured at 9th September was 2.0%, which was one-half of the content at 20th May. On the other hand, during autumnal season, from 9th September to 27th December, the content increased significantly and reached to 5.0% at 27th December. During winter and early spring, the content slightly decreased to 3.7% at the last sampling day (19 May, 1981). This percentage was almost same as 3.9% at the first sampling day (20th May, 1980). The annual average of the flavanol content by the fresh weight of the inner barks was 3.7%.

The seasonal changes of the total flavanol content by the oven-dried weight of the inner barks have shown a similar tendency as those by the fresh weight of the inner barks because the moistural content in the inner barks was almost constant around 60% throughout the year. The average of the total flavanol content by the oven-dried weight was 8.9% (see Fig. 2).

3. 2 Seasonal changes of the molecular weight distribution of the total flavanols

The mean molecular weight of the total flavanols was determined by gel permeation chromatography of the methylated crude methanol extracts by detection of UV absorption at 280 nm. The typical chromatogram is shown in Fig. 3. The molecular weight of the total flavanols distributed from 300 to 3,000 and this pattern was almost constant throughout the

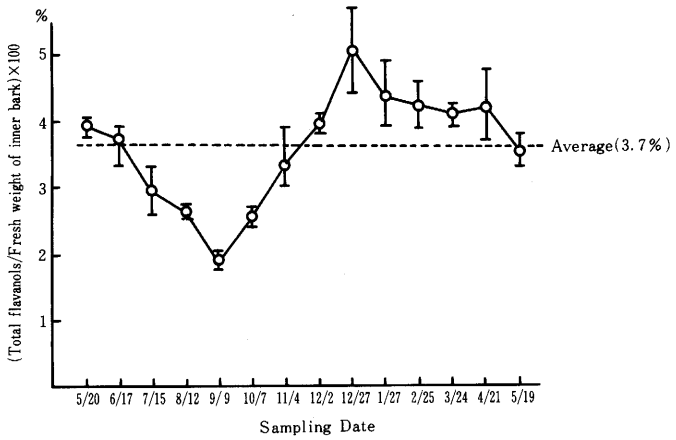

Fig. 1 Seasonal changes of total flavanol content by the fresh weight of the inner barks of *Cryptomeria japonica*

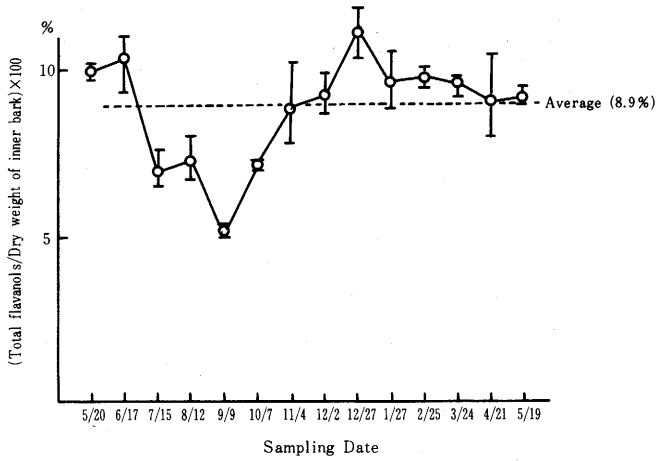

Fig. 2 Seasonal changes of total flavanol content by the oven-dry weight to the inner barks of *Cryptomeria japonica*

year. The number average weight of total flavanols was 880 and the mean polymerization degree of the flavanol unit was 3.0. Differences of the mean molecular weight among each sample at the same sampling day were very small (see Fig. 4).

3. 3 Seasonal changes of the composition of the stereoisomers of monomeric and dimeric flavanols

Monomeric flavanols in the inner barks are composed of two stereoisomers; i. e., (+) −catechin (1) and (−) −epicatechin (2). On the other hand, dimeric flavanols in the inner barks are composed mainly of procyanidin B-3 (3) and B-4 (4). The other

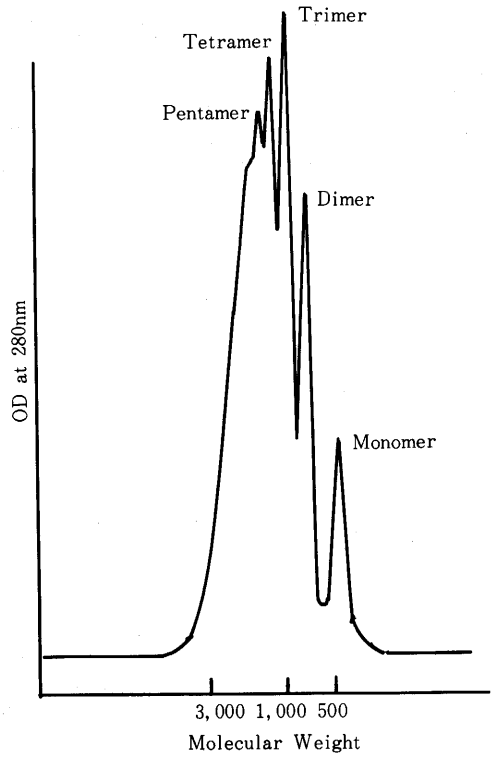

Fig. 3 Gel permeation chromatogram of the methylated extracts from the inner barks of *Cryptomeria japonica*

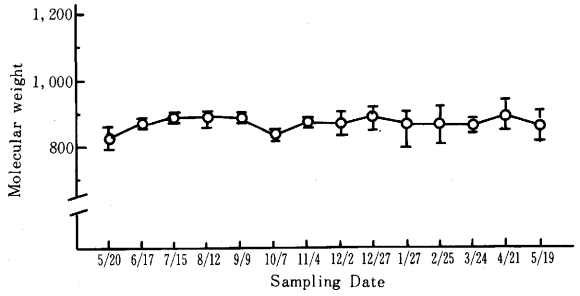

Fig. 4 Seasonal changes of the number average weight of total flavanols from the inner barks of *Cryptomeria japonica*

stereoisomers of the dimers were detected in only trace amounts. Concerning the composition of these compounds, no significant seasonal changes were observed (Fig. 6 and Fig. 7). However, some variations were detected among each sample at the same sampling

day and the variations were larger during the growth period (from late spring to early autumn) than during the rest season (from late autumn to early spring). The annual average ratio of (+) −catechin (1) to (−) −epicatechin (2) and those of procyanidin B-3 (3) to B-4 (4) were 78 : 22 and 67 : 33, respectively.

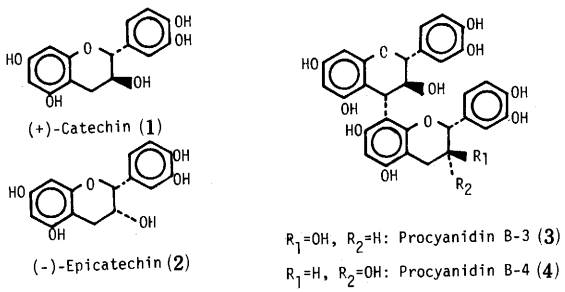

Fig. 5 Monomeric and dimeric flavanols from the inner barks of *Cryptomeria japonica*

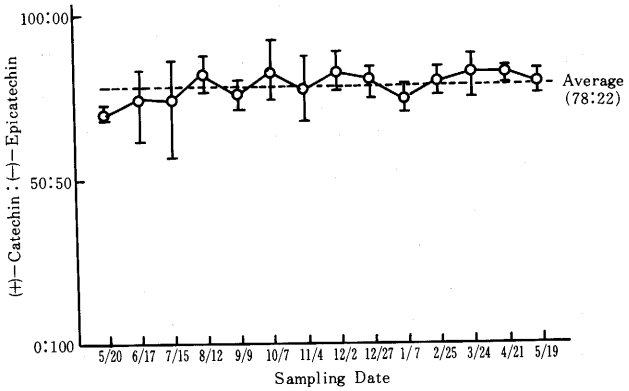

Fig. 6 Seasonal changes of the proportion of the stereoisomers of monomeric flavanols from the inner barks of *Cryptomeria japonica*

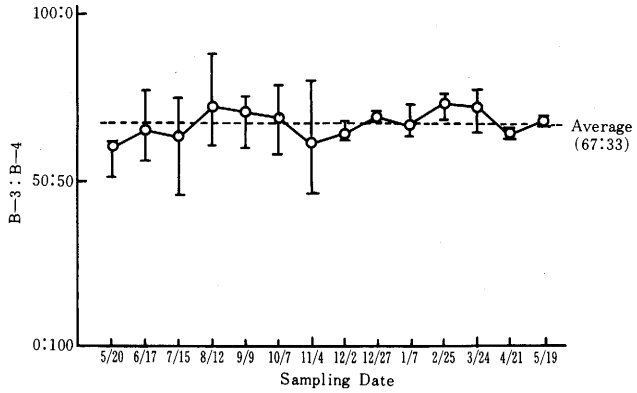

Fig. 7 Seasonal changes of the proportion of the stereoisomers of dimeric flavanols from the inner barks of *Cryptomeria japonica*

4 Discussion

Two possible reasons are considered to account for the significant decreasing of the total flavanol content in the inner barks during late spring and summer. The first reason is that only small amount of flavanols might be produced in the inner barks during this period whereas much amount of cell wall component is produced for cell division and for cell elongation because the cambial activities are very high in this period[11),12)]. Therefore, relative concentration of flavanols in the inner barks might be decreased. Another reason is that some amount of flavanols might be catabolized in this season. However, little is known about catabolism of flavanols in plant tissues.

During autumnal season, the total flavanol content in the inner barks increased rapidly. This phenomenon suggested that much amount of flavanols might be produced during this period whereas cell wall components not produced because cambial activities are very weak in this period[12)]. The pattern of seasonal changes of flavanol content in the inner barks is considered to be very similar to seasonal changes of early and late wood formation in xylem. From these observation, the control of flavanol production is considered to be one of most fundamental physiological phenomenon in the inner barks of coniferous trees.

No seasonal changes were observed on the molecular weight distribution of total flavanols in the inner barks. Therefore, the physiological factors is considered to be less effective to the polymerization degree of flavanols in the inner barks.

Furthermore, no seasonal changes were also observed on the compositions of the stereoisomers of monomeric and dimeric flavanols. Although considerable variations of the compositions were observed among each sample within the same sampling day, these variations are definitely smaller than those among different species or different tissues as

previously reported[4),6),8)].

Therefore, the structural characteristics of flavanols are considered to be determined mainly by genetic factors among species and in course of cell differentiation rather than by physiological factors in the same tissues.

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Summary

Seasonal changes of the flavanol content and the structural characteristics in the inner barks of *Cryptomeria japonica* D. Don were investigated and the following results were obtained.

i) Flavanol content in the inner barks decreased rapidly during late spring and summer (from 20th May to 9th September). On the other hand, the flavanol content increased rapidly during autumnal season (from 9th September to 27th December).

ii) The mean molecular weight of total flavanol are almost constant throughout the year.

iii) No seasonal changes were observed on the composition of stereoisomers of monomeric and dimeric flavanols in the inner barks.

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スギ内樹皮中でのフラバノール類の季節変化

鮫島正浩，善本知孝

要　　旨

スギ内樹皮中のフラバノール類の季節変化について調べた。得られた結果はつぎのようである。

i）内樹皮のフラバノール含有量は晩春から夏にかけて急速に減少する。これに対して，秋期において内樹皮中のフラバノール類は急速に増加する。内樹皮の生重量に対するフラバノール量は最高が12月27日の5.0%で最低が9月9日の2.0%であった。そして，年平均が3.7%であった（Fig. 1）。内樹皮の含水率は年間を通して約60%前後であるので，内樹皮の絶乾重量に対するフラバノール量の季節変化も生重量に対する場合とほぼ同様の傾向を示した（Fig. 2）

ii）内樹皮中に存在するフラバノール類の分子量分布は年間を通してほとんど変化しない。フラバノール類の数平均分子量は880，平均重合度は3.0であった（F_{1_6}　。

iii）内樹皮中に存在するフラバノール単量体および二量体を構成する立体異性体の構成比は年間を通じてめだった変化を示さなかったが，同一測定日においても検体によってやや構成比が異っていた（Fig. 6, Fig. 7）。

フラバノール類の量的な季節変化は形成層の活動の変化とよく類似した傾向を示しており，形成層の活動が活発な春から夏にかけては減少し，活動が弱まる秋期において増加する。このことはフラバノールの生成が樹木の最も基礎的な生理現象の変化とよく対応していることを示している。

フラバノール類の化学構造的特徴は内樹皮中の生理的因子の変化をあまり強く受けないようである。これに対して，フラバノール類の構造的特徴は樹種差あるいは同一樹種でも組織の差によって著しく異なる。このことはフラバノール類の構造的特徴が生理的因子よりもむしろ遺伝的因子によって決定されていることを示唆している。