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短報（Note）

A Heartwood Norlignan, Agatharesinol, was Generated in Sapwood during Withering of a Sugi (*Cryptomeria japonica* D. Don) Log

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Abstract

Sugi (*Cryptomeria japonica* D. Don) heartwood contains characteristic phenolic compounds called norlignans, which are synthesized during heartwood formation. The pathways of norlignan biosynthesis are unknown. Norlignans have been reported to generate in sapwood during withering of *C. japonica* logs. Assuming that this phenomenon would be helpful for studying the mechanisms of norlignan biosynthesis, we investigated the composition of norlignans generated in the withering sapwood of *C. japonica*, taking the changes of moisture content into consideration. Wood discs were collected over 70 days from a log stored indoors. The level of a single norlignan species gradually increased in the inner sapwood and reached a maximum on the 41st day after cutting, when the moisture contents of the sapwood had dropped to the level observed in the transition zone immediately after cutting. The norlignan was identified by gas chromatography-mass spectrometry as agatharesinol, a typical heartwood norlignan of *C. japonica*. These results show that the generation of norlignans in sapwood of cut logs provides a useful model system for studying the molecular mechanisms of norlignan biosynthesis.

Key words : *Cryptomeria japonica*, heartwood formation, norlignan, norlignan biosynthesis, sapwood, Sugi, withering process

Introduction

Heartwood formation is a characteristic event in the development of woody plants. It is accompanied by the accumulation of specific extractable compounds which affect the color, fragrance, and durability, and therefore the economic value of the wood.

Sugi (*Cryptomeria japonica* D. Don) wood is frequently used as a building and furnishing material in Japan. Its heartwood contains phenolic compounds with diphenylpentane carbon skeletons (C_6-C_5-C_6) called norlignans. Norlignans are assumed to influence the color of the heartwood of *C. japonica* (Kai and Teratani, 1977; Takahashi, 1981). The elucidation of norlignan biosynthetic pathways might contribute to a better understanding of the properties of *C. japonica* wood. Several hypothetical pathways have been suggested (Begley et al., 1973; Enoki et al., 1977; Erdtman and Harmatha, 1979). Recently, (*Z*)-hinokiresinol (nyasol) was reported to be synthesized from 4-coumaryl alcohol and 4-coumaroyl CoA in cultured cells of *Asparagus officinalis* after fungal elicitor treatment (Suzuki et al., 2001). In addition, it was also shown that an enzyme preparation from fungal-elicited asparagus cultured cells catalyzed the formation of the norlignan from 4-coumaryl alcohol and 4-coumaroyl CoA (Suzuki et al., 2002). However, little experimental evidence has been available for the biosynthesis of other norlignans, especially in woody plants, because norlignans are present in a limited number of species, and their biosynthesis in woody plants appears restricted to the xylem where it occurs in a particular season (Nobuchi et al., 1982).

Agatharesinol and sugiresinol, two norlignans commonly found in *C. japonica* heartwood, were generated in the sapwood during the withering process of *C. japonica* logs that were collected in different seasons and stored indoors (Ohashi et al., 1988, 1990). On the basis of these findings, we used a withering log as an experimental system for investigating the composition of norlignans generated in the sapwood during the withering process, taking the changes of moisture content into consideration.

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Materials and Methods

Preparation of samples

A 15-year-old *C. japonica* tree that grew in the nursery garden of the Forestry and Forest Products Research Institute (Tsukuba) was cut down in May 2001. A 0.8 m-long trunk section from a height of 1 m above the ground was excised and stored in a room at 20-25 ºC. Six cm-thick disks were cut off from the bottom end of the log immediately after cutting the tree, and on the 10th, 20th, 41st and 70th day of the withering process, respectively. The disks were divided into outer and inner sapwood, and transition zone (TZ). These samples were cut into small pieces which were soaked in methanol (MeOH).

Extraction and analysis of norlignans

The wood pieces were extracted with MeOH using Soxhlet's extractor. The extracts were evaporated under reduced pressure and were fractionated first with *n*-hexane and then with ethyl acetate (EtOAc). The EtOAc soluble fraction was applied to a column filled with 0.2 g silica gel (Wakogel C-200). The effluent was collected and analyzed by high performance liquid chromatography (HPLC) using a ZORBAX eclipse XDB-C8 column (5 µm, 4.6 x 140 mm) eluted with 19% acetonitrile at a flow rate of 1 ml min^{-1}. Norlignans were monitored at 265 nm and identified by comparing the retention times (Rt) with those of authentic compounds. Agatharesinol was identified by gas chromatography-mass spectrometry (GC-MS). Samples were trimethylsilylated according to common methods. The GC-MS conditions were as follows: column OV-1 (0.25 mm x 25 m), carrier gas He, flow rate 1 ml min^{-1}, temperature rising from 150 ºC by 4 ºC min^{-1} up to 280 ºC, and electron impact ionization (70 eV).

Measurement of moisture contents

A part of the wood pieces prepared as described above were weighed before and after drying at 105 ºC for 24 h. Percentage moisture content was calculated as (fresh weight – dry weight (DW)) * DW^{-1} * 100.

Results and Discussion

Generation of a norlignan in sapwood during the withering process

We chose the inner sapwood for the analysis of norlignans, because inner sapwood was reported to generate larger amounts of norlignans than outer sapwood during the withering process of *C. japonica* logs (Ohashi et al., 1990).

In the EtOAc soluble fraction from fresh inner sapwood, no prominent peaks corresponding to norligans were detected by HPLC (Fig. 1). On the 10th day of the withering process, a peak (Rt 4.6 min) appeared which did not correspond to any norlignan used as authentic compounds. On the 20th day, another peak (Rt 6.7 min) became dominant and increased markedly until the 41st day. Analysis of the sample fraction containing this peak by GC-MS showed that the compound was agatharesinol.

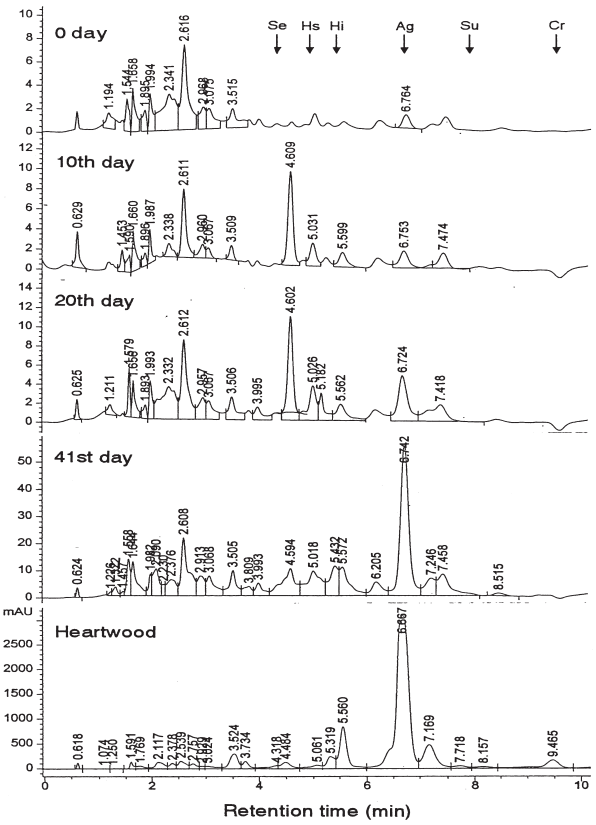

Fig. 1. HPLC chromatograms of ethyl acetate soluble fractions from inner sapwood (0, 10, 20 and 41 days after cutting a *C. japonica* tree) and heartwood. Vertical arrows indicate the retention times of authentic norlignans. Se, sequirin-C; Hs, hydroxysugiresinol; Hi, (*E*)-hinokiresinol; Ag, agatharesinol; Su, sugiresinol; Cr, cryptoresinol. The scales of vertical axes differ between the chromatograms.

The time-course of agatharesinol content during withering showed trace amounts of agatharesinol in fresh inner sapwood (Fig. 2), which was consistent with

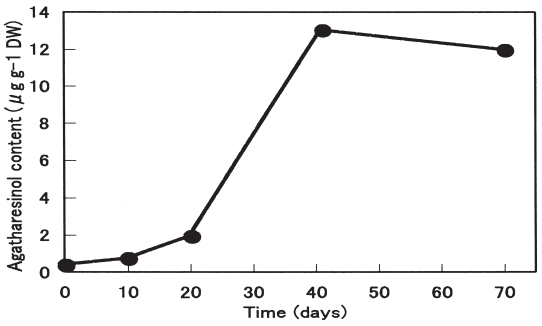

Fig. 2. Change of agatharesinol contents in the inner sapwood during the withering process of a *C. japonica* log.

a previous report (Ohashi et al., 1990). The agatharesinol content gradually increased until the 20th day, and then rose sharply to reach a maximum on day 41. This maximum content amounted to 0.7% of that (1.8 mg g^{-1} DW) in heartwood. On the 70th day the agatharesinol level was slightly decreased. These results suggest that the biosynthesis of agatharesiol was most active between the 20th and the 41st day.

Agatharesinol was the sole norlignan detected in this study, whereas Ohashi et al. (1990) reported the generation of some norlignans such as sugiresinol, in addition to dominant agatharesinol, during the withering process. A possible reason for this discrepancy is the lack of ability to produce most norlignans in *C. japonica* used in this study, since norligans other than agatharesinol were barely detectable in its heartwood (Fig. 1). The composition of norlignans in heartwood varies among races of *C. japonica* (Takahashi, 1981). The biosynthetic process of norlignans may stop after producing agatharesinol in the discolored *C. japonica* sapwood formed by the invasion of some insects (Takahashi and Ogiyama, 1986). Thus, another possibility is that norlignan biosynthesis pauses at the step of agatharesinol in injured sapwood. However, it is not known whether our experimental treatment and invasion of insects have the same effect on initiation of norlignan biosynthesis in sapwood.

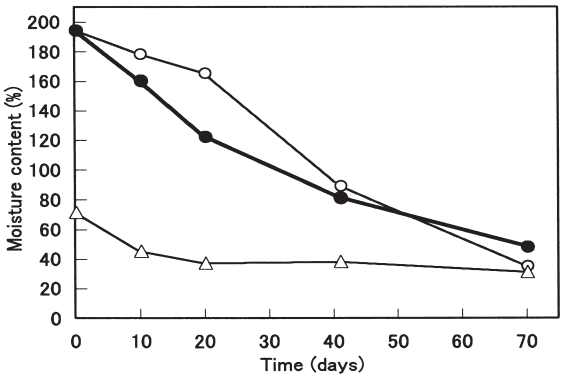

Fig. 3. Changes of moisture contents in the inner sapwood (closed circle), the outer sapwood (open circle), and the transition zone (open triangle) during the withering process of a *C. japonica* log.
Each data point represents the mean of two independent measurements.

Changes of moisture contents during the withering process

Heartwood extractives are thought to be synthesized in the TZ in standing trees (Magel, 2000). One of the characteristics of the TZ is a lower moisture content as compared to sapwood (Nobuchi and Harada, 1983). It has been suggested that entry of air into vessels or tracheids results in the induction of heartwood formation (Thomas, 2000).

The initial moisture content was 194% in inner and outer sapwood, and 71% in TZ (Fig. 3). The moisture content gradually decreased, and that of the sapwood dropped to the level of fresh TZ on the 41st day. During this period, agatharesinol is accumulated in the sapwood (Fig. 2). This process may mimic the initial stage of heartwood formation, characterized by the deposition of heartwood constituents occurring in standing trees.

The generation of heartwood norlignans in withering sapwood of *C. japonica* provides a useful system for investigating the molecular mechanism of norlignan biosynthesis. Future research will aim at identifying genes that encode enzymes involved in this process.

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スギ丸太の乾燥過程における辺材での心材ノルリグナンの生成

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要　旨

スギ（*Cryptomeria japonica* D. Don）は心材にノルリグナンと総称される特徴的なフェノール性成分を含有する。ノルリグナンは心材形成に伴って合成されるが、その生合成経路は明らかにされていない。スギ丸太の乾燥過程において辺材中にノルリグナンが生成するという報告があった。この現象をノルリグナンの生合成機構を調べる実験系として利用できるのではないかと考え、丸太の乾燥過程において辺材で生成するノルリグナンの組成を、含水率の変化に着目して分析した。スギを伐採後、丸太を切り出して室内に放置し、伐倒直後、10、20、41 および 70 日後にそれぞれ木口から円盤を切り取り、ノルリグナンや含水率の経時変化を調べた。辺材内方においてある種のノルリグナンが次第に増加し、含水率が伐倒直後の移行材と同程度まで低下した 41 日後に最多となった。ガスクロマトグラフィー - 質量分析によりこのノルリグナンをアガサレジノールと同定した。アガサレジノールはスギ心材に一般に含まれる。これらの結果は、乾燥過程における辺材でのノルリグナンの生成が、その生合成機構を研究するうえで有用な実験系であることを示している。

キーワード：*Cryptomeria japonica*、心材形成、ノルリグナン、ノルリグナン生合成、辺材、スギ、乾燥過程

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