J Wood Sci (2006) 52:134–139
DOI 10.1007/s10086-005-0733-8

ORIGINAL ARTICLE

Koetsu Takahashi · Keisuke Mori

Relationships between blacking phenomenon and norlignans of sugi (*Cryptomeria japonica*) heartwood III: coloration of norlignans with alkaline treatment

Received: July 28, 2004 / Accepted: April 12, 2005 / Published online: February 1, 2006

Abstract We investigated norlignan coloration with alkaline treatment to clarify the relationship between the blackening of sugi (*Cryptomeria japonica* D. Don) heartwood and norlignans in the heartwood. Of the four main norlignans (agatharesinol, sequirin-C, sugiresinol, hydroxysugiresinol) of sugi heartwood, only sequirin-C was clearly colored with alkaline treatment (0.4% potassium hydrogencarbonate solution, pH 8.6). Sequirin-C changed color to deep purple with alkaline treatment. The absorbance spectrum of colored sequirin-C had two peaks (450 and 525 nm) and one shoulder (626 nm). Coloration began at pH 6.2.). The spectrum of blackened sugi heartwood was similar to that of sequirin-C treated with alkali. A catechol nucleus and a double bond conjugated with a benzene nucleus play important roles in norlignan coloration with alkaline treatment. The number of hydroxyl groups is related to solubility in alkaline solution, and, therefore, whether a norlignan changes color also depends on the number of hydroxyl groups. Based on these results, we concluded that sugi heartwood turns black because sequirin-C, which is readily soluble in alkaline solution and can form a large intramolecular conjugation system when alkalized, is converted to products with a deep purple color as the heartwood is basified.

Key words *Cryptomeria japonica* · Heartwood · Blacking phenomenon · Norlignans · Alkaline treatment

Introduction

We reported the relationship between the blackening of sugi heartwood and norlignans in the first report of this study[1] based on a study using individuals in which the main components were agatharesinol and sequirin-C. In the second report,[2] which precedes the present report, the relationship was based on a study using individuals in which the main components were sugiresinol and hydroxysugiresinol.

In both studies, parts of heartwood that are normally red showed acidic pH values and a high content of sequirin-C, while the blackened parts of heartwood showed alkaline pH values and a low content of sequirin-C. The blackened parts had a significantly high content of potassium hydrogencarbonate.

Ishiguri et al. also reported that sequirin-C has a definite relationship with the stabilization of heartwood color based on a series of studies involving the smoke heating of sugi.[3–5]

These studies have suggested that changes in the quality of sequirin-C and the pH value in heartwood are important factors in the blackening of heartwood. Therefore, we investigated the coloration mechanism with alkaline treatment by using norlignans (known to exist in sugi) as the main samples.

K. Takahashi (✉) · K. Mori
Section of Forest Environment and Resource, Department of Bioenvironment, Faculty of Agriculture, Yamagata University, Tsuruoka 997-8555, Japan
Tel. +81-235-28-2926; Fax +81-235-28-2843
e-mail: tkoetsu@tds1.tr.yamagata-u.ac.jp

Materials and methods

Sample material

Five norlignans (agatharesinol, sequirin-C, sugiresinol, hydroxysugiresinol, and cryptoresinol) were isolated from sugi heartwood, and two norlignans (hinokiresinol, yateresinol), and 1,4-bis-(*p*-hydroxyphenyl)-butadiene (C_{16} compound) were isolated from damaged sugi wood as samples. Ferruginol, sugiol, and xantopherol were also isolated from sugi heartwood as samples of diterpene phenol.

Alkaline treatment of norlignans

Five milliliters of 0.4% potassium hydrogencarbonate solution (pH 8.6) was added to 1 mg of each sample.

pH adjustment

The pH of each sample was adjusted to specified values from 3 to 12 with acetic acid, potassium hydrogencarbonate, and potassium hydroxide.

Absorption spectrum measurement

The visible absorption spectra (370–750 nm) of each sample were measured using the Shimadzu spectrophotometer type UV-1600PC.

Gas chromatography analysis

Each sample was neutralized and ethyl acetate was then added to each. A separating funnel was used to extract and isolate the parts soluble in ethyl acetate. These parts were concentrated, then silylated and analyzed under the same conditions as described in the first report.[1] Moreover, the 0.4% potassium hydrogencarbonate solution used for alkaline treatment was analyzed at 15 min and at 1, 2, 4, 8, 12, 16, and 24 h after alkaline treatment.

Measurement of absorption spectrum of blackened heartwood

Blackened heartwood was collected from sugi in the seedling field at the Yamagata University Agriculture Faculty experimental plantation. After the pH (8.0) of the blackened heartwood was measured, 10 ml of potassium hydrogencarbonate solution (pH 8.0) was added to 2 g of sample. The 2-g sample was ground in a mortar and pestle, filtered by using suction, then measured for absorbance 1, 2, 4, 8, 12, 16, and 24 h later.

Results and discussion

Coloration of sugi norlignans with alkaline treatment (0.4% potassium hydrogencarbonate solution)

It is reported that norlignans change color through air oxidation and enzyme oxidation. Kai et al.[6] reported that a colored compound with an absorption spectrum similar to that of sugi forms from hydroxysugiresinol treated with an enzyme via an intermediate product (dehydrohydroxysugiresinol), which shows maximum absorbance at 410 nm. We also reported that hydroxysugiresinol and sequirin-C change to deep reddish-brown compounds, with maximum absorbance at 440 nm through air oxidation.[7] It is also known that hinokiresinol, yateresinol, 1,4-bis-(*p*-hydroxyphenyl)-butadiene, and cryptoresinol change to bright red, orange, yellow, and pink, respectively, through air oxidation.[6,8] There is no report, however, that details norlignan coloration with alkaline treatment.

We treated the main norlignans of sugi heartwood (agatharesinol, sequirin-C, sugiresinol, hydroxysugiresinol) with 0.4% potassium hydrogencarbonate solution to measure their absorption spectra 2 h later (Fig. 1) because sugi reportedly blackens rapidly for 1–2 h in potassium hydrogencarbonate (pH 8.6).[9,10] Agatharesinol and sugiresinol did not change color, but sequirin-C and hydroxysugiresinol did. Sequirin-C rapidly changed color to a deep purple and showed an absorption spectrum with peaks at 450 nm and 525 nm, and a shoulder at 625 nm. Hydroxysugiresinol colored slightly to pink, but did not show a clear spectrum, while cryptoresinol did not change color. Hydroxysugiresinol and agatharesinol showed weak absorption spectra (shown in Fig. 1) at or above pH 10 and pH 12, respectively.

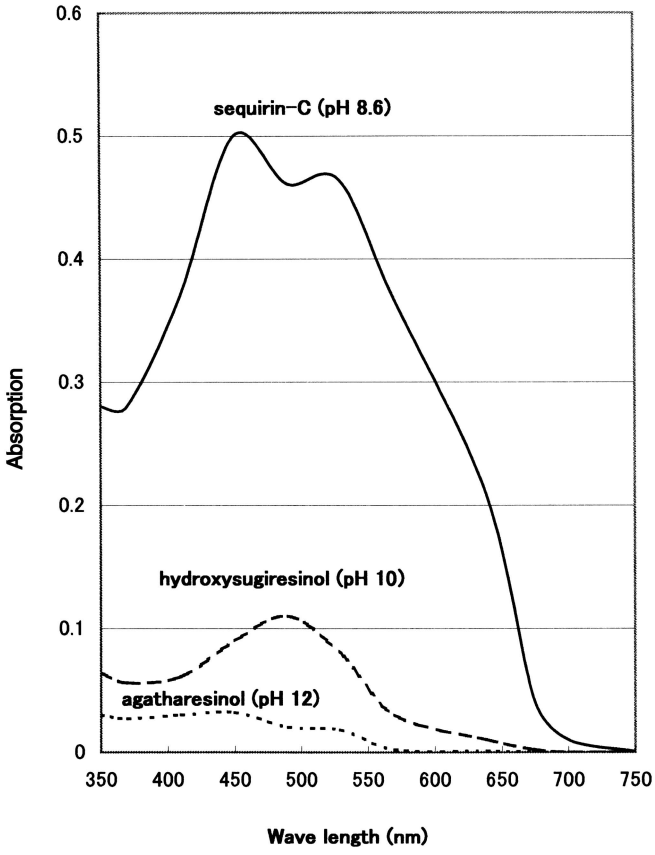

Fig. 1. Absorption spectra of colored norlignans 2 h after alkaline treatment

Hinokiresinol, yateresinol, and 1,4-bis-(*p*-hydroxyphenyl)-butadiene showed no clear absorption spectrum, although hinokiresinol slightly changed color to pink.

Figure 2 (taken 2 h after treatment) shows the coloration of norlignans with alkaline treatment, and clearly shows that only sequirin-C is clearly colored (deep purple).

Relationship between pH value and coloration

We investigated the coloration of three norlignans (sequirin-C, hydroxysugiresinol, hinokiresinol) in the pH range of 3–12. Sequirin-C did not color, but remained stable at and below pH 6. It started coloring at pH 6.2 and colored

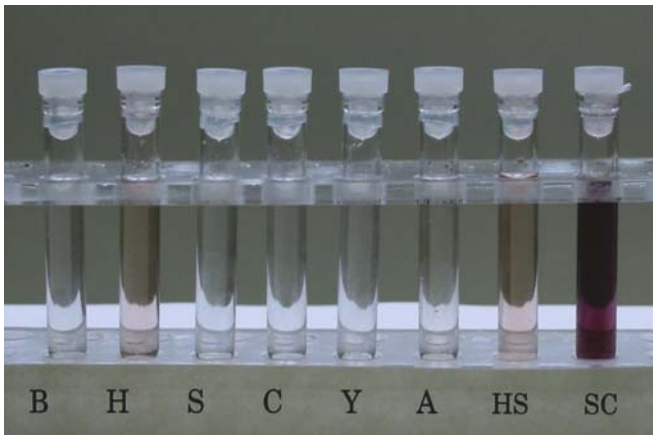

Fig. 2. Coloration of norlignans by alkaline treatment. *B*, 1,4-bis(*p*-hydroxyphenyl)-butadiene (C_{16} compound); *H*, hinokiresinol; *S*, sugiresinol; *C*, cryptoresinol; *Y*, yateresinol; *A*, agatharesinol; *HS*, hydroxysugiresinol; *SC*, sequirin-C

rapidly at and above pH 8. Hydroxysugiresinol clearly colored only at and above pH 10, while hinokiresinol did not clearly change color even at and above pH 9.

It was concluded that sequirin-C is very sensitive to changes in pH, while other norlignans hardly change color even at high pH.

Relationship between alkaline treatment time and coloration

The absorption spectrum of sequirin-C was measured at specified times. It gradually colored after alkaline treatment (with 0.4% potassium hydrogencarbonate solution), and the maximum absorbance occurred 2 h later. Then the absorbance decreased, but the sample remained colored (deep purple) up to 24 h after alkaline treatment (Fig. 3).

Thus, the time taken to blacken sugi heartwood with alkaline treatment was almost the same as the time that elapsed after alkaline treatment to reach the maximum absorbance of sequirin-C.

Decrease of main norlignans with alkaline treatment

We investigated how quantities of the main four norlignans of sugi heartwood decrease over time after alkaline treatment (pH 8.6). The results are shown in Fig. 4.

Sugiresinol and agatharesinol decreased by 6%–8% immediately after alkaline treatment, but subsequently showed little decrease. Hydroxysuginol and sequirin-C began decreasing immediately after treatment and continued to decrease. The decrease of sequirin-C was the most remarkable.

Absorption spectrum of blackened heartwood

The absorption spectrum of blackened heartwood was measured after alkaline treatment at specified times (Fig. 5).

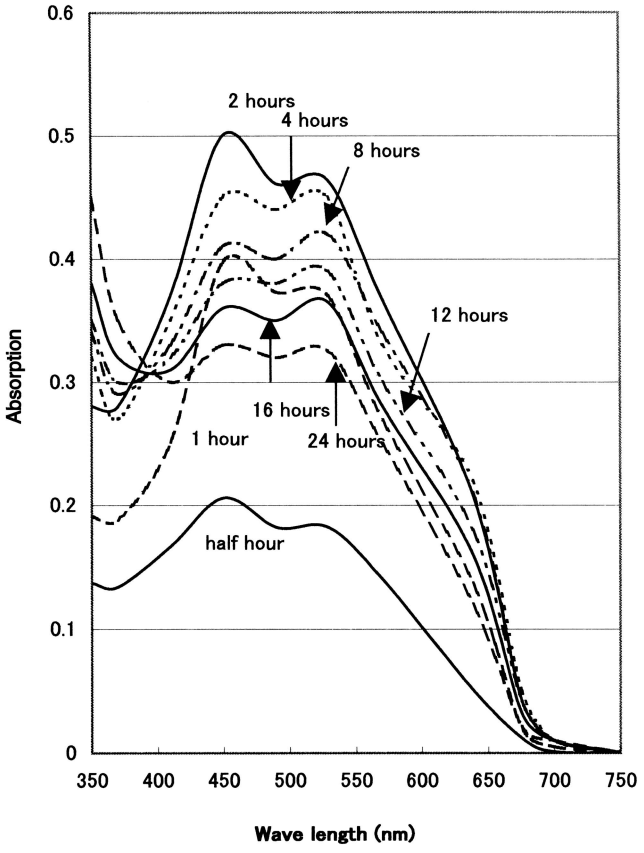

Fig. 3. Absorption spectra of sequirun-C for different times after alkaline treatment

The absorbance increased continuously and reached a maximum 2 h after the start of measurement, and then gradually decreased. The total absorbance of the absorption spectrum was low 24 h after the start of measurement. However, the spectrum (with peaks at 450 and 525 nm) is similar to that of sequirin-C treated with alkali.

Thus, it was confirmed that the absorption spectra of blackened heartwood and alkalized sequirin-C are similar to each other and change similarly as time elapses after alkaline treatment.

Coloration of diterpene phenol

It is known that the highest content of ferruginol is found in sugi heartwood as diterpene phenol (including other diterpene phenol, sugiol, and xantopherol). These were treated with alkali to investigate their coloration. Ferrginol and sugiol hardly dissolved in alkaline solution and were not colored, while xantopherol easily dissolved and changed color from light yellow to yellow.

We concluded that differences in solubility in alkaline solution influenced the coloration and that diterpene phenol coloration does not affect the blackening of sugi heartwood because no diterpene phenol changed to purple or black.

Relationship between structure and coloration of norlignans

For the relationship between pH and the coloration of polyphenols, anthocyanins and flavones have often been investigated.[11] Generally, anthocyanins are stable while acidic, but change to various colors as the pH increases. Flavones are stable while acidic or neutral, but cleave, become colored, and decompose when basified.

Only a few investigations have been conducted on norlignan coloration, except for air oxidation. Table 1 shows how norlignans are colored with alkaline treatment and air oxidation, and lists the structural features of each norlignan.

Sugiresinol and agatharesinol do not change color with alkaline treatment and air oxidation. All other norlignans change color through air oxidation, but only hinokiresinol, hydroxysugiresinol, and sequirin-C change color with alkaline treatment. The coloration of hinokiresinol and hydroxysugiresinol with alkaline treatment are not remarkable, only sequirin-C changes color significantly with alkaline treatment. There are two possible reasons for the difference in norlignan coloration with alkaline treatment: the structural difference and the difference in solubility.

A rough sketch of the structural features of sugi norlignans is shown in Fig. 6. The basic structure of

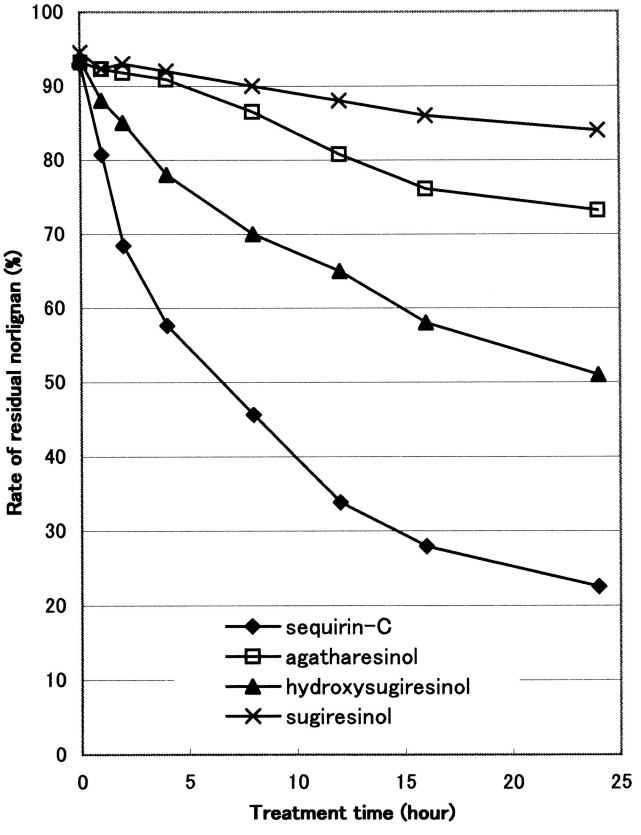

Fig. 4. Amount of residual norlignans after alkaline treatment

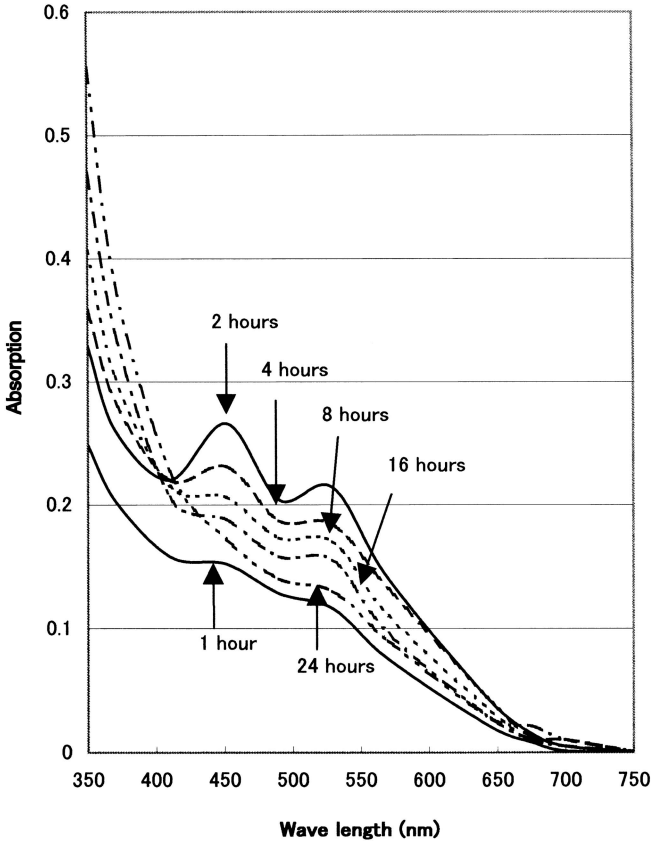

Fig. 5. Absorption spectra of the alkaline solution of blank heartwood for different times after alkaline treatment

Table 1. The structural features of norlignans and their coloration by air oxidation and alkaline treatment

Norlignan	Coloration		Structure		
	Air oxidation	Alkaline treatment	Hydroxyl group	Double bond	Catechol
Butadiene[a]	Yellow (420, 463 nm)	None	2	2	0
Hinokiresinol	Red (475, 510 nm)	Light pink	2	2	0
Sugiresinol	None	None	3	0	0
Cryptoresinol	Pink (490, 530 nm)	None	3	1	0
Yateresinol	Vermilion (435 nm)	None	4	1	0
Agatharesinol	None	None	4	1	0
Hydroxysugiresinol	Red-brown (440 nm)	Light pink	4	0	1
Sequirin-C	Red-brown (440 nm)	Deep purple (450, 525, 625 nm)	5	1	1

[a] 1,4-bis-(*p*-hydroxyphenyl)-butadiene (C_{16} compound)

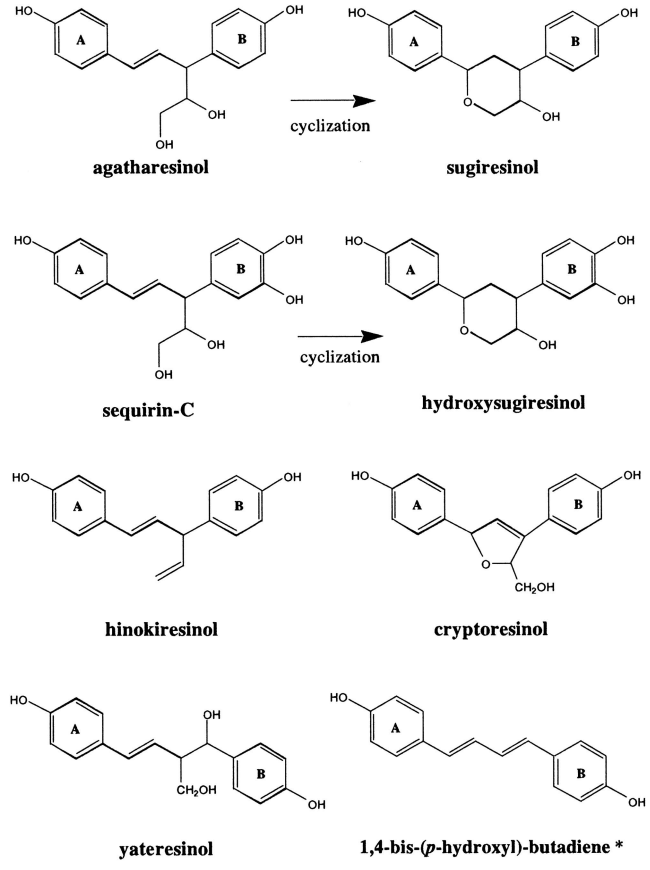

Fig. 6. Structures of norlignans in sugi (*Cryptomeria japonica* D. Don). *Asterisk* shows C_{16} compound

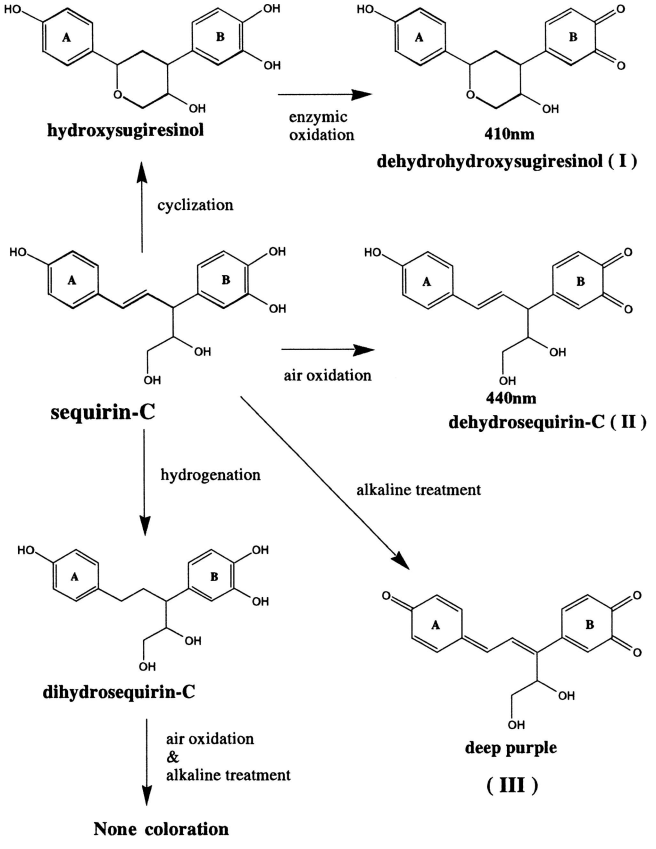

Fig. 7. Coloration mechanism of sequirin-C

norlignans is that ring A forms a *p*-hydroxyphenyl nucleus, ring B forms a *p*-hydroxyphenyl or catechol nucleus, and the side chain contains a double bond conjugated with a benzene nucleus or forms a ring. Also, there are two to five hydroxyl groups.

The structure of agatharesinol, which is not colored, is similar to that of sequirin-C, which does change to a dark color. The structural difference is the structure of ring B. Ring B of agatharesinol forms a *p*-hydroxyphenyl nucleus, while that of sequirin-C forms a catechol nucleus. The structural difference between hydroxysugiresinol and sequirin-C is the structure of the side chain. The side chain of hydroxysugiresinol is in the ring-closed form, while in sequirin-C the side chain is ring-open. Moreover, dihydrosequirin-C does not color,[12] showing that conjugation of the double bond in the side chain with a benzene nucleus is related to coloration. Thus, it is reasonable to assume that the structural features of a catechol nucleus, a ring-open side chain, and a double bond conjugated with a benzene nucleus have a significant effect on norlignan coloration.

Hinokiresinol with two hydroxyl groups changes to bright red through air oxidation, but hardly colors with alkaline treatment. This is because hinokiresinol is barely soluble in alkaline solution. Sequirin-C with five hydroxyl groups readily dissolves in alkaline solution and changes to deep purple with alkaline treatment. This suggests that the number of hydroxyl groups, which is related to solubility in alkaline solution, influences coloration.

The proposed coloration mechanism of sequirin-C treated with alkali is shown in Fig. 7. It is known that hydroxysugiresinol changes color through enzyme oxidation by changing into dehydrohydroxysugiresinol (**I**), of which ring B has an *o*-quinone structure, and shows maximum absorbance at 410 nm.[6] The colored matter (showing maximum absorbance at 440 nm) into which sequirin-C changes through air oxidation is thought to be dehydrosequirin-C (**II**). It is also conjectured that sequirin-C changes into dehydrosequirin-C (**III**) with the ring A structure changed to a *p*-quinone structure with alkaline treatment. Therefore, we believe that sequirin-C changes color to deep purple with alkaline treatment due to the formation of a large conjugated system.

It is thus suggested that sugi heartwood is mainly blackened because sequirin-C is chemically altered to give products with a deep purple color as the heartwood is alkalized. Various constituents[13–15] other than norlignans are found in sugi heartwood. Therefore, more detailed investigations must be conducted.

References

1. Takahashi K (1996) Relationships between the blacking phenomenon and norlignans of sugi (*Cryptomeria japonica* D. Don) heartwood I. A case of partially black heartwood (in Japanese). Mokuzai Gakkaishi 42:998–1005
2. Takahashi K (1998) Relationships between the blacking phenomenon and norlignans of sugi (*Cryptomeria japonica* D. Don) heartwood II. On blacking heartwood containing two main norlingans. Sugiresinol and hydroxysugiresinol (in Japanese). Mokuzai Gakkaishi 44:125–133
3. Maruyama S, Ishiguri F, Andoh M, Abe Z, Yokota S, Takahashi K, Yashizawa N (2001) Reddening by UV irradiation after smoke-heating in sugi (*Cryptomeria japonica* D. Don) black heartwood. Holzforschung 55:347–354
4. Ishiguri F, Maruyama S, Takahashi K, Abe Z, Yokota S, Andoh M, Yashizawa N (2003) Extractive relating to heartwood color changes in sugi (*Cryptomeria japonica* D. Don) by a combination of smoke-heating and UV radiation exposure. J Wood Sci 49:135–139
5. Ishiguri F, Maruyama S, Takahashi K, Andoh M, Yokota S, Abe Z, Yashizawa N (2003) Prevention of sugi (*Cryptomeria japonica* D. Don) from turning black by smoke heating. Wood Fiber Sci 35:209–216
6. Kai Y, Kuroda H, Teratani F (1972) On the phenolic constituents from *Cryptomeria japonica* D. Don VI. Hydroxysugiresinol and coloration of heartwood. Mokuzai Gakkaishi 18:315–321
7. Takahashi K (1981) Heartwood phenols and their significance to color in *Cryptomeria japonica* D. Don. Mokuzai Gakkaishi 27:654–657
8. Takahashi K (1988) The study of discolored wood in sugi (*Cryptomeria japonica* D. Don). The phenolic compounds relating to the discoloration of sugi wood (in Japanese). Faculty of Agriculture, Yamagata University, Tsuruoka, pp 110–112
9. Abe Z, Oda K, Matsumura J (1994) The color change of sugi (*Cryptomeria japonica*) heartwood from reddish brown to black I. The color change and its causes (in Japanese). Mokuzai Gakkaishi 40:1119–1125
10. Abe Z, Oda K (1994) The color change of sugi (*Cryptomeria japonica*) heartwood from reddish brown to black II. Identification of potassium hydrogencarbonate as one of the causative materials (in Japanese). Mokuzai Gakkaishi 40:1126–1130
11. Nakabayashi T (1995) Polyphenols and discoloration. In: Kimura S, Nakabayashi T, Kato H (eds) Chemistry of food discoloration (in Japanese). Kourin, Tokyo, pp 18–105
12. Takahashi K (1996) The relationships between the structural difference of norlignans and coloration. T MRS-J 20:159–162
13. Ogiyama K, Yasue M, Takahashi K (1983) Chemosystematic study on heartwood extractives of *Cryptomeria japonica* D. Don. Proceedings of International Symposium on Wood and Pulp Chemistry, Tsukuba, Japan 1:101–106
14. Nagahama S, Tazaki M, Sanetika T, Nishimura K, Tajima M (1998) Terpenoids of the wood oil of sugi (*Cryptomeria japonica*) V. Components of form. Ayasugi (in Japanese). Mokuzai Gakkaishi 44:282–286
15. Nagahama S, Fujii H, Sonoda T, Sakaki M (2002) Terpenoids of the wood oil of sugi (*Cryptomeria japonica*) VIII. Components of Kenkunisaki-5 and five other elite clones (in Japanese). Mokuzai Gakkaishi 48:380–386