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ORIGINAL ARTICLE

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# Photodiscoloration of western hemlock (*Tsuga heterophylla*) sapwood III*

# Early stage of photodiscoloration reaction with lignans

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**Abstract** The reaction during the early stage of photodiscoloration of constituents in western hemlock [*Tsuga heterophylla* (Raf.) Sarg., Pinaceae] sapwood was investigated with chemical methods. The main photodiscoloring constituents, hydroxymatairesinol, allohydroxymatairesinol, α-conidendrin, and oxomatairesinol, were used as substrates for light-irradiation experiments in vitro. The structures of photodiscoloration reaction products were elucidated by isolation and instrumental analyses and/or co-high-performance liquid chromatography analyses with authentic specimens. The experiment was undertaken to distinguish each series of liquid phases using chloroform, water (both including a trace of methanol), and methanol, and the solid phase. The reaction products allohydroxymatairesinol (**2**), oxomatairesinol (**3**), α-conidendrin (**4**), allo-7′-methoxymatairesinol (**5**), 7′-methoxymatairesinol (**6**), and vanillin (**7**) were isolated or detected in the reaction mixture of a hydroxymatairesinol system. The reaction products hydroxymatairesinol (**1**), **3**, **4**, **5**, **6**, and **7** were confirmed in the reaction system of allohydroxymatairesinol, which was an epimer of hydroxymatairesinol. Product **3** was confirmed from the α-conidendrin system, and reaction product **7** was confirmed from oxomatairesinol. The photodiscoloration reaction of western hemlock sapwood could be initiated by the formation of phenoxy radicals from the respective constituents. The reaction was then presumed to progress via formation of a quinonemethide intermediate in many of them. It was suggested that the reactive species, such as phenoxy radical or quinonemethide intermediate, formed by light-irradiation might be converted to quinone derivatives and colored oligomers. Products **1**, **2**, **3**, **4**, and **7**, formed from substrates such as hydroxymatairesinol, allohydroxymatairesinol, α-conidendrin, and oxomatairesinol, were the same as the original metabolic constituents of western hemlock. Therefore it was concluded that the photodiscoloration of western hemlock depends not on the quantitative level of a few respective metabolites but, rather, on the coexistence of many metabolites.

**Key words** *Tsuga heterophylla* sapwood · Photodiscoloration · Phenoxy radical · Quinonemethide intermediate · Lignan

## Introduction

The development of effective utilization methods of natural resources is an indispensable problem for humankind. Wood resources are a subject for investigation, as all difficulties with wood utilization must be overcome. For example, there is the problem of photodiscoloration on western hemlock wood, which is one of the main timber resources of North America.[1]

In general, it has been recognized that the phenolic constituents in extracts caused the photodiscoloration problems of wood.[2–4] Flavonoids, stilbenes, and quinones have been recognized as photodiscoloring constituents of wood.[4–6] Investigative work on the photodiscoloration mechanism of the extract is scarce, although its significance in the photodiscoloration of wood has been pointed out.[4,5,7] The photochemical reaction mechanism for quercetin pentamethyl ether by Waiss et al. was a rare report on this problem.[8]

It is known that western hemlock [*Tsuga heterophylla* (Raf.) Sarg., Pinaceae] sapwood markedly discolors with exposure to sunlight.[1,9] In previous papers we described the conditions causing the photodiscoloration of woody parts and the isolation and structural determination of constitu-

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ents causing the photodiscoloraton of western hemlock.[10–12] In addition, we demonstrated that catechin was not the main constituent causing photodiscoloration, although it had been suggested in several earlier papers that this constituent was a primary cause.[13,14] Photodiscoloration was then thought to be caused by lignans.[10–12] Furthermore, it was suggested that plural constituents interacted and caused photodiscoloration.[10,11]

This study investigated the reaction mechanism during the early stage of photodiscoloration of constituents in western hemlock. Reaction systems in liquid using nonpolar and polar solvents and solid phases were established, and light-irradiation examinations using lignans isolated from western hemlock were performed. The structures of reaction products were determined by instrumental analyses, and a reaction mechanism is proposed and discussed.

## Results and discussion

### Structural determination of photodiscoloration reaction products

The main photodiscoloring constituents, hydroxymatairesinol, allohydroxymatairesinol, α-conidendrin, and oxomatairesinol, were used as substrates for light-irradiation experiments. These substrates had been isolated from western hemlock sapwood by chromatographic methods. The specification, isolation, and identification of these lignans were described in our previous studies.[10–12] The structures of substrates and products on the photodiscoloration reaction are shown in Fig. 1. In model experiments, experimental systems were used to distinguish the solvents (chloroform with a trace of methanol, water with a trace of methanol, and 100% methanol) as liquid phases and the solid phase.

The substrate hydroxymatairesinol was light-irradiated in chloroform (including 0.3% methanol). Photodiscoloration reaction products were isolated and their structures determined. $^1$H-nuclear magnetic resonance (NMR) spectra of products **5** and **6** showed the same number of protons and their signal pattern as hydroxymatairesinol and allohydroxymatairesinol, respectively, except for the methoxyl group based on the alcoholic hydroxyl group ($C_{7'}$ position). Optical rotations of products **5** and **6** indicated +32.1° and −56.8° in methanol, and their sign (±) agreed with those of allohydroxymatairesinol and hydroxymatairesinol, respectively.[11] The structures of products **5** and **6** were then determined to be (8*R**,8′*R**,7′*S**)-(+)-allo-7′-methoxymatairesinol and (8*R**,8′*R**,7′*R**)-(−)-7′-methoxymatairesinol, respectively (Fig. 1). Note also that because products **5** and **6** were not formed in the experimental system using 100% chloroform the methyl moiety at $C_{7'}$ position of products appears to originate from methanol.

The $^1$H- and $^{13}$C-NMR spectra and optical rotation ($[\alpha]_D^{25}$ = −50.8°, $c$ = 0.14, acetone) of product **4** agreed with those of the authentic specimen.[11] Therefore product **4** was determined to be (8*R**,8′*R**,7′*S**)-(−)-α-conidendrin. Diacetate, reaction product **3a**, was isolated from the acetylated fraction and identified as oxomatairesinol diacetate by instrumental analyses. Co-HPLC (high-performance liquid chromatography) analyses of reaction products were performed using authentic specimens. The specified peaks were ultraviolet (UV) scanned. Reaction products **2** and **7** were identified as allohydroxymatairesinol and vanillin, respectively.

Hydroxymatairesinol was irradiated with light in water (including 0.3% methanol) as a polar solvent to investigated the photodiscoloration phenomenon near the actual phase of western hemlock. The photodiscoloration products were isolated and their structures determined as

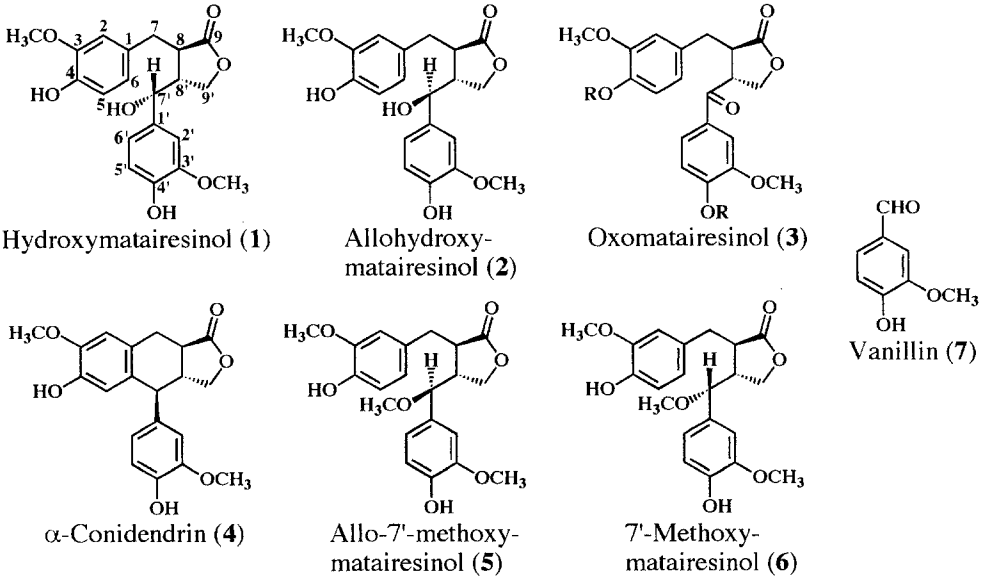


**Fig. 1.** Structures of substrates and products of the photodiscoloration reaction of constituents in westen hemlock sapwood. R = H, oxomatairesinol (**3**); R = Ac, oxomatairesinol diacetate (**3a**)

mentioned above. The formation of the main product, (8*R**,8′*R**,7′*S**)-(+)-allohydroxymatairesinol (**2**), was confirmed by direct comparison of its $^1$H-NMR spectrum and optical rotation with those of an authentic specimen. Furthermore, by instrumental analyses or co-HPLC analyses the formation of products allo-7′-methoxymatairesinol (**5**), 7′-methoxymatairesinol (**6**), α-conidendrin (**4**), oxomatairesinol (**3**), and vanillin (**7**) were confirmed.

We were also interested in the changes in reaction based on the difference in stereochemical structure for photodiscoloring constituents. Allohydroxymatairesinol, which corresponds to an epimer of hydroxymatairesinol, was light-irradiated in a 0.3% methanol/chloroform system. Co-HPLC analyses were performed using authentic specimens. The formation of products hydroxymatairesinol (**1**), oxomatairesinol (**3**), α-conidendrin (**4**), allo-7′-methoxymatairesinol (**5**), 7′-methoxymatairesinol (**6**), and vanillin (**7**) were confirmed.

α-Conidendrin was light-irradiated in a 0.3% methanol/chloroform system. Product **3** (oxomatairesinol) was detected by co-HPLC analyses and UV scans. Oxomatairesinol was light-irradiated and analyzed as mentioned above. Product **7** (vanillin) was detected.

Products on the photodiscoloration reactions, **1**, **2**, **3**, **4**, and **7**, were the same as the original constituents (secondary metabolites) of western hemlock.[13,15] Although these products began to form during light-irradiation for 5 h (data not shown), in this experiment we irradiated with light for 20 h to increase the yield of products during the early stage of the photodiscoloration reaction for the purpose of isolation.

## Reaction mechanism for photodiscoloration of lignans in western hemlock sapwood

The reaction mechanism for formation of products **2**, **4**, **5**, and **6** during irradiation of hydroxymatairesinol are summarized in Fig. 2. The photodiscoloration reaction during the formation of product **4** could be initiated by formation of a phenoxy radical with abstraction of a hydrogen from a phenolic hydroxyl group.[16–18] The following reaction was presumed to progress via formation of a quinonemethide intermediate (Fig. 2, i) by radical reaction. α-Conidendrin (**4**) was then presumed to be formed via intermediate (ii) by the indicated electron transfer [route (a) in Fig. 2]. It is thought that products **2**, **5**, and **6** were formed by the attack of a lone pair of methanol [route (b) in Fig. 2] or water [route (c) in Fig. 2] molecules on *si* site or *re* site of sp$^2$ carbon at the $C_{7'}$ position of quinonemethide intermediate (i).[19] The attack on the *si* site afforded products **5** and **2**. The attack on the *re* site formed product **6** and a return to the starting substrate (hydroxymatairesinol). The quantitative HPLC analysis resulted in the ratio of formation quantities for products 5/6: 2.8:1.0. We concluded that steric hindrance on the *re* site of the quinonemethide intermediate caused the difficulty of attack of methanol on this site (Fig. 3). In the reaction in the polar solvent system with water, the tendency of the quantity of epimeric products **1** (on *re* site and **2** (on *si* site) formed could be similar to that of products **5** and **6**. During the formation of α-conidendrin (**4**), the product that has an *S* configuration on the $C_{7'}$ position is similarly superior. A few instances had been recorded on the addition of solvents. The addition of acetone

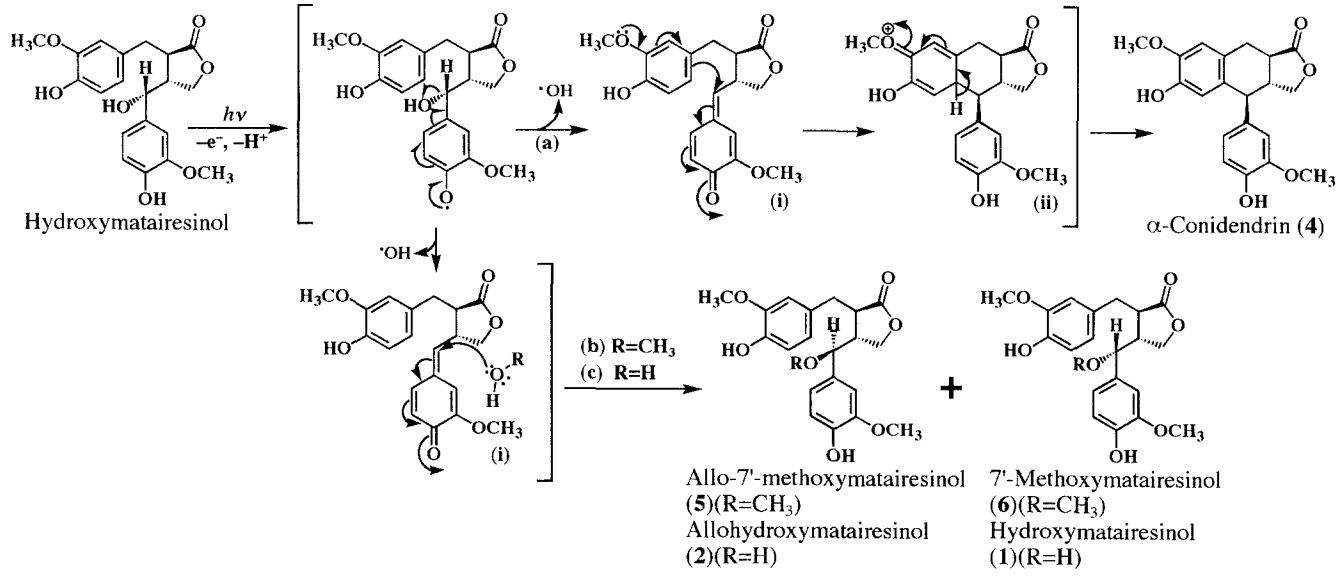


**Fig. 2.** Possible formation mechanism of products **1**, **2**, **4**, **5**, and **6** during the photodiscoloration reaction of hydroxymatairesinol. (*a*), (*b*), reaction in 0.3% MeOH/$CHCl_3$; (*c*), reaction in 0.3% MeOH/$H_2O$

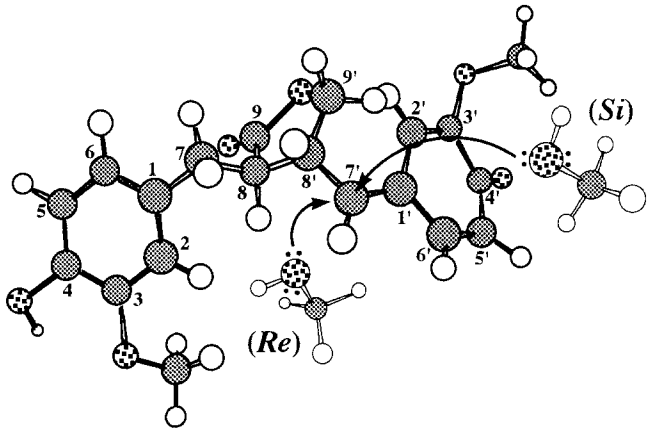


**Fig. 3.** Relation between stereochemical structure of the quinonemethide intermediate derived from hydroxymatairesinol and reactivity of the solvent molecule. *Si*, *Re*: *si* and *re* site of $sp^2$ carbon at the Cn-position, respectively

molecule to the aliphatic unsaturated bond of secosteroids,[20] addition of methanol molecule to quercetin pentamethyl ether,[8] and so on were reported. In the former case, the oxygen molecule further links to the product, and the product proceeds to epoxidation via the elimination of acetone molecule. Moreover, addition of a solvent molecule to the substrate was found during the steam explosion of lignin.[21]

Possible formation mechanisms of products **3** and **7** on the photodiscoloration reaction of hydroxymatairesinol are displayed in Fig. 4. It may be explained that a phenoxy radical is formed with the abstraction of one hydrogen from the phenolic hydroxyl group.[16–18] Accordingly, the disproportionation reaction of radicals $R_a$ and $R_b$ has occurred, and the cation intermediate (iii) is formed. Oxomatairesinol (**3**) is then formed by electron transfer via the quinonemethide intermediate (iv). Furthermore, vanillin (**7**) could be formed from the phenoxy radical by direct cleavage between the $C_{7'}$ and $C_{8'}$ positions. In another case, product **3** also may be formed by the reaction as follows: Radical $R_b$ is photooxygenated to a hydroperoxide intermediate,[22] quinonemethide intermediate (iv) is formed via leaving the OOH group, and product **3** is then formed by electron transfer in a manner similar to that seen in Fig. 4.

Similar light-irradiation tests on the hydroxymatairesinol derivatives, whose hydroxyl groups were protected, were performed (detailed data not shown). The quantity of products formed decreased remarkably. This result might be brought about by inhibition of phenoxy radical formation. The protection treatment of functional groups such as the hydroxyl group was confirmed to be effective for protection of photodiscoloration.

During the formation of products **1**, **4**, **5**, and **6** with the light-irradiation of allohydroxymatairesinol, the reaction was presumed to occur via the quinonemethide intermediate (i), same as for hydroxymatairesinol (Fig. 2). During formation of products **3** and **7** with the light-irradiation of allohydroxymatairesinol, the reaction was presumed to take place via quinonemethide intermediate (iv) and (ii), the same as for hydroxymatairesinol (Fig. 4).

It had been reported that the α-carbonyl group in lignin that excited by light-irradiation abstracted H$^{\bullet}$ from the phe-

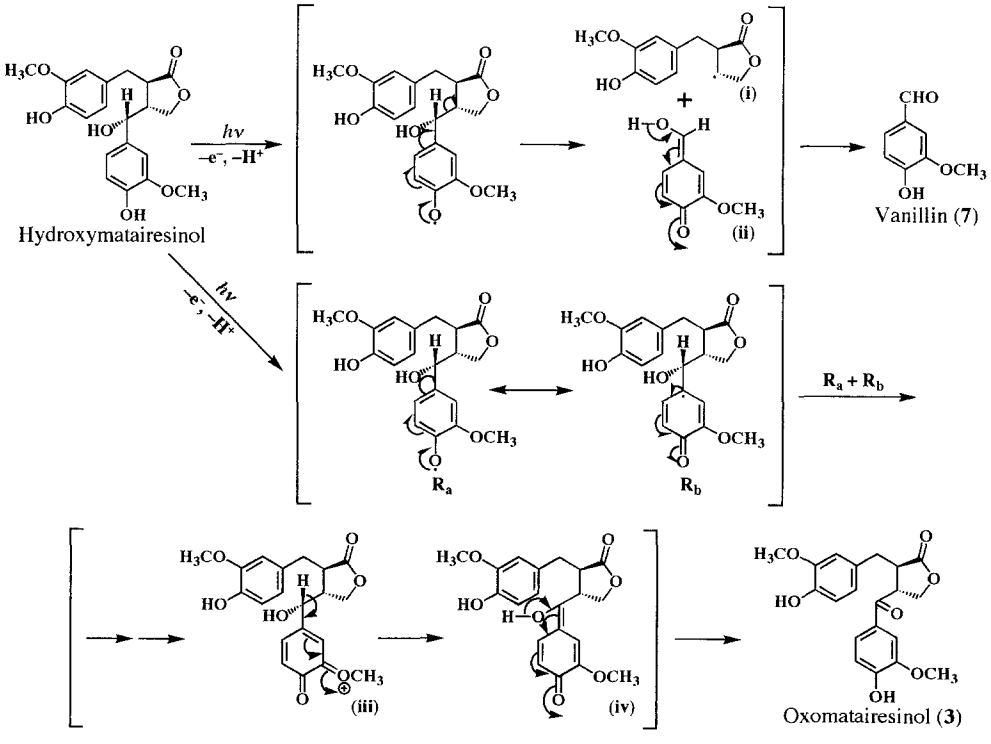


**Fig. 4.** Possible formation mechanism of products **3** and **7** during the photodiscoloration reaction of hydroxymatairesinol

nolic hydroxyl group and formed the phenoxy radical produced by the discoloration (yellowing) reaction of lignin.[16] In other reports, the mechanism of hydrogen abstraction was explained as follows: (1) α-carbonyl group plays a role of photosensitizer; (2) excited α-carbonyl group gives the energy to oxygen at ground state ($^3O_2$); (3)$^3O_2$ excites into singlet oxygen ($^1O_2$); and (4) $^1O_2$ abstracts hydrogen from the phenolic hydroxyl group.[17,18] In our study, one substrate, oxomatairesinol, which has an α-carbonyl group allows the explanation for the mechanism of hydrogen abstraction mentioned above. However, the above explanation cannot be applied to the other substrates – hydroxymatairesinol, allohydroxymatairesinol, and α-conidendrin – as these lignans do not possess the noteworthy α-carbonyl group. Further elucidation of the mechanism of hydrogen abstraction is a subject for future study.

## Effect of reaction systems in photodiscoloration

The photodiscoloration reaction products in each reaction system of the liquid and solid phases were analyzed quantitatively. The results are summarized in Table 1 (hydroxymatairesinol) and Table 2 (allohydroxymatairesinol). The ratio of products formed during photodiscoloration varied according to the reaction system.

**Table 1.** Effect of reaction systems on the photodiscoloration reaction of hydroxymatairesinol

| Reaction phase | Contents of reaction products[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| | **1**[b] | **2** | **3** | **4** | **5** | **6** | **7** |
| Liquid | | | | | | | |
| $CHCl_3$[c] | 34.0 | + | 6.7 | 24.8 | 2.8 | 1.0 | 0.7 |
| MeOH | 26.3 | 29.5 | 0.1 | 0.1 | 5.9 | 1.4 | 0.5 |
| $H_2O$[c] | 27.0 | 43.1 | 0.2 | 0.9 | 0.2 | + | + |
| Solid | 15.3 | 17.5 | 2.2 | 0.2 | – | – | 0.4 |

–, not detected; +, a minor value below 0.1%.
[a] Percent on substrate. Reaction product codes are the same as those in Fig. 1.
[b] Remaining ratio of substrate.
[c] Solvent includes a trace of MeOH.

**Table 2.** Effect of reaction systems on the photodiscoloration reaction of allohydroxymatairesinol

| Reaction phase | Contents of reaction products[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| | **1** | **2**[b] | **3** | **4** | **5** | **6** | **7** |
| Liquid | | | | | | | |
| $CHCl_3$[c] | 0.4 | 38.1 | 4.1 | 18.5 | 3.2 | 0.6 | 0.6 |
| MeOH | 18.6 | 50.5 | 1.1 | 0.5 | 1.7 | 0.4 | 0.8 |
| $H_2O$[c] | 17.9 | 52.0 | 0.4 | 0.4 | 0.1 | – | 1.0 |
| Solid | 16.3 | 51.1 | 2.4 | 1.1 | – | – | 0.7 |

Symbols are the same as those in Table 1.
[a] Percent. Reaction product codes are the same as those in Table 1.
[b] Remaining ratio of substrate.
[c] Solvent includes a trace of MeOH.

It had been reported that lignin produced vanillin with light-irradiation.[23,24] In the present study there was little vanillin (**7**) formed from hydroxymatairesinol and allohydroxymatairesinol in any reaction system. The quantities formed by reactions in the water and solid systems (which were close to the photodiscoloration conditions in nature) were particularly small. Accordingly, in the case of lignans, the significance of the photodiscoloration route that forms vanillin is expected to be small, as the lignan content in wood is less than that of lignin.

The quantities of epimeric products formed (**2** and **1**) from hydroxymatairesinol and allohydroxymatairesinol were largest in the irradiation in water system. These were also large quantities of these products formed with irradiation in methanol and the solid phase. With irradiation in methanol and the solid phase, the water that existed in trace amounts in the reaction system might react with the substrate. The quantities of products **3** and **4** formed were largest with irradiation in chloroform; the quantity was small with the other reaction systems. With irradiation in water and methanol, the formation of products **3** and **4** might be inhibited, as the reaction with sufficient existing solvents took precedence. The quantities of products **5** and **6** formed were largest with irradiation in methanol, as these products formed by the reaction of substrate and methanol molecules.

The influence of the reaction systems and solvents was almost the same for the two substrates (hydroxymatairesinol and allohydroxymatairesinol). The amount of products formed from hydroxymatairesinol, however, differed from that of allohydroxymatairesinol.

## Early stage of photodiscoloration reaction with constituents in western hemlock

Possible pathways in the photodiscoloration reaction of photodiscoloring constituents in western hemlock are shown in Fig. 5. With irradiation in the solvent system using chloroform, the main product was α-conidendrin (**4**) [case (a) in Fig. 5]. With irradiation in the solvent system using water, the reaction via formation of an epimer from hydroxymatairesinol and allohydroxymatairesinol was the main route [case (b) in Fig. 5].

It was proved that oxomatairesinol (**3**), α-conidendrin (**4**), vanillin (**7**), and the corresponding epimer (**2** and **1**) were formed from hydroxymatairesinol and allohydroxymatairesinol by light-irradiation. Oxomatairesinol (**3**) was formed from α-conidendrin (**4**), and vanillin (**7**) was formed from oxomatairesinol (**3**) by light-irradiation. These products were original constituents (secondary metabolites) of western hemlock. Specifically, during the photodiscoloration examination in vitro the reaction progressed via formation of the other original secondary metabolites. Generally speaking, few representative constituents have been found to cause discoloration during the photodiscoloration of wood.[4,5,7] With photodiscoloration of western hemlock, however, it might be considered that although a particular constituent could not cause remarkable photodiscoloration,

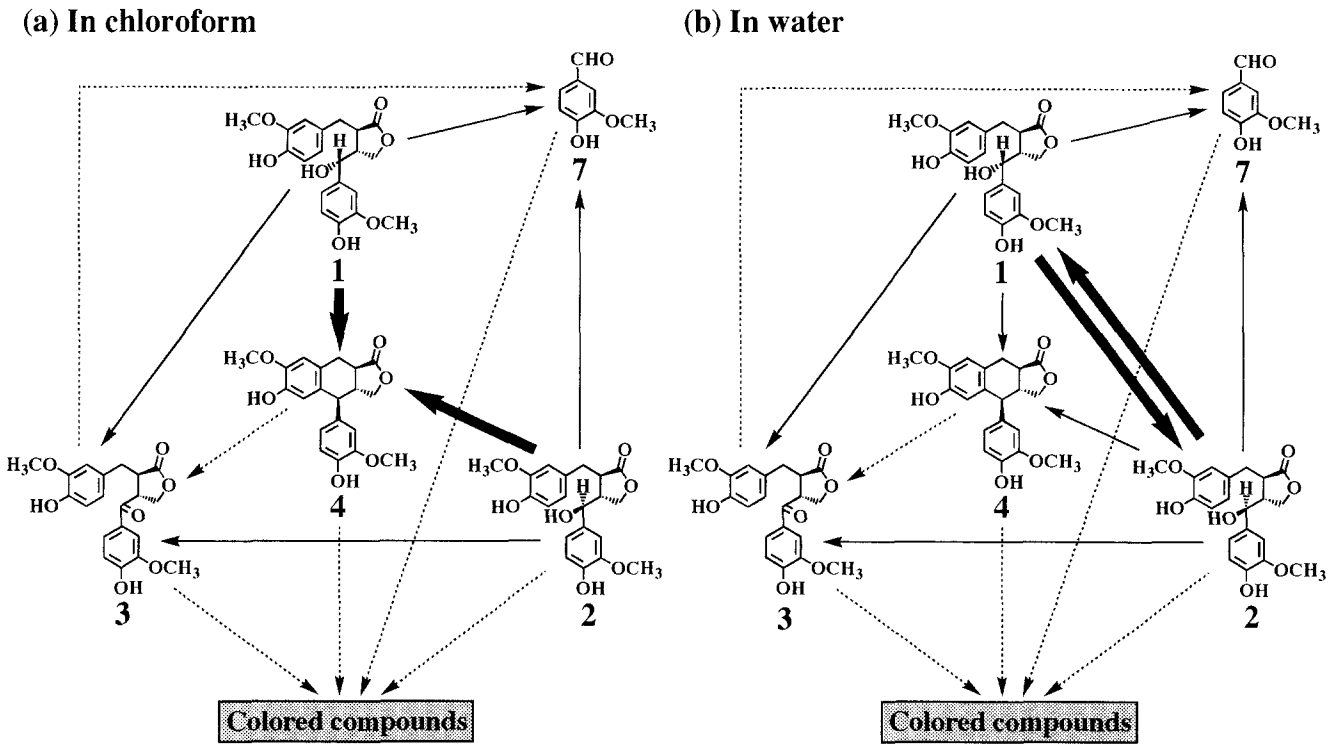


**Fig. 5.** Possible pathways in the photodiscoloration reaction of constituents in *Tsuga heterophylla* sapwood in chloroform (**a**) and water (**b**). *Heavy arrows*, main routes; *solid arrows*, normal routes; *broken arrows*, minor or not yet revealed routes. (**a**) Reaction in 0.3% MeOH/$CHCl_3$. (**b**) Reaction in 0.3% MeOH/$H_2O$

multiple constituents interacted to cause it, as indicated by the results mentioned above. These results were consistent with the presumption in our previous paper.[10,11]

We supplement this description as follows. All reaction mixtures obtained in a series of our photodiscoloration experiments in vitro were colored brown, although the products isolated from the mixture were colorless or pale compounds. It appears that the reactive species, such as phenoxy radical or quinonemethide intermediate, formed by light-irradiation may ultimately be converted to quinone derivatives and colored oligomers.[25,26] It was thought that only small quantities of the colored products were formed in the present experiment. In the next experiment, the colored products should be isolated to elucidate the whole photodiscoloration phenomena of western hemlock. Consequently, we offer the present study as the start of work on the mechanism of the photodiscoloration reaction of constituents in western hemlock sapwood.

## Experiment

The $^1H$- and $^{13}C$-NMR spectra were obtained with JEOL JNM-GX270 (270 MHz) and/or Barian Gemini-2000 (200M Hz) FT-NMR spectrometers. MS spectra were obtained with Shimadzu GCMS-QP1000 and/or GCMS-QP5000 spectrometers. HPLC was performed with systems of JASCO PU-980 pump with JASCO UV-970 UV/VIS detector using columns prepacked Waters μ Bondasphere 5μ $C_{18}$ 100 Å (150 × 3.9mm i.d., analytical; 150 × 19.0mm i.d., preparative). The other analytical equipments, plant materials, extraction, and fractionation were the same as in our previous report.[10–12]

### Isolation of constituents (substrates)

The ethyl acetate soluble fraction (6.07g) was chromatographed on column chromatography (benzene/ethyl acetate 8:2–0:10, v/v), and 95 fractions were collected in 100-ml portions. Eluates 48–53 were chromatographed on reverse-phase preparative HPLC (PHPLC) (flow rate 7.0ml/min, detection $UV_{265nm}$) [methanol (MeOH)/water ($H_2O$) 38:62, v/v) and obtained colorless amorphous hydroxymatairesinol (452.4mg). Eluates 18–21 were chromatographed on reverse-phase PHPLC (MeOH/$H_2O$ 55:45, v/v), and colorless crystal α-conidendrin (88.3mg) was obtained. The isolation procedure for allohydroxymatairesinol was the same as in our previous report.[11]

### Light-irradiation tests with isolated constituents

Light-irradiation tests were carried out by high-pressure mercury lamp (National, BHRF100). The light-irradiation

field was air-cooled. A quartz cell was used for the irradiation test in organic solvents, and sample solution was irradiated perpendicular to the cell surface. A pyrex beaker was used for the irradiation test in water, and the sample solution was irradiated perpendicular to the direct open side of the beaker. The distance between the light source and the sample solution was 20 cm, and the irradiation time was 20 h. In the experimental system for HPLC analyses of reaction products, the concentration of the sample solution was 50 μM. In the experimental system for isolating the reaction product, the concentrations of sample solution were 50 μM (in water) and 4 or 40 mM (in chloroform). The solvent was distilled before the irradiation test. After light-irradiation the solvent was evaporated in vacuo, and a mixture of photodiscoloration reaction products was obtained. For the irradiation test on the solid phase, the MeOH solution (20 mg/ml) of the sample was measured by microsyringe (5 μl), spread on an inner wall of the quartz cell, and air-dried, followed by drying in a desiccator in vacuo.

## HPLC analyses for photodiscoloration reaction products

The photodiscoloration products were specified on HPLC analyses, using hydroquinone diethyl ether as internal standard. Relative retention times (*RRt*) were determined as a value relative to that of the internal standard. Quantitative values were derived by the peak area percentage method. The analyses were repeated three times on each reaction system, and mean values were calculated. The eluents and conditions for the analyses were as follows: eluents, MeOH/$H_2O$ 45:55, 36:64, or both (v/v); flow rate 0.8 ml/min; detection $UV_{280nm}$. Reaction product (code no.) (*RRt*): vanillin (**7**) (0.075), hydroxymatairesinol (**1**) (0.083), allohydroxymatairesinol (**2**) (0.089), oxomatairesinol (**3**) (0.145), α-conidendrin (**4**) (0.172), allo-7′-methoxymatairesinol (**5**) (0.255), 7′-methoxymatairesinol (**6**) (0.307). Co-HPLC analyses were performed using authentic vanillin, allohydroxymatairesinol, and oxomatairesinol. UV wavelength scans (200–400 nm) were performed with a UV detector in the HPLC system, and the UV spectra of peak compounds **2**, **3**, and **7** were recorded.

## Isolation and structural determination of photodiscoloration reaction products in organic solvent

A 30% MeOH/chloroform solution of hydroxymatairesinol (40 mM) was light-irradiated. A mixture of reaction products (49.0 mg) was chromatographed on repeated PHPLC (MeOH/$H_2O$ 60:40, v/v) to elute fractions (Fr.) 1–15. Fr. 5 and Fr. 6 were repeated PHPLC (MeOH/$H_2O$ 50:50, v/v) to afford the colorless amorphous products **5** (17.1 mg) and **6** (5.2 mg). Hydroxymatairesinol solution (4 mM) was irradiated with light the same as mentioned above. A mixture of reaction products (94.3 mg) were chromatographed on repeated PHPLC (MeOH/$H_2O$ 65:35, v/v) to prepare Fr. 16–23. Fr. 4 and Fr. 19 were combined and repeated PHPLC (MeOH/$H_2O$ 47:53, v/v) to afford colorless product **4** (13.3 mg). Fr. 3 and Fr. 18 were combined (8.0 mg) and acetylated with acetic anhydride and pyridine at room temperature. A mixture of acetates was chromatographed on repeated semi-PHPLC (*n*-hexane/chloroform/MeOH 79:12:9, v/v) to prepare Fr. 24–27. Diacetate **3a** (2.0 mg) was purified by semi-PHPLC (*n*-hexane/chloroform/MeOH 85:6:9, v/v) from Fr. 25.

(8*R**,8′*R**,7′*S**)-(+)-Allo-7′-methoxymatairesinol (product **5**). Colorless amorphous gum, $[\alpha]_D^{25} = +32.1°$ (*c* = 1.53, MeOH). UV $\lambda_{max}^{MeOH}$nm (log ε): 282.0 (3.77), 231.4 (4.10), and 206.8 (4.61). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3432 (OH), 2940, 1763 (γ-lactone), 1605, 1517, 1465, 1453, 1432, 1371, 1274, 1242, 1210, 1155, 1123, and 1033. $^1$H-NMR ($CDCl_3$): δ 2.07 (1H, *m*, H-8′), 2.56–2.70 (2H, *m*, H-$7_a$, H-8), 2.75 (1H, *dd*, *J* = 13.2, 4.8 Hz, H-$7_b$), 3.10 (3H, *s*, alc. OMe), 3.75–3.76 (4H, PhOMe, H-7′), 3.78 (3H, *s*, PhOMe), 4.16 (1H, *dd*, *J* = 9.53, 7.70 Hz, H-9′$_a$), 4.37 (1H, *dd*, *J* = 9.53, 5.49 Hz, H-9′$_b$), 5.58 (1H, *s*, PhOH), 5.68 (1H, *s*, PhOH), 6.41 (1H, *d*, *J* = 1.8 Hz, H-2), 6.44 (1H, *d*, *J* = 1.8 Hz, H-2′), 6.47 (1H, *dd*, *J* = 8.1, 1.8 Hz, H-6′), 6.60 (1H, *dd*, *J* = 8.1, 1.8 Hz, H-6), 6.76 (1H, *d*, *J* = 8.1 Hz, H-5′), and 6.84 (1H, *d*, *J* = 8.1 Hz, H-5). $^{13}$C-NMR ($CDCl_3$) (DEPT): δ 35.06 ($CH_2$, C-7), 43.82 (CH, C-8), 45.91 (CH, C-8′), 55.69 ($CH_3$, 2 × OMe), 56.71 ($CH_3$, OMe), 68.68 ($CH_2$, C-9′), 83.68 (CH, C-7′), 108.19 (CH, C-2′), 111.15 (CH, C-2), 113.91 (CH, C-5), 114.04 (CH, C-5′), 120.48 (CH, C-6′), 122.04 (CH, C-6), 129.38 (C, C-1), 130.49 (C, C-1′), 144.51 (C, C-4), 145.71 (C, C-4′), 146.70 (C, C-3), 147.04 (C, C-3′), and 178.86 (C, C-9). MS *m/z* [rel. int. (%)]: 388 (16, $M^+$), 167 (100), 152 (13), and 137 (20).

(8*R**,8′*R**,7′*R**)-(−)-7′-Methoxymatairesinol (product **6**). Colorless amorphous gum, $[\alpha]_D^{25} = -56.8°$ (*c* = 0.46, MeOH). UV $\lambda_{max}^{MeOH}$nm (log ε): 282.0 (3.74), 231.6 (4.08), and 206.4 (4.58). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3432 (OH), 2939, 1760 (γ-lactone, 1605, 1517, 1465, 1453, 1432, 1386, 1371, 1275, 1240, 1209, 1155, 1123, and 1032. $^1$H-NMR ($CDCl_3$): δ 2.56 (1H, *m*, H-8′), 2.86–2.93 (2H, *m*, H-$7_a$, H-8), 3.04 (1H, *dd*, *J* = 15.4, 7.0 Hz, H-$7_b$), 3.23 (3H, *s*, alc. OMe), 3.79–3.91 (8H, 2 × PhOMe, H-9′), 4.01 (1H, *d*, *J* = 7.0 Hz, H-7′), 5.52 (1H, *s*, PhOH), 5.63 (1H, *s*, PhOH), 6.60 (1H, *dd*, *J* = 8.1, 1.8 Hz, H-6), 6.62 (1H, *d*, *J* = 1.8 Hz, H-2), 6.64 (1H, *d*, *J* = 1.8 Hz, H-2′), 6.67 (1H, *dd*, *J* = 8.1, 1.8 Hz, H-6′), 6.78 (1H, *d*, *J* = 8.1 Hz, H-5), and 6.87 (1H, *d*, *J* = 8.1 Hz, H-5′). $^{13}$C-NMR ($CDCl_3$)(DEPT): δ 34.96 ($CH_2$, C-7), 44.14 (CH, C-8), 44.89 (CH, C-8′), 55.84 ($CH_3$, OMe), 55.89 ($CH_3$, OMe), 56.86 ($CH_3$, OMe), 68.34 ($CH_2$, C-9′), 84.69 (CH, C-7′), 108.59 (CH, C-2′), 112.06 (CH, C-2), 113.96 (CH, C-5′), 114.37 (CH, C-5), 120.03 (CH, C-6′), 122.65 (CH, C-6), 129.51 (C, C-1), 130.47 (C, C-1′), 144.42 (C, C-4), 145.68 (C, C-4′), 146.49 (C, C-3), 146.96 (C, C-3′), and 179.14 (C, C-9). MS *m/z* (%): 388 (17, $M^+$), 177 (20), 167 (100), 152 (15), and 137 (24).

(8*R**,8′*R**,7′*S**)-(−)-α-Conidendrin (product **4**). Colorless amorphous powders, $[\alpha]_D^{25} = -50.8°$ (*c* = 1.02, acetone) [lit.[11] − 53.1°, *c* = 0.14, acetone]. UV $\lambda_{max}^{MeOH}$nm (log ε): 284.0 (3.88), 231.0 (*sh*) (4.23), and 209.2 (4.68). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3435 (OH), 1760 (γ-lactone), 1605, 1513, 1468, 1434, 1367, 1355, 1272, 1217, 1157, 1110, 1029, 996, and 749.

$^1$H-NMR ($CDCl_3$): δ 2.51–2.64 (2H, *m*, H-8, H-8′), 2.97 (1H, *dd*, *J* = 16.3, 10.6 Hz, H-7$_a$), 3.21 (1H, *dd*, *J* = 16.3, 4.4 Hz, H-7$_b$), 3.82–3.89 (7H, 2 × OMe, H-7′), 4.01 (1H, *dd*, *J* = 8.8, 10.6 Hz, H-9′$_a$), 4.23 (1H, *dd*, *J* = 8.8, 6.2 Hz, H-9′$_b$), 5.42 (1H, *s*, PhOH), 5.58 (1H, *s*, PhOH), 6.40 (1H, *s*, H-5), 6.54 (1H, *d*, *J* = 1.8 Hz, H-2′), 6.64–6.68 (1H, *dd*, *J* = 8.1, 1.8 Hz, H-6′), 6.67 (1H, *s*, H-2), and 6.87 (1H, *dd*, *J* = 8.1 Hz, H-5′). $^{13}$C-NMR ($CD_3OD$): δ 30.17 (C-7), 42.97 (C-8′), 48.55 (C-7′), 50.73 (C-8), 56.44 (2 × OMe), 73.34 (C-9′), 112.90 (C-2), 113.48 (C-2′), 116.40 (C-5′), 117.03 (C-5), 122.40 (C-6′), 127.56 (C-1), 133.33 (C-6), 135.85 (C-1′), 146.00 (C-4), 146.79 (C-4′), 147.91 (C-3), 149.42 (C-3′), and 179.91 (C-9). MS *m/z* (%): 356 (100, $M^+$), 271 (9), 255 (12), 241 (22), and 173 (12).

Oxomatairesinol diacetate (product **3a**). Colorless amorphous gum. $^1$H-NMR ($CDCl_3$): δ 2.29 (3H, *s*, OAc), 2.34 (3H, *s*, OAc), 3.07 (2H, *d*, *J* = 6.0 Hz, H-7), 3.56 (1H, *m*, H-8), 3.67 (3H, *s*, OMe), 3.87 (3H, *s*, OMe), 4.08 (2H, *m*, H-8′, H-9′$_a$), 4.45 (1H, *m*, H-9′$_b$), 6.64 (1H, *dd*, *J* = 8.0, 1.9 Hz, H-6), 6.72 (1H, *d*, *J* = 1.9 Hz, H-2), 6.87 (1H, *d*, *J* = 8.0 Hz, H-5), 7.10 (1H, *d*, *J* = 8.2 Hz, H-5′), 7.21 (1H, *dd*, *J* = 8.2, 1.9 Hz, H-6′), and 7.44 (1H, *d*, *J* = 1.9 Hz, H-2′). MS *m/z* (%); 456 (1, $M^+$), 414 (51), 372 (10), 356 (4), 221 (11), 194 (100), 151 (49), and 137 (36).

## Isolation and structural determination for photodiscoloration products in water

A 0.3% MeOH/$H_2O$ solution of hydroxymatairesinol (50 μM) was light-irradiated. Reaction products were isolated by repeating PHPLC (MeOH/$H_2O$ 48:52, v/v) (MeOH/$H_2O$ 38:62, v/v) and/or. The products allohydroxymatairesinol (**2**) (3.5 mg), oxomatairesinol (**3**) (0.6 mg), and α-conidendrin (**4**) (1.2 mg) were identified by comparing the $^1$H-NMR, EI-MS, and UV spectra with those of authentic specimens.

(8*R**,8′*R**,7′*S**)-(+)-Allohydroxymatairesinol (product **2**). Colorless amorphous powders, $[\alpha]_D^{25}$ = +4.2° (*c* = 0.24, MeOH) (lit.[11] + 4.9°, *c* = 0.82, MeOH]. UV $\lambda_{max}^{MeOH}$nm (log ε): 282.0 (3.81), 231.2 (4.15), and 206.6 (4.63). $^1$H-NMR ($CDCl_3$): δ 1.91 (1H, *d*, *J* = 2.6 Hz, alc. OH), 2.51 (1H, *m*, H-8′), 2.63 (1H, *m*, H-8), 2.70 (1H, *dd*, *J* = 12.9, 7.2 Hz, H-7$_a$), 2.79 (1H, *dd*, *J* = 12.9, 5.3 Hz, H-7$_b$), 3.76 (1H, *s*, OMe), 3.79 (1H, *s*, OMe), 4.18 (1H, *dd*, *J* = 9.5, 7.4 Hz, H-9′$_a$), 4.39 (1H, *dd*, *J* = 7.8, 2.6 Hz, H-7′), 4.43 (1H, *dd*, *J* = 9.5, 5.5 Hz, H-9′$_b$), 5.51 (1H, *s*, PhOH), 5.62 (1H, *s*, PhOH), 6.43 (1H, *d*, *J* = 2.0 Hz, H-2), 6.49 (1H, *dd*, *J* = 7.9, 2.0 Hz, H-6), 6.55 (1H, *d*, *J* = 2.0 Hz, H-2′), 6.63 (1H, *dd*, *J* = 8.1, 2.0 Hz, H-6′), 6.78 (1H, *d*, *J* = 7.9 Hz, H-5), and 6.83 (1H, *d*, *J* = 8.1 Hz, H-5′). MS *m/z* (%): 374 (26, $M^+$), 237 (9), 219 (8), 194 (3), 180 (5), 177 (7), 153 (100), 137 (59), and 93 (34).

Oxomatairesinol (product **3**). Pale yellowish amorphous powders, UV $\lambda_{max}^{MeOH}$nm (log ε): 310.2 (3.98), 282.4 (4.10), 232.2 (4.35), and 205.4 (4.72). $^1$H-NMR spectrum of product **3** was identical with that of authentic specimen, oxomatairesinol.[11] MS *m/z* (%): 372 (31, $M^+$), 221 (9), 194 (100), 177 (14), 151 (59), and 137 (64).

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