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Lignans causing photodiscoloration of *Tsuga heterophylla*: 8-hydroxy-oxomatairesinol from sapwood

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Abstract

A lignan, (8*S*,8′*S*,)-(+)-8-hydroxy-oxomatairesinol, has been isolated from the sapwood of *Tsuga heterophylla* (western hemlock, Pinaceae). The known lignans matairesinol, lariciresinol and secoisolariciresinol were also obtained. The structure of the compound was established by 1D and 2D NMR spectroscopy. Results of the light-irradiation test of the lignans from *T. heterophylla* are also reported.

Keywords: *Tsuga heterophylla*; Pinaceae; Western hemlock; Sapwood; Photodiscoloration; Lignan; (+)-8-Hydroxy-oxomatairesinol

1. Introduction

The sapwood of *Tsuga heterophylla* (Raf.) Sarg. (western hemlock, Pinaceae) is known to be susceptible to photodiscoloration (Barton, 1968; Hrutfiord et al., 1985). In our previous paper, five lignans [(+)-pinoresinol, (+)-*allo*-hydroxymatairesinol, (−)-hydroxymatairesinol, (+)-oxomatairesinol, (−)-α-conidendrin] and a neolignan [(+)-cedrusin], the main constituents causing photodiscoloration, were isolated and identified (Kawamura et al., 1996a, 1996b, 1998). Furthermore, we isolated four new sesquilignans and lignans, (−)-7′-hydroxylappaol E (**5**), *epi*-7′-hydroxylappaol E, (−)-8-hydroxy-α-conidendrin and (+)-8-hydroxy-α-conidendric acid methyl ester (Kawamura et al., 1997). In a continuation of this work, a new lignan, (+)-8-hydroxy-oxomatairesinol (**1**) was isolated. Herein, we report on the structural elucidation of this new compound. Furthermore, the photodiscoloration of lignans from *T. heterophylla* is discussed.

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2. Results and discussion

Compounds **1**–**4** were isolated from *T. heterophylla* sapwood by chromatographic methods. By comparing the spectroscopic properties ($[\alpha]_D^{25}$, UV, IR, MS, ^{1}H- and ^{13}C-NMR spectra), the known lignans were identified as matairesinol (**2**), lariciresinol (**3**) and secoisolariciresinol (**4**) (Umezawa et al., 1991; Abe and Yamauchi, 1989). The structure of the new lignan **1** was determined as follows.

Compound **1** ($C_{20}H_{20}O_8$; $[\alpha]_D^{25}$ +97.3° (MeOH)), showed an M^+ at *m*/*z* 388.1168 in its EI mass spectrum. IR absorptions, at 1772 and 1659 cm^{-1} indicated the presence of a γ-lactone and conjugated C=O, respectively. The ^{13}C-NMR spectrum (Table 1) of **1** exhibited the presence of two carbonyl groups, two phenolic methoxyl groups, two methylene groups, a methine group, an oxygenated quaternary carbon and two phenyl groups. The ^{1}H-NMR resonances (Table 1) were assigned with ^{1}H–^{1}H COSY (Table 2). It was observed that two double doublets at δ 4.29 and 4.51 ($J = 9.0$, 6.3 Hz, H-9′a; $J = 9.0$, 2.4 Hz, H-9′b, in acetone-d_6) were mutually coupled with the signal at δ 4.45 (*dd*, $J = 6.3$, 2.4 Hz, H-8′). The methylene protons on C-7, appearing as two doublets at δ 3.04 ($J =$

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Table 1
^{1}H- and ^{13}C-NMR spectral data for compound **1** (^{1}H: 399.7 MHz, ^{13}C: 100.4 MHz)

Position	^{13}C ($CDCl_3$)[a]	^{1}H ($CDCl_3$)[b]	^{1}H (acetone-d_6)[b]
1	126.2 *s*		
2	112.7 *d*	6.79 *d* (1.7)	7.10 *d* (1.7)
3	146.6 *s*		
4	145.2 *s*		
5	114.3 *d*	6.81 *d* (7.8)	6.84 *d* (8.1)
6	123.1 *d*	6.70 *dd* (7.8, 1.7)	6.94 *dd* (8.1, 1.7)
7a	42.9 *t*	3.03 *d* (14.1)	3.04 *d* (13.7)
7b		3.14 *d* (14.1)	3.07 *d* (13.7)
8	78.1 *s*		
9	176.0 *s*		
1′	129.1 *s*		
2′	109.8 *d*	7.33 *d* (2.0)	7.25 *d* (1.7)
3′	146.9 *s*		
4′	151.7 *s*		
5′	113.9 *d*	6.87 *d* (8.3)	6.72 *d* (8.3)
6′	124.2 *d*	7.09 *dd* (8.3, 2.0)	7.02 *dd* (8.3, 1.7)
7′	196.9 *s*		
8′	47.0 *d*	4.20 *m*	4.45 *dd* (6.3, 2.4)
9′a	67.1 *t*	4.22 *m*	4.29 *dd* (9.0, 6.3)
9′b		4.46 *m*	4.51 *dd* (9.0, 2.4)
3-OMe	55.9 *q*	3.80 *s*	3.80 *s*
4-OH		5.59 *s*	
8-OH		4.38 *s*	
3′-OMe	56.1 *q*	3.90 *s*	3.77 *s*
4′-OH		6.20 *s*	

[a] Assignment and multiplicities were determined with HMQC and DEPT spectra.
[b] *J* in Hz.

13.7 Hz, H-7a) and 3.07 ($J = 13.7$ Hz, H-7b), did not correlate with any other peaks. Therefore, the corresponding methylene group was between two quaternary carbons, as revealed by ^{13}C-NMR spectroscopy. The signal pattern of the protons in the aromatic region showed two 1,3,4-trisubstituted phenyl groups. In the long-range ^{1}H–^{1}H COSY (Table 2) spectrum of **1**, the cross peaks of H-7 were observed on H-2, H-6, H-8′ and 8-OH. The assignment of the carbon atoms was established by a combination of HMQC and HMBC (Table 2) spectroscopies. In the HMBC spectrum of **1**, the proton at δ 4.20 (H-8′) showed two correlations to quaternary carbons, C-8 and C-7′. The proton at δ 4.38 (8-OH), showed four correlations to C-8′, C-8, C-9 and C-7′. The results of the other long-range C–H couplings were also substantiated by the assignment of the ^{1}H-NMR spectrum. The NOE difference spectrum of **1** exhibited an NOE between H-8′ and H-7. This result suggested a *cis*-relationship between H-8′ and C-7. In this way, the structure for the new lignan **1**, (8*R*,8′*R*)-(+)-8-hydroxy-oxomatairesinol [2-(4-hydroxy-3-methoxybenzoyl)-3-hydroxy-(4-hydroxy-3-methoxybenzyl)-butyrolactone] was proposed.

Table 3
Contents of lignans in the sapwood of *T. heterophylla*

Lignans	Contents in sapwood (ppm)[a]
(+)-8-Hydroxy-oxomatairesinol (**1**)	6.7
(−)-Matairesinol (**2**)	73.5
(+)-Lariciresinol (**3**)	31.7
(−)-Secoisolariciresinol (**4**)	4.5

[a] Calculated based on oven-dried matter.

The content of lignans **1**–**4** were quantitatively determined by HPLC (Table 3). The concentrations of lignans **1** and **4** were very low. Secoisolariciresinol is a precursor of matairesinol in biosynthesis, and it was

Table 2
^{1}H-NMR, ^{1}H–^{1}H COSY, long-range ^{1}H–^{1}H COSY, HMBC and NOE spectral data for compound **1** (^{1}H: 399.7 MHz)

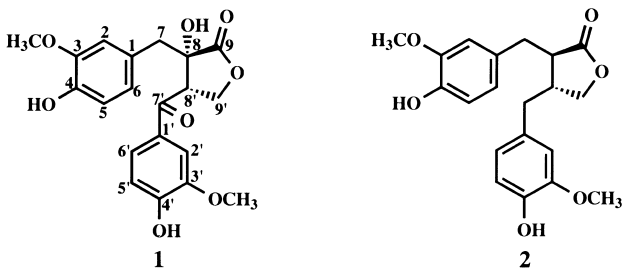
Position	^{1}H	^{1}H–^{1}H COSY	Long-range ^{1}H–^{1}H COSY	HMBC	NOE
2	6.79	H-6	H-5, H-6, 3-OMe	C-7, C-6, C-4	H-7a, H-7b
5	6.81	H-6	H-2, H-6	C-1, C-4, C-3	
6	6.70	H-2, H-5	H-2, H-5, 4-OH	C-2, C-4	H-7a, H-7b
7a	3.03	H-7b	H-7b, H-2, H-6, 8-OH	C-8′, C-8, C-2, C-6, C-1, C-9	H-7b, H-2, H-6, H-8′ (w)[a]
7b	3.14	H-7a	H-7a, H-2, H-6, H-8′	C-8′, C-8, C-2, C-6, C-1, C-9	H-7a, H-2, H-6, H-8′ (w)[a]
2′	7.33	H-6′	H-5′, H-6′, 3′-OMe	C-6′, C-4′, C-7′	
5′	6.87	H-6′	H-2′, H-6′	C-6′, C-1′, C-3′, C-4′	
6′	7.09	H-2′, H-5′	H-2′, H-5′, 4′-OH	C-2′, C-4′, C-7′	
8′	4.20	H-9′a, H-9′b	H-6′, H-7b	C-8, C-7′	H-7a (w), H-7b (w), H-9′a[a]
9′a	4.22	H-9′b	H-9′b	C-8, C-9, C-7′	H-9′b, H-8′
9′b	4.46	H-9′a	H-9′a	C-8, C-9, C-7′, C-8′	H-9′a
3-OMe	3.80		H-2	C-3	
4-OH	5.59		H-6	C-5, C-3, C-4	
8-OH	4.38		H-7a	C-8′, C-8, C-9, C-7′	
3′-OMe	3.90		H-2′	C-3′	
4′-OH	6.20		H-6′	C-5′, C-3′, C-4′	

[a] w = weak.

found that the amount of secoisolariciresinol was very small.

We have previously isolated and identified (+)-pinoresinol (**6**), (+)-*allo*-hydroxymatairesinol (**7**), (−)-hydroxymatairesinol (**8**), and (+)-oxomatairesinol (**9**) from the sapwood of *T. heterophylla* (Kawamura et al., 1996b). In the present study, chiral HPLC analysis for lignans **1**–**4** and **6**–**9** was carried out, and all these lignans were suggested to be optically pure. Davin et al. reported that a combination of a dirigent protein (78 kDa) and an oxidase catalyzed the enantioselective coupling of bimolecular phenoxy radicals from coniferyl alcohol to afford optically pure (+)-pinoresinol in *Forsythia intermedia* (Davin et al., 1997). The metabolic steps to produce optically pure lignans differ among different plant species. In contrast to the lignan biosynthesis in *Forsythia* sp., (−)-pinoresinol, (−)-lariciresinol and (−)-secoisolariciresinol isolated from *Wikstroemia sikokiana* are not optically pure and not racemic (Umezawa et al., 1997). The results of enantiomeric composition analysis of the lignans from *T. heterophylla* imply that the stereochemical mechanisms of lignan biosynthesis in the plant could be similar to that in *Forsythia* spp., and optically pure lignans (e.g. (−)-hydroxymatairesinol) might be formed in the later metabolic steps, although the detailed mechanism is unknown.

The quantity of photodiscoloration (expressed by color difference, ΔE^*) of lignans in paper matrix was investigated by light-irradiation test with a high-pressure mercury lamp. The influence of concentration of lignan **8** on the ΔE^* of photodiscoloration was investigated (Fig. 1). As the concentration of sample solution increased, ΔE^* increased, leveling it off at 20 mg/ml. Fig. 2 shows the time course of ΔE^* change of lignans **1**, **8** and **12**. ΔE^* of **1** was highest at 48-h irradiation. In the case of **12**, irradiation for 12 h showed maximum ΔE^*, followed by reduction by half at 72 h. It was presumed that the conjugated system formed in the photodiscoloration products of **12** was cleaved as the irradiation time was extended. ΔE^* of 13 lignans/neolignan were displayed in Fig. 3. Lignans **1**, **9**, **10**, **11** and **12**, and a neolignan **13** photodiscolored as well. The large values of ΔE^* observed for **1** and **9** appear to be caused by excitation of the 7′-carbonyl group (Kringstad and Lin, 1970). The results obtained from the ΔE^* of **10**, **11** and **12** also suggest a preliminary structure–photodiscoloration relationship, that is, the presence of tetrahydronaphthalene skeleton increases the ΔE^*. The neolignan **13** possesses a phenylcoumaran skeleton. Regarding the mechanochemical reactions of lignin, stilbenoids and phenylcoumarones were isolated from a mixture of reaction products of phenylcoumaran models, and a radical reaction mechanism was proposed (Lee et al., 1990). Assuming that stilbenoids or phenylcoumarones are formed by light-irradiation of **13**, the product is considered to contribute to photodiscoloration, since it has a stilbenoid skeleton including the conjugated system (Morgan and Orsler, 1968).



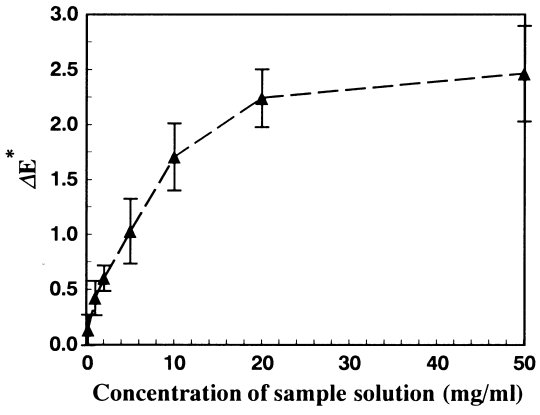


Fig. 1. The influence of sample **8** concentration on the extent of photodiscoloration (ΔE^*). Conditions for light-irradiation: temperature, 36°C; irradiation time, 24 h. Results are expressed as mean ± SD ($n = 3$).

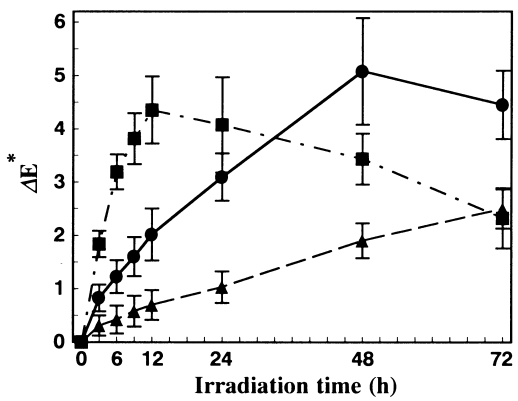
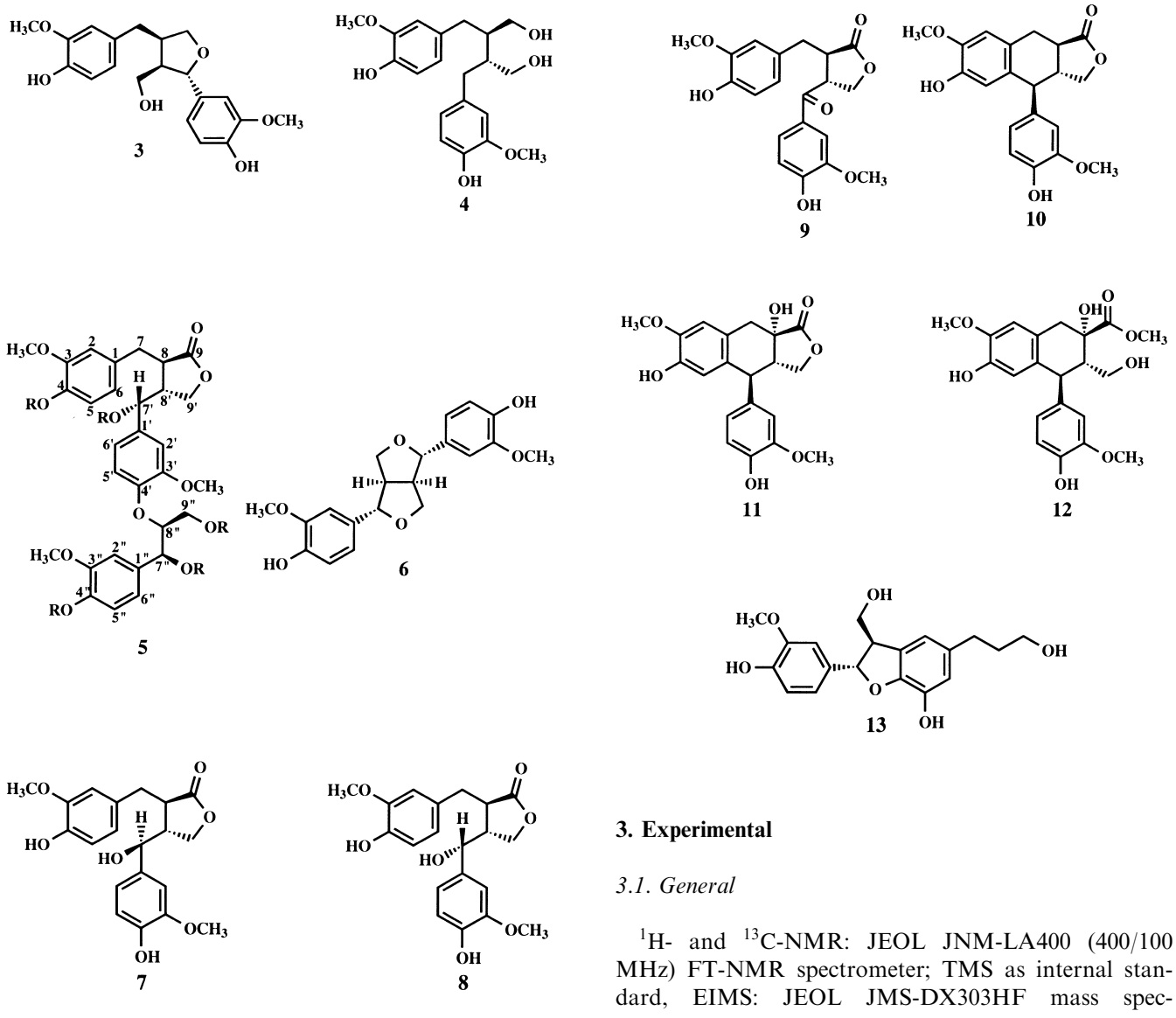


Fig. 2. Time course of ΔE^* change of lignans **1** (●), **8** (▲) and **12** (■). Conditions for light-irradiation: temperature, 36°C; sample concentration, 5 mg/ml. Results are expressed as mean ± SD ($n = 3$).



3. Experimental

3.1. General

^{1}H- and ^{13}C-NMR: JEOL JNM-LA400 (400/100 MHz) FT-NMR spectrometer; TMS as internal standard, EIMS: JEOL JMS-DX303HF mass spec-

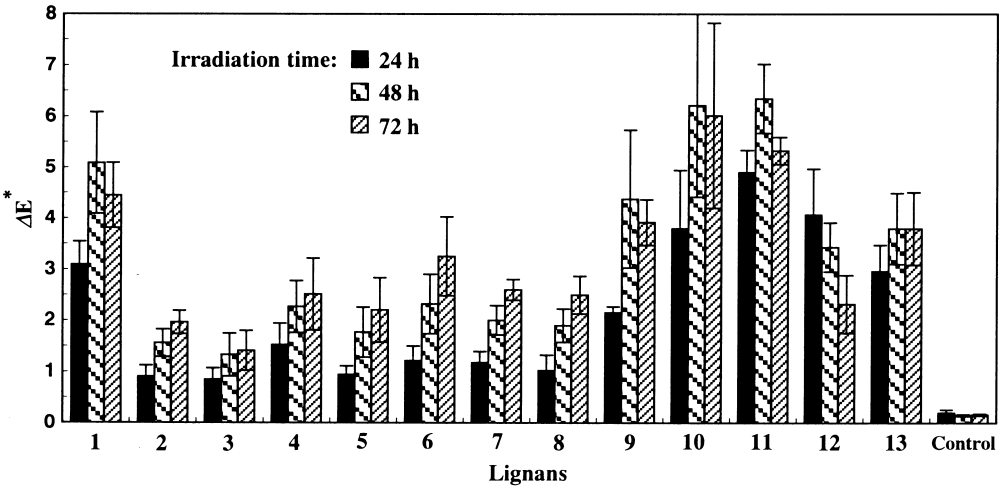


Fig. 3. ΔE^* of lignans **1**–**12** and a neolignan **13**. Conditions for light-irradiation: temperature, 36°C; sample concentration, 5 mg/ml. Results are expressed as mean ± SD ($n = 3$).

trometer; UV: Shimadzu UV-3100PC UV–Vis–NIR scanning spectrophotometer; IR: Perkin–Elmer spectrum 2000 FT-IR spectrometer; optical rotations: Horiba SEPA-300 polarimeter; Other analytical equipment, plant materials, extraction, and fractionation were the same as in our previous report (Kawamura, et al., 1997).

3.2. Isolation

The EtOAc-soluble fraction obtained from MeOH extracts of *T. heterophylla* sapwood was subjected to CC (C_6H_6–EtOAc (8:2–0:10, v/v)) and 95 fractions were collected in 100 ml portions. A mixture of eluates (frs. 33–34) was subjected to reverse phase prep. HPLC (MeOH–H_2O (38:62, v/v)) to afford crude compound **1**. Crude compound **1** was purified by repeated prep. HPLC (MeOH–H_2O (32:68, v/v)) to afford pale yellow amorphous 8-hydroxy-oxomatairesinol (**1**). Three mixtures of eluates (frs. 18–21, 65–82, and 83–96) were subjected to reverse phase prep. HPLC (MeOH–H_2O (55:45, 42:58, and 44:66, v/v)) to afford amorphous matairesinol (**2**), lariciresinol (**3**), and secoisolariciresinol (**4**), respectively.

3.3. (8S,8′S)-(+)-8-Hydroxy-oxomatairesinol (*1*)

Pale yellow amorphous powder, $[\alpha]_D^{25}$ +97.3° (MeOH; *c* 0.38);. $[\alpha]_D^{25}$ +95.1° (THF; *c* 0.38), >99% e.e.; UV λ_{max}^{MeOH} nm (log ε): 308.6 (3.94), 281.2 (4.08), 231.6 (4.29), and 201.0 (4.62); IR ν_{max}^{film} cm^{-1}: 3421 (OH), 1772 (γ-lactone), 1659 (conjugated C=O), 1591, 1516, 1429, 1274, 1198, 1129, and 1032; ^1H-, ^{13}C-NMR special data: see Table 1; ^1H–^1H COSY, long-range ^1H–^1H COSY, HMBC, and NOE difference spectra: see Table 2; EIMS *m/z* (rel. int. (%)): 388 (17, M^+), 370 (4), 210 (10), 178 (28), 151 (83), 137 (100), 123 (14); HR-EIMS *m/z*; found: 388.1168, calcd. for $C_{20}H_{20}O_8$: 388.1158.

3.4. (8R,8′R)-(−)-Matairesinol (*2*)

Colorless amorphous powder, >99% enantiomeric excess (e.e.), M^+ 358. NMR: identical with literature. (Umezawa et al., 1991).

3.5. (7′S,8R,8′R)-(+)-Lariciresinol (*3*)

Colorless amorphous powder, >99% e.e., M^+ 360; NMR: identical with literature (Abe and Yamauch, 1989).

3.6. (8R,8′R)-(−)-Secoisolariciresinol (*4*)

Pale yellow amorphous powder, >99% e.e., M^+ 362; NMR: identical with literature (Umezawa et al., 1991).

3.7. (8R,8′R,7′R,8′S,7′R)-(−)-7′-Hydroxylappaol E pentaacetate (pentaacetate of *5*)

In a previous paper, the assignment of carbon atoms for 7′-hydroxylappaol E pentaacetate was incomplete (Kawamura et al., 1997). By the DEPT, HMQC, and HMBC spectral data in the present study, it was completely assigned as follows: ^{13}C-NMR spectral data ($CDCl_3$): δ: 20.62 (PhOAc), 20.65 (PhOAc), 20.73 (9″-OAc), 21.03 (7′-OAc), 21.06 (7″-OAc), 34.75 (C-7), 43.33 (C-8′), 43.67 (C-8), 55.77 (3-OMe), 55.93 (3″-OMe), 55.96 (3′-OMe), 62.88 (C-9″), 67.78 (C-9′), 74.36 (C-7″), 75.59 (C-7′), 80.08 (C-8″), 110.74 (C-2′), 111.69 (C-2′), 113.64 (C-2), 118.37 (C-5), 118.44 (C-6′), 119.54 (C-6″), 121.85 (C-6), 122.79 (C-5″), 123.26 (C-5′), 131.97 (C-1), 135.27 (C-1″), 135.97 (C-1′), 139.89 (C-4′), 139.97 (C-4″), 146.78 (C-4), 150.72 (C-3), 151.11 (C-3″), 151.41 (C-3′), 168.72 (7′-OAc), 168.79 (4-OAc), 169.65 (4″-OAc), 169.70 (7″-OAc), 170.56 (9″-OAc), 178.00 (C-9).

3.8. Relative configurations of (−)-8-hydroxy-α-conidendrin (*11*) and (+)-8-hydroxy-α-conidendric acid methyl ester (*12*)

In a previous paper, the relative configurations of **11** and **12** were not clear (Kawamura et al., 1997). The NOE difference spectra of **11** and **12** exhibited an NOE between H-8′ and H-7, suggesting a *cis*-relationship between H-8′ and C-7. Therefore, the relative configurations of **11** and **12** were confirmed to be (7′*S*,8*S*,8′*S*).

3.9. Quantitative determination of lignans in the sapwood of T. heterophylla

Solvents for HPLC: HPLC grade (Wako, Osaka, Japan). The quantitative determination by HPLC was based on calibration curves prepared previously with authentic specimens. The eluent and condition for the analysis were as follows: eluent, MeOH–H_2O (30:70, v/v, for **1** and **3**), MeOH–H_2O (38:62, v/v, for **2**), MeOH–MeCN–H_2O (13:13:74, v/v, for **4**); flow rate, 0.5 ml/min; detection, UV at 280 nm; column oven temperature: 40°C. The retention times (min) of the lignans were **1** (24.6), **3** (37.4), **2** (29.0), and **4** (28.7).

3.10. Chiral HPLC method

Solvents for HPLC: HPLC grade (Wako, Osaka, Japan). All lignans were isolated from *T. heterophylla* without recrystallization. Chiral columns: Daicel Chiralcel OD and Chiralcel OC (each 250 × 4.6 mm i.d.).

The eluents and conditions for chiral HPLC analysis [No. of lignan, eluent (v/v), flow rate (ml/min), chiral column (Chiralcel OD or OC)]: **1**, *n*-hexane–EtOH (70:30), 0.5, OD; **9**, *n*-hexane-EtOH (80:20), 0.5, OD; **2** (Umezawa and Shimada, 1996), **7** and **8**, *n*-hexane–1% AcOH in EtOH (85:15), 1.0, OD; **3**, *n*-hexane–EtOH (20:80), 0.4, OC (Katayama et al., 1997); **4**, *n*-hexane–EtOH (70:30), 0.8, OD (Umezawa et al.,1994); **6**, EtOH, 0.4, OD (Umezawa et al., 1994). Detection: UV at 280 nm. The retention volumes (ml) of the lignans were as follows: (+)-**1** (37.2), (+)-**9** (23.6), (−)-**2** (32.7), (+)-**3** (8.0), (−)-**4** (7.4), (+)-**6** (13.5), (+)-**7** (23.6), and (−)-**8** (26.3). Lignans **2**, **3**, **4** and **6** were optically pure (>99% e.e.). We confirmed the retention volumes (ml) of (+)-**6** (5.7) and (−)-**6** (13.5) by chiral HPLC analysis of synthesized racemic pinoresinol. The additional chiral HPLC analyses of lignans **1**, **7**, **8** and **9** were carried out with both Daicel Chiralcel OD and Chiralcel OC chiral columns, and the ratio of solvents (*n*-hexane–1% AcOH in ethanol or isopropanol) as eluents was varied from 10:90 to 100:0. All the eluents showed a single peak, and it was considered that these lignans were optically pure.

3.11. Phtotodiscoloration tests with the lignans isolated from the sapwood of T. heterophylla

The photodiscoloration tests were carried out by light-irradiation with a high-pressure mercury lamp (Matsushita Electric Industrial Inc., BHRF100, 300 W). L^*, a^* and b^* values (CIE1976 L*a* b*color system) were measured by a color difference meter (Minolta CR-200b, Tokyo) using standard light source D_{65}. Quantitative filter papers (No. 7, Advantec Toyo Inc., Tokyo, 12 × 12 mm) were soaked in acetone solutions of the isolated lignans (0.2–50 mg/ml) for 1 min and air-dried. Lignan free filter papers were used as control. The irradiation conditions were as follows: distance between the sample and light source, 20 cm; temperature of irradiation field, 36°C. L^*, a^* and b^* values of the sample filter papers were measured before and after light-irradiation, and the color differences (ΔE^*) of the samples were calculated on the basis of the following formula: $\Delta E^* = [(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2]^{1/2}$. In the formula, L^*, a^* and b^* were expressed in measured values after exposure, and L_0^*, a_0^* and b_0^* were expressed in measured values of unexposed samples. The photodiscoloration tests were repeated three times for each data point.

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