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SESQUILIGNANS AND LIGNANS FROM *TSUGA HETEROPHYLLA*

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Key Word Index—*Tsuga heterophylla*; Pinaceae; western hemlock; plant chemotaxonomy; sapwood; sesquilignan; lignan.

Abstract—Two epimeric sesquilignans and two lignans were isolated from the sapwood of *Tsuga heterophylla* (western hemlock, Pinaceae). By spectroscopic analyses their structures were deduced to be (8*R*,8′*R*,7′*R*,8″*S*,7″*R*)-7′-hydroxylappaol E and (8*R*,8′*R*,7′*R*,8″*S*,7″*S*)-*epi*-7′-hydroxylappaol E, and (8′*R*,7′*S*)-8-hydroxy-α-conidendrin and (8′*R*,7′*S*)-8-hydroxy-α-conidendric acid methyl ester. Their presence in several samples of western hemlock sapwood was confirmed by quantitative analyses. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Western hemlock (*Tsuga heterophylla* (Raf.) Sarg., Pinaceae) is a conifer native to North America. Many of its phenolic constituents have been identified, including α-conidendrin, hydroxymatairesinol, oxomatairesinol, pinoresinol, leucocyanidin, catechin, *epi*-catechin, quinic acid, shikimic acid, vanillic acid, ferulic acid, dehydrodiconiferyl alcohol and coniferin [1, 2].

We have previously reported chemotaxonomic analyses of minute coniferous constituents [3–5]. With regard to western hemlock, we have also reported studies of its photodiscoloration [6, 7]. Photodiscoloring constituents isolated from sapwood include (+)-cedrusin, (+)-*allo*-hydroxymatairesinol, (−)-hydroxymatairesinol, (+)-oxomatairesinol, (−)-α-conidendrin, (+)-pinoresinol and (−)-matairesinol [7]. (+)-Cedrusin and (+)-*allo*-hydroxymatairesinol were first isolated from the sapwood of *T. heterophylla*. In continuation of this work, four additional new constituents (two sesquilignans and two lignans), which also cause photodiscoloration, were isolated. In this paper we describe the elucidation of their structures and discuss their presence from the standpoint of chemotaxonomy.

RESULTS AND DISCUSSION

Two new sesquilignans (**1** and **2**) and two new lignans (**3** and **4**) were isolated from *T. heterophylla* sapwood by chromatographic methods. Their structures were elucidated mainly by analysing their ^1H and ^{13}C NMR spectra.

Compound **1**, $C_{30}H_{34}O_{11}$, $[\alpha]_D^{25}-3.7°$ (MeOH), showed a $[M-H_2O]^+$ at *m/z* 552 in its EI mass spectrum. The IR absorption of 1751 cm^{-1} indicated the presence of a γ-lactone. The ^1H NMR spectrum of **1** did not reveal any acetoxyl groups. Because **1** co-eluted with **2**, the mixture was acetylated to give **1a** and **2a**. The detailed structure of **1** was determined using **1a**, as described below.

The EI mass spectrum of compound **1a** exhibited $[M]^+$ at *m/z* 780. Its ^1H NMR spectrum established the presence of three alcoholic acetoxyl groups, two phenolic acetoxyl groups, three methoxyl groups, three methylene groups, five methine groups and nine aromatic protons. The ^1H and ^{13}C NMR spectra of **1a** contained signals which were almost identical to those of hydroxymatairesinol triacetate (see (**5a**) in Experimental) [7]. The remaining signals corresponded to an additional phenylpropanoid moiety. The assignment of the protons was confirmed by a ^1H–^1H COSY analysis. It was established that two double doublets at δ 3.88 and 3.93 (*J* = 9.2, 7.3 Hz, H-9′$_a$; *J* = 9.2, 7.9 Hz, H-9′$_b$) and one doublet at δ 5.83 (*J* = 6.7 Hz, H-7′) were mutually coupled with the resonance at δ 2.76 (*m*, H-8′). A multiplet at δ 2.80 (H-8) was coupled with a multiplet at δ 2.85 (H-7$_a$) and a double doublet δ 3.11 (*J* = 14.0, 4.9 Hz, H-7$_b$), together with another multiplet (H-8′). These signals corresponded to the hydroxymatairesinol moiety. It was also found that two double doublets at δ 4.02, 4.33 (*J* = 11.6, 5.5 Hz, H-9″$_a$; *J* = 11.6, 4.9 Hz, H-9″$_b$) and a doublet at δ 6.11 (*J* = 6.1 Hz, H-7″) were mutually coupled with the signal at δ 4.59 (*m*, H-8″), with no further coupling

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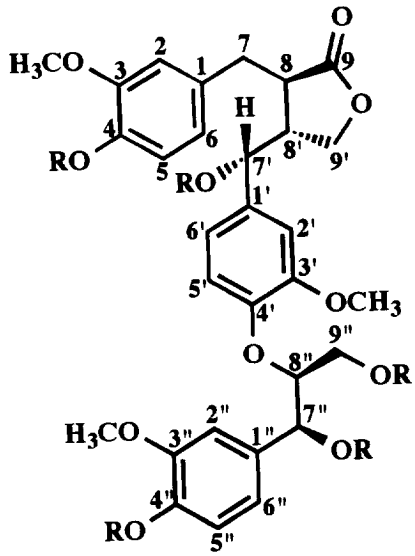

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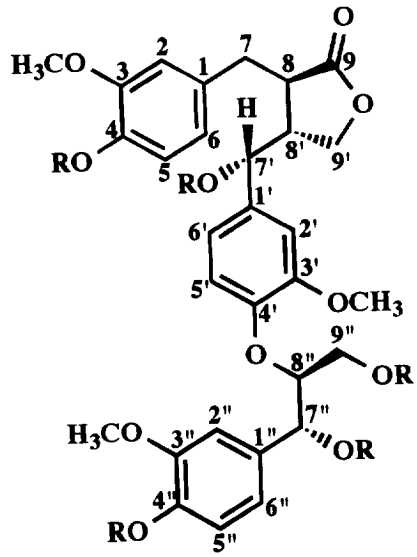

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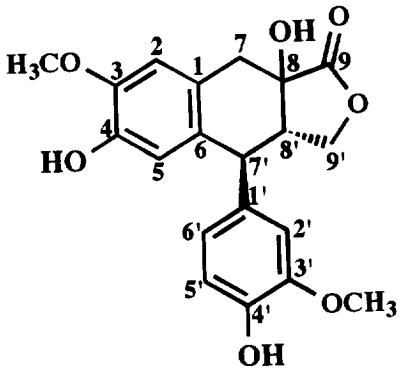

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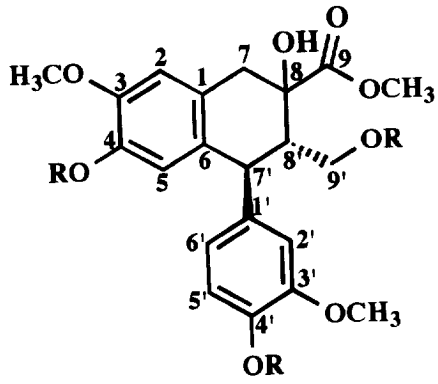

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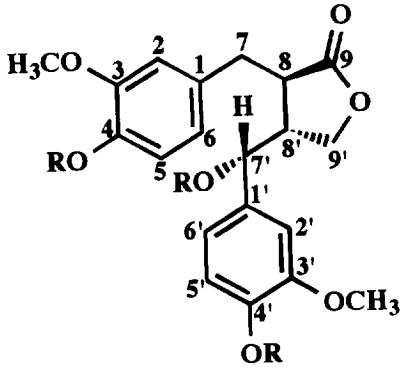

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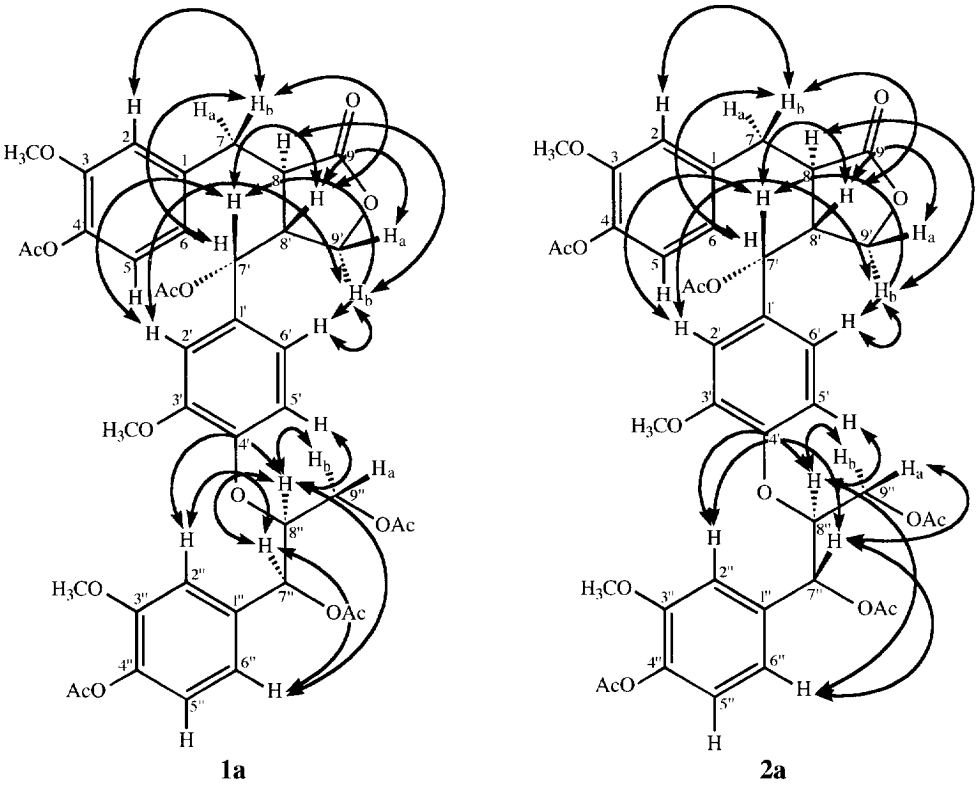

Fig. 1. NOE interactions in compounds **1a** and **2a**. Note: the NOE between H-7$_a$ and H-8 could not be confirmed because of partial saturation, which is responsible for their very approximate chemical shifts.

relationship. These signals belong to an additional phenylpropanoid moiety (1″–9″). Therefore, this moiety was linked with an hydroxymatairesinol moiety through an ether linkage. Furthermore, nuclear Overhauser enhancement (NOE) difference and NOESY experiments were performed to determine the spatial relationships of **1a** (Fig. 1). The NOEs (illustrated by arrows in Fig. 1) clearly revealed the presence of a *trans* relationship between H-8/H-8′ and *cis* relationships between H-7′ and H-8′ and H-7″ and H-8″. In this way, the stereochemical structure for the new sesquilignan (**1**, (8*R*, 8′*R*, 7′*R*, 8″*S*, 7″*S*)-7′-hydroxylappaol E) was deduced. Lappaol E, which lacks one hydroxyl group (C-7′–OH) has been isolated previously from *Arctium lappa* L. (Compositae) [8].

The EI mass spectrum and ^{13}C NMR spectrum of the acetate (**2a**) of **2** were very similar to those of **1a**. However, **2a** differed from **1a** in its chromatographic behaviour, and **2a** has an $[\alpha]_D^{25}$ value of +48.0° in chloroform, compared to $[\alpha]_D^{25}$ +13.7° for **1a**. In the ^1H NMR spectrum of **2a**, the number of protons was the same as in **1a**, but some differences were observed in the chemical shifts and coupling constants between **2a** and **1a**. In particular, differences were noted in the chemical shifts of the phenylpropanoid moiety, including the *vicinal* coupling constants of H-9″$_a$ (**1a**, 5.5 Hz; **2a**, 4.3 Hz), and of H-9″$_b$ (**1a**, 4.9 Hz; **2a**, 5.5 Hz) and the coupling constants of H-7″ (**1a**, 6.1 Hz; **2a**, 5.5 Hz). NOE difference and NOESY experiments were performed to reveal the spatial relationships of **2a** (Fig. 1). The confirmed NOEs revealed *trans* relationships between H-8 and H-8′ and H-7″ and H-8″ and a *cis* relationship between H-7′ and H-8′. Finally, the stereochemical structure of the new sesquilignan (**2**, (8*R*, 8′*R*, 7′*R*, 8″*S*, 7″*R*)-*epi*-7′-hydroxylappaol E) was elucidated. *epi*-7′-Hydroxylappaol E is the epimer of naturally occurring sesquilignan (**1**). This represents the first example of sesquilignan epimers but, interestingly, no *allo*-hydroxymatairesinol was detected.

Previously, we had isolated another pair of epimers, hydroxymatairesinol and *allo*-hydroxymatairesinol, from the sapwood of *T. heterophylla* [6, 7]. The isolation of two pairs of epimeric mono- and sesquilignans from western hemlock called our attention to their biosynthetic pathways, apart from the original photodiscoloration problem. Recently, the incisive studies on the mechanism of stereochemical control in lignan biosynthesis has progressed rapidly. For example, the route from coniferyl alcohol to (+)-pinoresinol, (+)-lariciresinol and/or (−)-secoisolariciresinol (→(−)-matairesinol) has been established in *Forsythia* plants (Oleaceae) [9–11]. However, details of the co-existence and biosynthesis of such epimers in certain species await clarification. It may be considered that, together with reactions from matairesinol to hydroxymatairesinol, conversion from hydroxymatairesinol (**5**) to the epimeric sesquilignans

1 and **2** proceed in *T. heterophylla* with stereochemical control. This important biosynthetic problem awaits clarification for the final characterization of how lignans differ from lignins.

Compound **3** ($C_{20}H_{20}O_7$; $[\alpha]_D^{25}$ −55.4° (MeOH)), showed a $[M]^+$ at *m/z* 372.4339 in its high-resolution EI mass spectrum. The IR absorption at 1749 cm^{-1} indicated the presence of a γ-lactone. The ^{13}C NMR spectrum showed the presence of a carbonyl group, two phenolic methoxyl groups, two methylene groups, two methine groups, an oxygenated quaternary carbon atom and two phenyl groups. The assignment of the carbon atoms was established by ^1H–^{13}C COSY, and the ^1H NMR resonances were assigned using ^1H–^1H COSY. It was revealed that two double doublets at δ 4.11 and 4.34 (J = 8.4, 7.0 Hz, H-9′$_a$; J = 8.4, 10.6 Hz, H-9′$_b$) and a doublet at δ 4.15 (J = 11.4 Hz, H-7′) were mutually coupled with the signal at δ 2.53 (*m*, H-8′). Two signals at δ 3.09 (J = 16.9 Hz, H-7$_a$) and δ 3.16 (J = 16.9 Hz, H-7$_b$) appeared as doublets, respectively. Therefore, the corresponding methylene group was considered to be sandwiched between two quaternary carbon atoms, as revealed by ^{13}C NMR spectroscopy. In the heteronuclear multiple bond correlation (HMBC) spectrum of **3**, the methylene proton at δ 3.09 (H-7$_a$) showed five correlations (to C-1, C-2, C-6, C-8 and C-8′), as did the methine proton at δ 3.15 (H-7′) (to C-6, C-1′, C-2′, C-6′ and C-8′). The results of the other long-range C–H couplings were also substantiated by the assignment of the ^1H NMR spectrum. The spatial relationships of **3** were determined from the NOE difference spectra. The confirmed NOEs revealed a *trans* relationship between H-7′ and H-8′; however, the configuration at the singlet carbon atom (C-8) could not be elucidated. In this way, the structure of the new lignan (**3**, (8′*R*,7′*S*)-8-hydroxy-α-conidendrin) is proposed.

Compound **4** ($C_{21}H_{22}O_7$, $[\alpha]_D^{25}$ +65.4° (MeOH)) showed a $[M]^+$ at *m/z* 404 in its EI mass spectrum. The IR absorption at 1729 cm^{-1} indicated the presence of a carbonyl group. The ^{13}C NMR (off-resonance, DEPT and ^1H–^{13}C COSY) spectra revealed the presence of a carbonyl group, two aromatic methoxyl groups, an aliphatic methoxyl group, two methylene groups, two methine groups, an oxygenated quaternary carbon atom and two phenyl groups. The ^1H NMR resonances were assigned using a ^1H–^1H COSY analysis. It was revealed that a doublet at δ 4.08 (1H, J = 11.4 Hz, H-7′) and a doublet at δ 3.58 (2H, J = 4.0 Hz, H-9′) were mutually coupled with the signal at δ 2.40 (1H, *dt*, J = 11.4, 4.4 Hz, H-8′). The signals at δ 2.83 (J = 16.1 Hz, H-7$_a$) and δ 3.50 (J = 16.1 Hz, H-7$_b$) appeared as two doublets. Therefore, the corresponding methylene group was considered to be between two quaternary carbon atoms, similar to the situation in **3**. However, it was suggested that the lactone ring was not present in **4**, as judged from the signal pattern of the H-9′. The signal of C-9′–OMe appeared at low field (δ 3.85) compared with the usual resonance of an aliphatic OMe group, because of its ester coupling. The EI mass spectrum of the acetate (**4a**) of **4** showed a $[M]^+$ at *m/z* 530. The ^1H NMR spectrum of **4a** exhibited signals for three acetoxyl groups. These results suggested that three hydroxyl groups, except the C-8–OH (tertiary alcohol hydroxyl group), were acetylated. In comparison with **4**, the signal for H-9′ of **4a** was shifted to lower field (δ 3.84–3.91, H-9′$_a$; δ 4.11, H-9′$_b$) on acetylation. NOE difference experiments were performed to show the spatial relationships in **4**. The confirmed NOEs revealed a *trans* relationship between H-7′ and H-8′; however, the configuration of the quaternary carbon atom (C-8) could not be elucidated. Thus, the structure, (8′*R*, 7′*S*)-8-hydroxy-α-conidendric acid methyl ester was proposed for the new lignan (**4**). In connection with the structure of **4**, thomasic acid from *Ulmus thomasii* (Ulmaceae) [12] and α-conidendric acid from *Picea abies* (Pinaceae) [13] have been reported previously.

Table 1. Content* of new minor lignans in 13 sapwood samples of *Tsuga heterophylla*

Sample no.	Lignan (ppm)			
	1	**2**	**3**	**4**
1	35.4	16.6	39.9	–†
2	15.5	6.5	18.8	19.6
3	24.4	–	–	–
4	6.6	6.3	13.1	–
5	10.0	7.8	–	–
6	17.7	3.3	22.1	–
7	–	–	44.2	15.3
8	–	–	–	–
9	–	–	9.4	–
10	37.6	–	–	–
11	25.5	5.5	–	–
12	–	–	19.0	21.2
13	26.6	8.9	55.0	38.4

*Calculated based on air-dried sapwood samples.

†Not determined with reliability under the established analysis conditions.

The presence of the four minor constituents, 7′-hydroxylappaol E (**1**), *epi*-7′-hydroxylappaol E (**2**), 8-hydroxy-α-conidendrin (**3**) and 8-hydroxy-α-conidendric acid methyl ester (**4**), were confirmed by additional quantitative HPLC experiments, using 13 individual samples of *T. heterophylla* sapwood (Table 1). In their ethyl acetate soluble fractions, **1** was determined in nine samples with good reliability. Similarly, **2** was determined in seven, **3** was found in eight and **4** was present in four samples. Therefore, it was concluded that these compounds are secondary metabolites in *T. heterophylla*, although they are present in small amounts.

EXPERIMENTAL

General. ^1H and ^{13}C NMR: JEOL JNM-GX270 (270M Hz) and/or JEOL JNM-α-500 (500M Hz) (for

^{13}C NMR and NOESY) FT-NMR spectrometers; TMS as int. stand. EIMS: Shimadzu GCMS-QP1000 spectrometer. HR-EIMS: Hitachi M-80 B spectrometer (direct insertion probe, 70 eV). UV spectra: JASCO V-550 UV/VIS spectrophotometer. IR spectra: Perkin Elmer system 2000 FT-IR spectrophotometer. Optical rotations: Union PM-201 polarimeter. HPLC: Gasukuro Kogyo Model 572P pump and Hitachi 655A UV detector and/or JASCO PU-980 pump and JASCO UV-970 UV/VIS detector; Waters Radial-Pak 8NVC$_{18}$ (100 × 8.0 mm ID), Waters μ Bondasphere 5μ C$_{18}$ 100Å (150 × 3.9, 150 × 19.0 mm ID) and Zorbax BP-SIL (250 × 4.6, 250 × 9.4 mm ID) columns; UV detection at 280 nm. TLC and prep. TLC: silica gel (Merck, Kieselgel 60 F_{254}, 0.5 mm); after development, compounds were located by exposure to UV light (254 and/or 365 nm) and recovered from the adsorbent using CH_2Cl_2–MeOH (8:2). CC: silica gel (Wako-gel C-200).

Extraction and isolation. Sample blocks of western hemlock were provided by a timber dealer in Shimizu, imported from the U.S.A. The air-dried sapwood meals (e.g. sample No. 13, 1.00 kg) were extracted with MeOH at room temp. for 72 hr and filtered (× 3). The combined MeOH soln was evaporated *in vacuo* to give the dry extract (22.19 g), which was next successively extracted with *n*-hexane, EtOAc and *n*-BuOH (× 5). After removing solvent from the combined extracts, *n*-hexane (2.15 g), EtOAc (9.07 g) and *n*-BuOH (3.03 g) soluble fractions and a *n*-BuOH insoluble fraction (7.92 g) were obtained, respectively. The EtOAc soluble fraction (3.00 g) was chromatographed on prep. TLC (C_6H_6–$CHCl_3$–acetone, 1:1:1, v/v), and 12 bands were separated. A mixt. of compounds **1** and **2** (fr. 1) from band 3 was obtained by repeated prep. TLC (C_6H_6–diisopropyl ether–MeOH, 10:78:12 (v/v)). Amorphous compound **1** (3.2 mg) from fr. 1 (11.0 mg) was purified by repeating prep. TLC (C_6H_6–$CHCl_3$–MeOH, 28:56:16 (v/v); 26.6 ppm yield, based on the air-dried sample powder). Apart from the above, fr. 1 (19.2 mg) was acetylated with acetic anhydride and pyridine at room temp. Pentaacetates **1a** (5.9 mg) and **2a** (2.0 mg) were purified by repeated normal phase semi-prep. HPLC (*n*-hexane–$CHCl_3$–MeOH, 81:10:9 (v/v); **1a**, 25.4 ppm yield, **2a**, 8.6 ppm yield, based on the air-dried sample powder). Another EtOAc soluble fr. (6.07 g) was chromatographed on CC (C_6H_6–EtOAc, 8:2–0:10, (v/v)) and 95 frs were collected in 100-ml portions. A mix. of eluates (Nos 33 and 34) was chromatographed on reversed-phase prep. HPLC (MeOH–water, 38:62 (v/v)) to afford the amorphous compounds **3** (36.8 mg) and **4** (25.7 mg) (**3**, 55.0 ppm yield, **4**, 38.4 ppm yield, based on the air-dried sample powder).

(8R, 8′R, 7′R, 8″S, 7″S)-(−)-7′-*Hydroxylappaol E* (**1**). Amorphous powder; $[\alpha]_D^{25}$ −3.7° (MeOH; *c* 0.27). UV λ_{max}^{MeOH} nm (log ε): 280.8 (4.01), 230.0 (4.40), 208.0 (4.63). IR ν_{max}^{KBr} cm^{-1}: 3433 (OH), 2919, 1751 (γ-lactone), 1606, 1516, 1466, 1454, 1431, 1386, 1271, 1232, 1157, 1127, 1032. ^1H NMR (CD_3OD) δ: 2.58 (1H, *m*, H-8′), 2.74 (1H, *m*, H-8), 2.89 (2H, *m*, H-7), 3.47 (1H, *dd*, *J* = 12.1, 5.1 Hz, H-9′$_a$), 3.79, 3.80, 3.81 (9H, each *s*, 3 OMe), 4.09 (2H, *m*, H-9′$_b$, H-9″$_a$), 4.30 (2H, *m*, H-9″$_b$, H-8″), 4.65 (1H, *d*, *J* = 5.13 Hz, H-7′), 4.81 (1H, *d*, *J* = 5.9 Hz, H-7″), 6.48–7.03 (9H, aromatic H). EIMS *m/z* (rel. int. (%)): 552 $[M-H_2O]^+$ (1), 534 (4), 522 (5), 504 (4), 382 (15), 374 (35), 356 (22), 298 (11), 232 (9), 180 (39), 153 (32), 137 (100), 124 (64).

(8R, 8′R, 7′R, 8″S, 7″S)-(+)-7′-*Hydroxylappaol E pentaacetate* (**1a**). Acetylation of **1** with acetic anhydride and pyridine gave corresponding penta-acetate (**1a**). Amorphous powder, $[\alpha]_D^{25}$ +13.7° ($CHCl_3$; *c* 0.49). ^1H NMR ($CDCl_3$) δ: 2.01, 2.07, 2.14 (9H, each *s*, 3 × alc. OAc), 2.30 (6H, *s*, 2 × PhOAc), 2.76 (1H, *m*, H-8′), 2.80 (1H, *m*, H-8), 2.85 (1H, *m*, H-7$_a$), 3.11 (1H, *dd*, *J* = 14.0, 4.9 Hz, H-7$_b$), 3.77, 3.79, 3.81 (9H, each *s*, 3 OMe), 3.88 (1H, *dd*, *J* = 9.2, 7.3 Hz, H-9′$_a$), 3.93 (1H, *dd*, *J* = 9.2, 7.9 Hz, H-9′$_b$), 4.02 (1H, *dd*, *J* = 11.6, 5.5 Hz, H-9″$_a$), 4.33 (1H, *dd*, *J* = 11.6, 4.9 Hz, H-9″$_b$), 4.59 (1H, *m*, H-8″), 5.83 (1H, *d*, *J* = 6.7 Hz, H-7′), 6.11 (1H, *d*, *J* = 6.1 Hz, H-7″), 6.57 (1H, *dd*, *J* = 7.9, 1.8 Hz, H-6), 6.70 (1H, *d*, *J* = 1.8 Hz, H-2), 6.74 (1H, *d*, *J* = 1.8 Hz, H-2′), 6.79 (1H, *dd*, *J* = 10.4, 1.8 Hz, H-6′), 6.83 (1H, *d*, *J* = 7.9 Hz, H-5), 7.02 (4H, *m*, H-5′, H-2″, H-5″, H-6″). ^{13}C NMR ($CDCl_3$) δ: 20.6, 20.7, 20.7 (each alc. OAc), 21.1 (2 × PhOAc), 34.8 (C-7), 43.4 (C-8′), 43.7 (C-8), 55.8 (OMe), 56.0 (2 OMe), 62.9 (C-9″), 67.8 (C-9′), 74.4 (C-7″), 75.6 (C-7′), 80.1 (C-8″), 110.8, 111.7, 113.7, 118.4, 118.5, 119.6, 121.9, 122.8, 123.3 (C-2, 5, 6, 2′, 5′, 6′, 2″, 5″, 6″), 132.0, 135.3, 136.0 (C-1, 1′, 1″), 139.9, 140.0 (C-4, 4″), 146.8 (C-4′), 150.7, 151.1, 151.4 (C-3, 3′, 3″), 168.7, 168.8, 169.65, 169.70, 170.6 (each OAc), 178.0 (C-9). EIMS *m/z* (%): 780 $[M]^+$ (4), 618 (3), 576 (4), 543 (3), 516 (4), 500 (3), 483 (7), 458 (44), 416 (33), 356 (31), 323 (14), 321 (39), 179 (44), 153 (45), 137 (100).

(8R, 8′R, 7′R)-(−)-*Hydroxymatairesinol triacetate* (**5a**). Hydroxymatairesinol [7] was acetylated for elucidation of the structure of **1a**. Amorphous powder, $[\alpha]_D^{25}$ −9.3° ($CHCl_3$; *c* 0.64), ^1H NMR ($CDCl_3$) δ: 2.13 (3H, *s*, alc. OAc), 2.31 (6H, *s*, 2 × PhOAc), 2.82 (1H, *m*, H-8′), 2.89 (2H, *m*, H-7$_a$, H-8), 3.13 (1H, *dd*, *J* = 15.8, 7.0 Hz, H-7$_b$), 3.79 (6H, *s*, 2 OMe), 3.91 (1H, *dd*, *J* = 9.5, 7.3 Hz, H-9′$_a$), 4.00 (1H, *dd*, *J* = 9.5, 8.1 Hz, H-9′$_b$), 5.83 (1H, *d*, *J* = 6.6 Hz, H-7′), 6.63 (1H, *dd*, *J* = 8.1, 1.8 Hz, H-6), 6.74 (1H, *d*, *J* = 1.8 Hz, H-2), 6.77 (1H, *dd*, *J* = 7.9, 1.8 Hz, H-6′), 6.79 (1H, *d*, *J* = 1.8 Hz, H-2′), 6.94 (1H, *d*, *J* = 8.1 Hz, H-5), 7.01 (1H, *d*, *J* = 7.9 Hz, H-5′). ^{13}C NMR ($CDCl_3$) δ: 20.61, 20.64, 21.00 (each OAc), 35.0 (C-7), 43.4 (C-8), 43.7 (C-8′), 55.9, 56.0 (each OMe), 67.7 (C-9′), 75.5 (C-7′), 110.8 (C-2′), 113.7 (C-2), 118.6 (C-5′), 121.7 (C-5), 122.8 (C-6), 123.3 (C-6′), 135.9 (C-1′), 135.9 (C-1), 138.8 (C-4′), 140.1 (C-4), 151.2 (C-3′), 151.5 (C-3), 168.7, 168.9, 169.6 (each OAc), 177.9 (C-9). EIMS *m/z* (%): 500 $[M]^+$ (2), 458 (48), 416 (27), 398 (18), 356 (30), 232 (14), 220 (15), 177 (50), 153 (43), 137 (100).

(8R, 8′R, 7′R, 8″S, 7″R)-epi-7′-*Hydroxylappaol E* (**2**) *and* (8R, 8′R, 7′R, 8″S, 7″R)-epi-7′-*hydroxylappaol*

E penta-acetate (**2a**). Amorphous powder. $[\alpha]_D^{25}$ +48.0° ($CHCl_3$; *c* 0.17). ^1H NMR ($CDCl_3$) δ: 2.03, 2.10, 2.13 (9H, each *s*, 3 × alc. OAc), 2.300, 2.304 (6H, each *s*, 2 × PhOAc), 2.64–2.90 (3H, *m*, H-8′, H-8, H-7_a), 3.10 (1H, *dd*, *J* = 13.4, 4.3 Hz, H-7_b), 3.74, 3.78, 3.81 (9H, each *s*, 3 OMe), 3.90 (2H, *m*, H-9′), 4.25 (1H, *dd*, *J* = 11.6, 4.3 Hz, H-$9''_a$), 4.44 (1H, *dd*, *J* = 11.6, 5.5 Hz, H-$9''_b$), 4.63 (1H, *m*, H-8″), 5.81 (1H, *d*, *J* = 7.3 Hz, H-7′), 6.07 (1H, *d*, *J* = 5.5 Hz, H-7″), 6.55 (1H, *dd*, *J* = 7.9, 1.8 Hz, H-6), 6.68 (1H, *d*, *J* = 1.83 Hz, H-2), 6.72 (1H, *d*, *J* = 7.9 Hz, H-5), 6.75 (1H, *d*, *J* = 1.8 Hz, H-2′), 6.77 (1H, *dd*, *J* = 7.9, 1.8 Hz, H-6′), 7.02 (4H, *m*, H-5′, H-2″, H-5″, H-6″). EIMS *m/z* (%): 780 $[M]^+$ (6), 618 (2), 576 (4), 516 (5), 483 (6), 458 (33), 416 (20), 356 (28), 323 (42), 221 (94), 179 (67), 153 (38), 137 (100).

(8′ R,7′ S)-(−)-8-*Hydroxy-α-conidendrin* (**3**). Pale yellow amorphous powder, $[\alpha]_D^{25}$ −55.4° (MeOH; *c* 0.56). UV λ_{max}^{MeOH} nm (log ε): 283.8 (3.89), 229.2 (*sh*) (4.25), 210.0 (4.71). IR ν_{max}^{KBr} cm^{-1}: 3422 (OH), 2926, 1749 (γ-lactone), 1637, 1517, 1458, 1385, 1276, 1212, 1158, 1117, 1033. ^1H NMR ($CDCl_3$) δ: 2.31 (1H, *s*, alc. OH), 2.53 (1H, *m*, H-8′), 3.09 (1H, *d*, *J* = 16.9 Hz, H-7_a), 3.16 (1H, *d*, *J* = 16.9 Hz, H-7_b), 3.83 (3H, *s*, 3′-OMe), 3.89 (3H, *s*, 3-OMe), 4.11 (1H, *dd*, *J* = 8.4, 7.0 Hz, H-$9'_a$), 4.15 (1H, *d*, *J* = 11.4 Hz, H-7′), 4.34 (1H, *dd*, *J* = 8.4, 10.6 Hz, H-$9'_b$), 5.42, 5.57 (2H, each *s*, 2 × PhOH), 6.44 (1H, *s*, H-5), 6.60 (1H, *d*, *J* = 1.83 Hz, H-2′), 6.66 (1H, *s*, H-2), 6.69 (1H, *dd*, *J* = 8.1, 1.8 Hz, H-6′), 6.88 (1H, *d*, *J* = 8.1 Hz, H-5′). NOE: H-7_a → H-7_b, H-7_a → H-2, H-7′ → H-$9'_b$, H-7′ → H-5, H-7′ → H-2′, H-7′ → H-6′, H-8′ → H-7_b, H-8′ → H-$9'_a$, H-8′ → H-2′, 3-OMe → H-2, 3′-OMe → H-2′. ^{13}C NMR ($CDCl_3$) (off-resonance) δ: 37.2 (*t*, C-7), 43.2 (*d*, C-7′), 49.9 (*d*, C-8′), 56.0 (*q*, 2-OMe), 70.9 (*t*, C-9′), 71.7 (*s*, C-8), 109.6 (*d*, C-2′), 111.9 (*d*, C-2), 114.7 (*d*, C-5′), 115.1 (*d*, C-5), 121.6 (*d*, C-6′), 123.5 (*s*, C-1), 131.3 (*s*, C-6), 133.4 (*s*, C-1′), 144.4 (*s*, C-4), 144.9 (*s*, C-4′), 145.7 (*s*, C-3), 147.0 (*s*, C-3′), 176.2 (*s*, C-9). HMBC: H-7_a/C-1, H-7_a/C-2, H-7_a/C-6, H-7_a/C-8, H-7_a/C-8′, H-$9'_a$/C-8, H-$9'_a$/C-9, H-7′/C-6, H-7′/C-1′, H-7′/C-2′, H-7′/C-6′, H-7′/C-8′, H-5/C-1, H-5/C-3, H-5/C-4, H-5/C-7′, H-2′/C-4′, H-2/C-3, H-2/C-4, H-2/C-6, H-2/C-7, H-5′/C-1′, H-5′/C-3′, 3-OMe/C-3, 3′-OMe/C-3′. EIMS *m/z* (%): 372 $[M]^+$ (100), 354 (10), 337 (16), 323 (17), 309 (14), 293 (16), 277 (22), 261 (12), 247 (13). HR-EIMS *m/z*: found, 372.4339; calcd. for $C_{20}H_{20}O_7$, 372.3746.

(8′R, 7′S)-(+)-8-*Hydroxy-α-conidendric acid methyl ester* (**4**). Amorphous powder, $[\alpha]_D^{25}$ +65.4° (MeOH; *c* 2.14). UV λ_{max}^{MeOH} nm (log ε): 284.6 (3.89), 231.2 (*sh*) (4.24), 208.6 (4.67). ν_{max}^{KBr} cm^{-1}: 3423 (OH), 2952, 1729 (C=O), 1612, 1516, 1450, 1434, 1366, 1275, 1254, 1215, 1119, 1031. ^1H NMR ($CDCl_3$) δ: 2.40 (1H, *dt*, *J* = 11.4, 4.4 Hz, H-8′), 2.83 (1H, *d*, *J* = 16.1 Hz, H-7_a), 3.50 (1H, *d*, *J* = 16.1 Hz, H-7_b), 3.58 (2H, *d*, *J* = 4.0 Hz, H-9′), 3.836, 3.843 (6H, each *s*, 2 × PhOMe), 3.85 (3H, *s*, (C=O)–OMe), 4.08 (1H, *d*, *J* = 11.4 Hz, H-7′), 5.34, 5.53 (2H, each *s*, 2 × PhOH), 6.35 (1H, *s*, H-5), 6.52 (1H, *s*, H-2), 6.66 (1H, *d*, *J* = 1.83 Hz, H-2′), 6.71 (1H, *dd*, *J* = 8.1, 1.8 Hz, H-6′), 6.85 (1H, *d*, *J* = 8.1 Hz, H-5′). NOE: H-7_a → H-7_b, H-7_a → H-2, H-7′ → H-5, H-7′ → H-2′, H-7′ → H-6′, H-9′ → H-8′. ^{13}C NMR ($CDCl_3$) (off-resonance) δ: 40.3 (*t*, C-7), 43.3 (*d*, C-7′), 47.8 (*d*, C-8′), 52.9 (*q*, (C=O)–OMe), 55.9, 56.0 (*q* each, 2 × PhOMe), 61.8 (*t*, C-9′), 76.3 (*s*, C-8), 110.5 (*d*, C-2), 111.7 (*d*, C-2′), 114.4 (*d*, C-5′), 115.5 (*d*, C-5), 122.4 (*d*, C-6′), 123.4 (*s*, C-1), 131.5 (*s*, C-6), 136.0 (*s*, C-1′), 144.0 (*s*, C-4), 144.4 (*s*, C-4′), 145.2 (*s*, C-3), 146.7 (*s*, C-3′), 177.02 (*s*, C-9). HMBC: H-7_a/C-1, H-7_a/C-2, H-7_a/C-6, H-7_a/C-8, H-7_a/C-8′, H-9′/C-8, H-7′/C-6, H-7′/C-1′, H-7′/C-8′, H-5/C-1, H-5/C-3, H-5/C-4, H-5/C-7′, H-2/C-3, H-2/C-4, H-2/C-6, H-2′/C-4′, H-5′/C-1′, H-5′/C-3′. EIMS *m/z* (%): 404 $[M]^+$ (3), 386 (2), 372 (100), 354 (14), 337 (18), 323 (24), 309 (25), 293 (29), 277 (46), 261 (25), 247 (26), 181 (24), 137 (28), 115 (30).

(8′R′, 7′S)-8-*Hydroxy-α-conidendric acid methyl ester triacetate* (**4a**). Acetylation of compound **4** with acetic anhydride and pyridine gave the corresponding triacetate **4a**. Gum. ^1H NMR ($CDCl_3$) δ: 1.92 (3H, *s*, alc. OAc), 2.21, 2.30 (6H, each *s*, 2 × PhOAc), 2.66 (1H, *m*, H-8′), 2.91 (1H, *d*, *J* = 16.5 Hz, H-7_a), 3.54 (1H, *d*, *J* = 16.5 Hz, H-7_b), 3.78, 3.79 (6H, each *s*, 2-OMe), 3.84–3.91 (4H, OMe, H-$9'_a$), 3.93 (1H, *d*, *J* = 12.1 Hz, H-7′), 4.11 (1H, *dd*, *J* = 11.7, 8.1 Hz, H-$9'_b$), 6.40 (1H, *s*, H-5), 6.63 (1H, *s*, H-2), 6.73 (1H, *d*, *J* = 1.8 Hz, H-2′), 6.76 (1H, *dd*, *J* = 11.7, 8.1 Hz, H-6′), 6.98 (1H, *d*, *J* = 8.1 Hz, H-5′). EIMS *m/z* (%): 530 $[M]^+$ (5), 488 (16), 470 (29), 452 (10), 439 (46), 428 (36), 411 (65), 397 (79), 368 (100), 351 (95), 327 (51), 309 (98), 284 (41), 137 (63).

Quantitative determination of sesquilignans and lignans in 13 *western hemlock sapwoods.* The presence of sesquilignans and lignans in 13 western hemlock sapwoods was confirmed by HPLC, in addition to TLC. RR_t values were determined with acetovanillone as internal standard; the amounts of the sesquilignans and lignans were determined. The eluent and conditions for analysis were as follows: eluent, MeOH–H_2O (3:7, v/v); flow rate, 1.0 ml min^{-1}; detection, UV 280 nm. Constituent (code No.) (RR_t): 7′-hydroxylappaol E (**1**) (1.97), *epi*-7′-hydroxylappaol E (**2**) (1.93), 8-hydroxy-α-conidendrin (**3**) (1.47), 8-hydroxy-α-conidendric acid methyl ester (**4**) (1.45), (−)-hydroxymatairesinol (**5**) (1.27), (+)-cedrusin (1.14), (+)-*allo*-hydroxymatairesinol (1.43), (+)-oxomatairesinol (3.34), (−)-α-conidendrin (4.34), (+)-pinoresinol (5.20). Although the original compound **2** could not be isolated, compounds **1** and **2** were determined based on their acetates (**1a** and **2a**) in the normal-phase HPLC analyses.

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