New Neolignans and Lignans from Vietnamese Medicinal Plant *Machilus odoratissima* NEES

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Four new natural neolignans and lignans, which were given the trivial names odoratisols A—D (1—4), together with (−)-licarin A, kachirachirol B, obovatifol, and machilin-I were isolated from the air-dried bark of the Vietnamese medicinal plant *Machilius odoratissima* NEES (Lauraceae). Their absolute structures were determined on the basis of spectroscopic analyses including circular dichroism spectra.

Key words *Machilus odoratissima*; Lauraceae; neolignan; lignan; odoratisol; absolute structure

Machilus is a genus in the Lauraceae family which includes twelve species distributed throughout Vietnam. They are *Machilus odoratissima* NEES, *M. thunbergii* SIEB. *et* ZUCC., *M. velutina* CHAMP. ex. BENTH., *M. oreophila* HANCE, *M. robutus* J. J. SON., *M. bonii* LEC., *M. coriacea* A. CHEV., *M. thunbergii* var. *condorensis* LEC., *M. parviflora* MEISSN., *M. platycarpa* CHUN., *M. macrophyla* HEMSLEY, and *M. cochinchinensis* LEC.[1—3] *Machilus odoratissima* NEES (Vietnamese name *Khao nham*) is a timber tree growing up to a height of 8—10 m. The tree bark is used in the folk medicine as antiseptic and anti-inflammatory remedies. The leaves are used to treat snake bite and burn wounds.[4] We carried out the first systematic study on *M. odoratissima* dealing with the isolation and structural elucidation of four new and four known neolignans and lignans.

The bark of *M. odoratissima* was air-dried in the shadow, powdered, and extracted with MeOH at room temperature. The MeOH extract was partitioned between H_2O and solvents of increasing polarities to afford *n*-hexane-, CH_2Cl_2-, EtOAc-, and 1-BuOH-soluble fractions. Column-chromatographic separation of the *n*-hexane- and CH_2Cl_2-soluble fractions led to the isolation of four new neolignans and lignans, named odoratisol A—D (**1**—**4**) and four known ones, (−)-licarin A, kachirachirol B, obovatifol, and machilin-I, which were determined by comparing their physical ($[\alpha]_D$) and spectroscopic data with the literature values.[5—9]

Odoratisol A (**1**) was isolated as an oil and its molecular formula was deduced to be $C_{21}H_{24}O_5$ from negative-ion high-resolution (HR)-FAB-MS. The IR spectrum indicated the presence of hydroxyl groups (3450 cm^{-1}) and aromatic rings (1609, 1517, 1458 cm^{-1}). The ^{1}H- (Table 1) and ^{13}C-NMR (Table 2) spectroscopic data indicated that **1** had a planar structure of 5-methoxydehydrodiisoeugenol. The *trans* geometry of 1′-propenyl group was determined on the basis of the large coupling constant between H-7′ and H-8′ (J=15.6 Hz). The *trans* relationship of H-7 and H-8 was established based on ^{1}H-NMR spectroscopic data characteristic of *trans*-7-aryl-8-methyl-7,8-dihydro-benzofuranoid-type neolignans [δ_H 5.0 (1H, d, J=9.5 Hz, H-7), 3.37 (1H, dq, J=9.5, 6.6 Hz, H-8), 1.32 (3H, d, J=6.6 Hz, H-9)].[10,11] The positions of the methoxyl groups at δ_H 3.81 (3H, s) and 3.82 (6H, s) were assigned using nuclear Overhauser effect spectroscopy (NOESY) (Fig. 2). 5-Methoxydehydrodiisoeugenol was reported previously from *Myristica fragrans*,[12] however, its stereochemistries at C-7 and C-8 have not been determined at that time. In this study the circular dichroism (CD) spectrum was used to determine the absolute configuration of **1** to be the 7*S*,8*S* stereoisomer of 5-methoxydehydrodiisoeugenol from a positive Cotton effect at 242 nm (+4.35) and a negative one at 269 nm (−5.93), which were similar to those exhibited by (−)-licarin A.[6] This configuration agreed well with the same sign of the optical rotations of **1** ($[\alpha]_D^{25}$ −35.1°) and (−)-licarin A ($[\alpha]_D^{25}$ −44.0°).[6] Thus **1** was isolated for the first time from Nature and its absolute stereostructure was concluded to be (7*S*,8*S*)-7,8-dihydro-7-(4-hy-

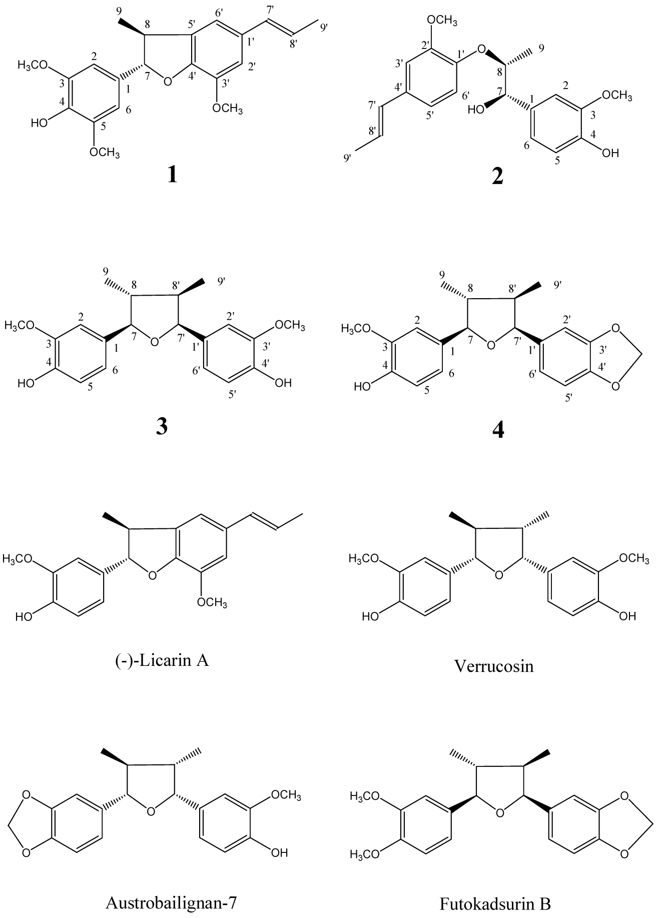

Fig. 1. Absolute Structures of Compounds **1**—**4**, (−)-Licarin A, Verrucosin, Austrobailignan-7, and Futokadsurin B

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Table 1. ^{1}H-NMR Spectroscopic Data of **1**—**4** (δ in ppm, J in Hz in Parentheses, 400 MHz, CDCl$_3$)

H	**1**	**2**	**3**	**4**
2	6.59 s	6.84 d (2.0)	6.97 d (1.7)	6.95 d (1.7)
5		6.78 d (8.5)	6.84 d (8.3)	6.83 d (8.3)
6	6.59 s		6.90 dd (8.3, 1.7)	6.87 dd (8.3, 1.7)
7	5.0 d (9.5)	4.75 d (3.2)	4.31 d (9.3)	4.27 d (9.3)
8	3.37 dq (9.5, 6.6)	4.27 dq (3.2, 6.4)	1.70 br dq (9.3, 6.6)	1.68 br dq (9.3, 6.6)
9	1.32 d (6.6)	1.09 d (6.4)	0.98 d (6.6)	0.95 d (6.6)
2′	6.72 s	6.91 d (2.0)	6.78 d (1.7)	6.70 d (1.7)
5′		6.88 d (8.3)	6.80 d (8.0)	6.71 d (8.0)
6′	6.69 s	6.84 dd (8.3, 2.0)	6.74 dd (8.0, 1.7)	6.79 dd (8.0, 1.7)
7′	6.29 d (15.6)	6.28 d (15.6)	5.03 d (8.8)	5.01 d (8.8)
8′	6.03 dq (15.6, 6.3)	6.08 dq (15.6, 6.6)	2.14 br dq (8.8, 6.8)	2.13 br dq (8.8, 7.1)
9′	1.79 d (6.3)	1.80 d (6.6)	0.58 d (6.8)	0.59 d (7.1)
MeO-3	3.82 s	3.819 s	3.82 s	3.82 s
MeO-5	3.82 s			
MeO-3′	3.81 s	3.822 s	3.77 s	
OCH$_2$O				5.85 s

All assignments were made on the basis of heteronuclear single quantum correlation (HSQC) and NOESY experiments.

Table 2. ^{13}C-NMR Spectroscopic Data of **1**—**4** (δ in ppm, 100 MHz, CDCl$_3$)

C	**1**	**2**	**3**	**4**
1	133.2	133.8	133.2	132.5
2	103.6	109.0	109.5	109.3
3	147.1	146.5	146.6	146.55
4	134.9	144.9	145.2	145.3
5	147.1	113.9	114.2	114.3
6	103.6	119.9	119.3	119.5
7	94.0	82.4	87.4	82.8
8	45.7	73.7	47.8	47.8
9	17.6	13.4	14.9	15.0
1′	132.3	131.9	132.8	135.2
2′	109.5	109.5	109.8	120.2
3′	144.2	145.6	146.2	147.4
4′	146.6	151.6	144.6	146.59
5′	136.4	119.1	113.9	107.7
6′	113.3	118.8	119.9	107.5
7′	131.0	130.5	83.1	87.5
8′	123.5	125.0	46.0	45.9
9′	18.3	18.5	15.0	14.9
MeO-3	56.4	55.8	55.86	55.9
MeO-5	56.4			
MeO-3′	56.0	55.9	55.9	
OCH$_2$O				100.9

droxy-3,5-dimethoxyphenyl)-3′-methoxy-8-methyl-1′-*trans*-propenylbenzofuran.

Odoratisol B (**2**) was isolated as an oil and had the molecular formula $C_{20}H_{24}O_5$ based on negative-ion HR-FAB-MS. The IR spectrum indicated the presence of hydroxyl groups (3448 cm^{-1}) and aromatic rings (1603, 1511, 1458 cm^{-1}). The ^{1}H- (Table 1) and ^{13}C-NMR (Table 2) spectroscopic data of **2** were superimposable with those of machilin C,[13] including the *erythro* stereochemistry between H-7 and H-8 as shown by a small coupling constant (J=3.2 Hz) between them. However, the optical rotation of **2** ($[\alpha]_D^{25}$ +18.6°) was almost of the same in value but reverse in sign in comparison with that of machilin C ($[\alpha]_D^{25}$ −16.5°) showing the need to determine the absolute stereochemistry at two stereogenic elements at C-7 and C-8. The CD spectrum of **2** showed positive Cotton effect at 258 nm (+0.38) established the configurations at C-7 an C-8 are 7*S* and 8*R* as in the cases of analogous neolignans of *erythro* series.[14] On the basis of these data, the structure of **2** was concluded to be 7*S*,8*R*-*erythro*-4-hydroxy-3,2′-dimethoxy-4′-*trans*-propenyl-neolignan.

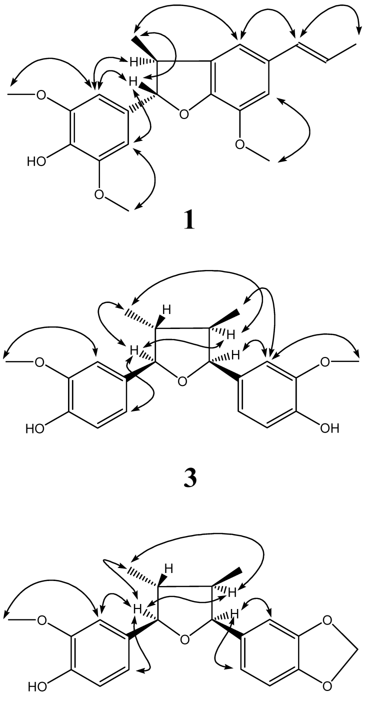

Fig. 2. NOESY Correlations of Compounds **1**, **3**, and **4**

Odoratisol C (**3**) was isolated as an oil, $[\alpha]_D^{25}$ −26.0°, and its molecular formula was characterized to be $C_{20}H_{24}O_5$ in negative-ion HR-FAB-MS. The IR spectrum indicated the presence of hydroxyl groups (3450 cm^{-1}) and aromatic rings (1607, 1514, 1457 cm^{-1}). The ^{1}H- (Table 1) and ^{13}C-NMR (Table 2) spectroscopic data showed the structural resemblance of **3** and verrucosin,[15] which, however, displayed a positive optical rotation ($[\alpha]_D$ +14.8°). The *trans* H-7/H-8, *trans* H-8/H-8′, and *cis* H-7′/H-8′ relative stereochemistries of the tetrahydrofuran ring were determined by comparing the ^{1}H-NMR data [δ_H 0.98 (3H, d, J=6.6 Hz, H-9), 1.70 (1H, br dq, J=9.3, 6.6 Hz, H-8), 4.31 (1H, d, J=9.3 Hz, H-7); 0.58 (3H, d, J=6.8 Hz, H-9′), 2.14 (1H, br dq, J=8.8, 6.8 Hz, H-8′), 5.03 (1H, d, J=8.8 Hz, H-7′)] with those reported in literature for 7,8-*trans*-8,8′-*trans*-7′,8′-*cis*-configurated tetrahydrofuran-type lignans.[15—17] The *trans* H-7/H-8 and *cis* H-7′/H-8′ configurations were in agreement with the upfield shift ($\Delta\delta_H$ −0.4) of methyl proton signals on going from C-9 to C-9′ due to the anisotropic effect of the aromatic group in the case of the *cis*-configuration of the aryl group at C-7′ and methyl substituent at C-8′. The stereochemical assignments were supported by the NOESY spectrum (Fig. 2) of **3**, which showed NOEs between H_3-9 (δ_H 0.98) and H-7 (δ_H 4.31), between H_3-9 and H-8′ (δ_H 2.14), and between H-7 and H-8′, between H_3-9′ (δ_H 0.58) and H-2′ (δ_H 6.78).[17,18] The locations of two 4-hydroxy-3-methoxyphenyl moieties were also confirmed by NOEs between H-6 [δ_H 6.90 (dd, J=8.3, 1.7 Hz)] and H-7 and between H-2′ [δ_H 6.78 (d, J=1.7 Hz)] and H-7′. Since **3** had the same relative stereochemistry but opposite optical rotation in comparison with those of verrucosin, the absolute configurations at the C-7, C-8, C-7′, and C-8′ were deduced to be opposite to those of verrucosin. Thus the absolute structure of **3** was determined to be (7*R*,8*R*,7′*S*,8′*R*)-4-hydroxy-3-methoxy-4′-hydroxy-3′-methoxy-7,7′-epoxylignan.

Odoratisol D (**4**) was isolated as an oil and its molecular formula was determined $C_{20}H_{22}O_5$ by means of negative-ion HR-FAB-MS. The IR spectrum indicated the presence of hydroxyl groups (3450 cm^{-1}) and aromatic rings (1608, 1517, 1442 cm^{-1}). The ^{1}H-NMR (Table 1) spectrum showed the presence of a 4-hydroxy-3-methoxyphenyl and a monosubstituted 3,4-methylenedioxyphenyl systems, which contained an aromatic methoxyl group [δ_H 3.82 (3H, s)] and a methylenedioxy group [δ_H 5.85 (2H, s)], and a 2,5-disubstituted 3,4-dimethyltetrahydrofuran ring [δ_H 0.95 (3H, d, J=6.6 Hz, H-9), 1.68 (1H, br dq, J=9.3, 6.6 Hz, H-8), 4.27 (1H, d, J=9.3 Hz, H-7); 0.59 (3H, d, J=7.1 Hz, H-9′), 2.13 (1H, br dq, J=8.8, 7.1 Hz, H-8′), 5.01 (1H, d, J=8.8 Hz, H-7′)]. The ^{1}H- and ^{13}C-NMR (Table 2) spectroscopic data of **4** resembled those of futokadsurin B[18] except for the lack of an additional methoxyl group. The *trans* H-7/H-8, *trans* H-8/H-8′, and *cis* H-7′/H-8′ relative stereochemistry of the tetrahydrofuran ring were conclusive on the basis of the comparison of the ^{1}H-NMR data with those reported in literature.[16—19] Upfield shift ($\Delta\delta_H$ −0.36) of methyl proton signals on going from C-9 to C-9′ agreed with *trans* H-7/H-8 and *cis* H-7′/H-8′ configurations. NOEs observed between H_3-9 (δ_H 0.95) and H-7 (δ_H 4.27), between H_3-9 and H-8′ (δ_H 2.13), and between H-7 and H-8′, but not between H_3-9′ (δ_H 0.59) and H-7′ (δ_H 5.01) supported the stereochemical assignments.[17] Thus **4** was concluded to have the same relative stereochemistry as that of **3**. NOESY spectrum (Fig. 2) of **4** showed the correlations between the methoxyl group (δ_H 3.82) and H-2, between H-2 (δ_H 6.95) and H-7 (δ_H 4.27), between H-6 (δ_H 6.87) and H-7, between H-2′ (δ_H 6.70) and H-7′ (δ_H 5.01), and between H-6′ (δ_H 6.79) and H-7′ confirmed the assignments of the position of the 4-hydroxy-3-methoxyphenyl moiety at C-7 and the 3′,4′-methylenedioxyphenyl moiety at C-7′ as shown in Fig. 2. To establish the absolute structure of **4** the CD spectra of **3** and **4** were measured and compared. Similar CD curves of **3** and **4** were seen, namely, **4** displayed the Cotton effects at 213 nm (−1.25), 240 nm (+0.95), and 285 nm (−0.40) assuring the absolute structure of **4** to be (7*R*,8*R*,7′*S*,8′*R*)-4-hydroxy-3-methoxy-3′,4′-methylenedioxy-7,7′-epoxylignan.

Experimental

General Procedure Optical rotations were measured on a JASCO P-1030 polarimeter. FT-IR spectra were recorded on a Horiba FT-710 spectrophotometer. ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded using a JEOL JNM-α 400 NMR spectrometer with tetramethylsilane as an internal standard. Negative-ion HR-FAB-MS were measured on a JEOL SX-102 mass spectrometer with PEG-400 as a calibration matrix. HPLC was carried out with a JASCO PU-1580 pump and an UV-2075 Plus detector (set at 210 nm) on YMC ODS columns (150×4.6 mm i.d. in analytical and 150×20 mm i.d. in preparative scales) at the corresponding flow rates of 0.5 and 5 ml/min. Silica (Si) gel 60 (0.063—0.200 mm, Merck, Germany) and reversed-phase octadecyl Si (ODS) gel (YMC, Japan) were used for open-column chromatography. TLC was carried out on Merck TLC plates (Si gel 60 F_{254}), and detected by spraying with 10% H_2SO_4 in 50% EtOH, followed by heating on a hot plate at 200 °C.

Plant Material The air-dried bark (2.0 kg) of *M. odoratissima* was collected in Province Thai Nguyen, Vietnam, and identified by Dr Nguyen Hoanh Coi of the Military Institute of Drug Controls (Hanoi, Vietnam), in June 2000. A voucher specimen (no. HCTN 2000-6) is deposited in the Laboratory of Chemistry of Natural Products, Faculty of Chemistry, Vietnam National University, Hanoi, Vietnam.

Extraction and Isolation of 1—10 The powdered air-dried bark of *M. odoratissima* (2.0 kg) was extracted with MeOH by percolation at room temperature (3 times, for 3 d each). After concentration by evaporation under reduced pressure, the resultant MeOH extract was suspended in H_2O and sequentially extracted with *n*-hexane, CH_2Cl_2, EtOAc, and 1-BuOH. The *n*-hexane-soluble fraction (5.9 g) was separated on a Si gel open column using mixtures of *n*-hexane in EtOAc (10 : 1, 4 : 1, 2 : 1, 1 : 1). Five pooled fractions were collected on the basis of TLC pattenrs. Fraction 2 (1.8 g), fraction 3 (0.5 g), and fraction 4 (0.4 g) underwent the same treatment, first separation on an ODS gel open column (MeOH–H_2O, 3 : 1, 4 : 1), then purification on ODS preparative HPLC (MeOH–H_2O, 3 : 1) to give odoratisol A (**1**, 12.2 mg), odoratisol B (**2**, 7.0 mg), odoratisol C (**3**, 27.9 mg), odoratisol D (**4**, 40.2 mg), (−)-licarin A (0.48 g), machilin-I (13.4 mg), kachirachirol B (16.1 mg), and obovatifol (17.8 mg). Similar procedure was used to separate the CH_2Cl_2-soluble fraction yielding **1** (7.9 mg), (−)-licarin A (55.8 mg), and kachirachirol B (56.4 mg).

Odoratisol A (**1**): Oil, $[\alpha]_D^{25}$ −35.1° (c=1.22, CHCl$_3$). UV λ_{max} (MeOH) nm (log ε): 270 (4.18), 219 (4.43). IR ν_{max} (film) cm^{-1}: 3450, 2957, 2926, 2854, 1609, 1517, 1493, 1458, 1375, 1078. CD (MeOH): $\Delta\varepsilon$ (nm): −2.14 (218), +4.35 (242), −5.93 (269) (c=2.0×10^{-5} M). ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. Negative-ion HR-FAB-MS: *m/z* 355.1544 [M−H]$^-$ (Calcd for $C_{21}H_{23}O_5$: 355.1545).

Odoratisol B (**2**): Oil, $[\alpha]_D^{25}$ +18.6° (c=0.70, CHCl$_3$). UV λ_{max} (MeOH) nm (log ε): 258 (3.68), 218 (3.87). IR ν_{max} (film) cm^{-1}: 3448, 2957, 2925, 2854, 1603, 1511, 1458, 1377, 1061. CD (MeOH): $\Delta\varepsilon$ (nm): −0.74 (219), +0.38 (258), +0.18 (350) (c=4.2×10^{-5} M). ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. Negative-ion HR-FAB-MS: *m/z* 343.1523 [M−H]$^-$ (Calcd for $C_{20}H_{23}O_5$: 343.1545).

Odoratisol C (**3**): Oil, $[\alpha]_D^{25}$ −26.0° (c=2.79, CHCl$_3$). UV λ_{max} (MeOH) nm (log ε): 280 (3.71), 232 (4.05). IR ν_{max} (film) cm^{-1}: 3450, 2958, 2926, 2854, 1607, 1514, 1457, 1377, 1033. CD (MeOH): $\Delta\varepsilon$ (nm): −4.37 (211), +0.31 (258), −0.38 (281) (c=4.3×10^{-5} M). ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. Negative-ion HR-FAB-MS: *m/z* 343.1542 [M−H]$^-$ (Calcd for $C_{20}H_{23}O_5$: 343.1545).

Odoratisol D (**4**): Oil, $[\alpha]_D^{25}$ −12.8° (c=4.0, CHCl$_3$). UV λ_{max} (MeOH) nm (log ε): 282 (3.80), 234 (3.97). IR ν_{max} (film) cm^{-1}: 3450, 2959, 2927, 2873, 1608, 1517, 1488, 1442, 1377, 1036. CD (MeOH): $\Delta\varepsilon$ (nm): −1.25 (213), +0.95 (240), −0.40 (285) (c=8.8×10^{-5} M). ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. Negative-ion HR-FAB-MS: *m/z* 341.1401 [M−H]$^-$ (Calcd for $C_{20}H_{21}O_5$: 341.1389).

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