New Constituents from the Heartwood of *Picea morrisonicola* HAYATA

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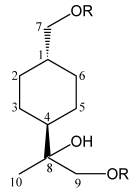
Three new constituents, including a *p*-menthane type monoterpene, *trans*-*p*-menthane-7,8,9-triol (1), an aromatic, 2,6-dihydroxy-3,4-dimethylbenzoic acid methyl ester (2), and a lignan, (+)-morrisonicolanin (3), were isolated from the low polar layer of heartwood extracts of *Picea morrisonicola*, and their structures were elucidated on the basis of spectroscopic analysis. Of these compounds identified, 1 was obtained as its diacetylated derivative 1a, and 2 was the chemical entity first isolated from a natural source.

Key words *Picea morrisonicola*; Pinaceae; heartwood; monoterpene; lignan; aromatic

A total of six species of the genus *Picea* (Pinaceae), including five transplantation (*P. abies*, *P. glauca*, *P. glehnii*, *P. asperata*, *P. orientalis*) and one endemic species (*P. morrisonicola*), were grown in Taiwan. Among them, cumulative phytochemical examinations have revealed that *P. abies*,[1—3] *P. glauca*,[4] and *P. glehnii*[5] contained lignans, flavonoids and their glucosides, and diterpenoids. *P. morrisonicola* HAYATA, a large tree, is distributed at high altitudes of about 2000—2500 m in the central range, and scattered in ravines and mountain slopes.[6] So far its wood has been used only for building materials, but not for any other purpose locally. In our previous papers, bundles of novel compounds, including four abietane-type diterpenes[7] and two norditerpenes,[8] were isolated and identified from the low polar layer of acetonic extracts of its heartwood. However, many constituents in other fractions were still not fully investigated. Therefore, further studies on different fractions of the same extracts yielded three new components including a monoterpene **1**, an aromatic **2**, and a lignan **3**. The following describes the structural assignments of these compounds.

The acetonic extract of the heartwood of *P. morrisonicola* was concentrated to give a residue which was subjected to partitioning with EtOAc and water. The combined EtOAc soluble layer was then separated sequentially using Si-gravity column chromatography and HPLC to yield three new components, **1**—**3**.

Compound **1** was purified as its diacetylated derivative **1a**. Compound **1a** was obtained as a colorless oil, whose molecular formula was confirmed to be $C_{14}H_{24}O_5$ as deduced from HR-EI-MS and ^{13}C-NMR spectra. Its IR spectrum indicated the presence of a hydroxyl group (3429 cm^{-1}) and a carbonyl group (1719 cm^{-1}). The ^{1}H-NMR spectrum of **1a** showed signals due to one tertiary methyl group at δ_H 1.09 (3H, s), two methine protons at δ_H 1.39 (1H, m, H-4α) and 1.55 (1H, m, H-1β), one pair of acetoxymethylene protons at δ_H 3.86 (2H, d, J=6.4 Hz, H-7), one pair of AB type acetoxymethylene protons at δ_H 3.96 (1H, d, J=11.2 Hz, H-9) and 4.03 (1H, d, J=11.2 Hz, H-9), and four pairs of methylene protons at [δ_H 0.95 (2H, m, H-2α,-6α), 1.85 (2H, m, H-2β, -6β)], [δ_H 1.15 (1H, m, H-3β), 1.92 (1H, m, H-3α)], and [δ_H 1.24 (1H, m, H-5β), 1.87 (1H, m, H-5α)] as further evidenced by ^{13}C-NMR, distortionless enhancement by polarization transfer (DEPT) and ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) spectra. The ^{1}H-NMR spectrum of **1a** also revealed two acetyl methyl singlets at δ_H 2.02 (3H, s) and 2.09 (3H, s), suggesting that there existed two acetoxyl and one hydroxyl (3429 cm^{-1}) groups in **1a**. Based on the molecular formula $C_{14}H_{24}O_5$, the degree of unsaturation of **1a** was three, including two acetyl carbonyls. Thus, the number of rings of **1a** should be one. In the heteronuclear multiple bond connectivity (HMBC) spectrum of **1a**, the long-range ^{13}C–^{1}H correlations, including one acetyl carbonyl with H_2-9, the other acetyl carbonyl with H-7, and one hydroxy-bearing carbon C-8 with H_3-10, established the locations of two acetyl groups and one residual hydroxyl group. All the above spectroscopic data, especially the symmetrical H-2 and H-6 signals and nuclear Overhauser and exchange spectroscopy (NOESY) correlations of **1a**, suggested that **1a** was a *p*-menthane type skeleton, except that both H_3-7 and H_3-9 were converted to CH_2OAc functionalities, and a hydroxyl group was attached to C-8. In the NOESY spectrum of **1a**, mutual correlations were as follows: H_2-7/H_2-2, -6; H-



R
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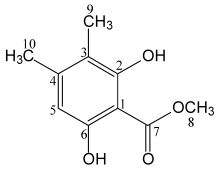

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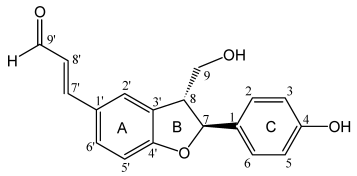

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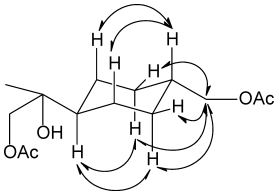

Fig. 1. Key NOE Correlations Observed in NOESY Spectrum of **1a**

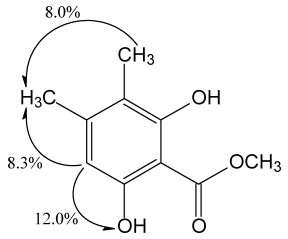

Fig. 2. NOE Correlations Observed in NOE Difference Spectra of **2**

4α/H-2α, -6α; H-1/H-3β, -5β, which demonstrated that the acetoxymethylene and 1-acetoxy-2-hydroxyisopropyl are both in equatorial orientation, as shown in Fig. 1. Further evidence of the relative configuration was the distinguishing features in the ^{1}H-NMR data, especially its $\delta_{H\text{-}7}$. In contrast to the lower field shift of $\delta_{H\text{-}7}$ (δ_H 4.03) in axial-oriented CH_2OAc of a *cis*-(4-*t*-butylcyclohexyl)methyl acetate analogue,[9,10] **1a** with a relative higher field shift of $\delta_{H\text{-}7}$ (δ_H 3.86), closely compatible with that (*ca.* δ_H 3.81—3.88) of a *trans*-(4-*t*-butylcyclohexyl)methyl acetate analogue,[9,10] suggested that its CH_2OAc should be equatorial-oriented, the same as its hydroxyl isopropyl group. Accordingly, compound **1a** was concluded to be *trans*-7,9-diacetoxy-*p*-menthan-7-ol.

The molecular formula for **2**, $C_{10}H_{12}O_4$, was determined by ^{13}C-NMR and HR-EI-MS data. The IR spectrum of **2** indicated the presence of a hydroxyl group (3409 cm^{-1}), a conjugated ester carbonyl with strong hydrogen bonding (1633 cm^{-1}), and a benzene ring (1608, 1493 cm^{-1}). The ^{1}H-NMR spectrum of **2** showed signals for a phenyl proton at δ_H 6.19 (1H, s), aryl methyl protons at δ_H 2.08 (3H, s) and 2.43 (3H, s), methoxyl protons at δ_H 3.98 (3H, s), and two phenolic protons including one normal signal at δ_H 5.51 (1H, s) and another signal at 12.01 (1H, s) due to strong hydrogen bonding, suggesting the presence of a benzene ring with five substituents. The two methyls, two hydroxys, and one carbonyl methoxy functionalities were determined to be located at C-3, -4, C-2, -6, and C-7, respectively, since correlations between H_3-9 and C-2, -3, -4; H_3-10 and C-3, -4, -5; OH-6 and C-1, -5, -6; and H_3-8 and C-7 were observed in the HMBC spectrum. Two aromatic carbon signals at δ_C 158.0 and 163.1 in the ^{13}C-NMR spectrum further supported the oxygenation of the benzene ring at C-2 and -6, respectively. All the above assignments were also supported by mutual correlations of H_3-9 (δ_H 2.08) and H_3-10 (δ_H 2.43, 8.0% enhancement), and H-5 and H_3-10 (8.3% enhancement), and OH-6 (δ_H 12.01, 12.8% enhancement) from the NOE difference experiments, as shown in Fig. 2. Thus, the structure of **2** was confirmed to be 2,6-dihydroxy-3,4-dimethylbenzoic acid methyl ester. To our knowledge, **2** has ever been synthesized *via* the methylation of 2,6-dihydroxy-3,4-dimethylbenzoic acid using diazomethane previously.[11] However, **2** was found to be new from a natural source.

(+)-Morrisonicolanin (**3**) was isolated as an amorphous solid. Its molecular formula $C_{18}H_{16}O_4$ was deduced from ^{13}C-NMR data and an $[M]^+$ ion at *m*/*z* 296.1121 in the HR-EI-MS. Analysis of the IR spectrum of **3** suggested that it contained a hydroxyl group (3387 cm^{-1}), a conjugated aldehyde (2760, 1660 cm^{-1}), and a benzene ring (1594, 1515 cm^{-1}). The ^{1}H-NMR spectrum of **3** showed a conjugated *trans*-propenal group [δ_H 6.68 (1H, dd, J=15.8, 7.8 Hz, H-8′), 7.40 (1H, d, J=15.8 Hz, H-7′), 9.62 (1H, d, J=7.8 Hz, H-9′)], three ABX type phenyl protons [δ_H 6.88 (1H, d, J=8.5 Hz, H-5′), 7.41 (1H, d, J=8.5 Hz, H-6′), 7.45 (1H, s, H-2′)], two methine protons [δ_H 3.59 (1H, m, H-8), 5.60 (1H, d, J=6.3 Hz, H-7)], and a hydroxymethylene group [δ_H 3.93 (2H, m, H-9)], similar to those of the A and B rings (phenyldihydrobenzofuran) of the neolignan skeleton with two branches,[12] and a hydroxymethylene and a *trans* propenal, attached at C-8 (δ_C 52.3) and C-1′; (δ_C 126.7), respectively. The remaining signals, four A_2X_2 type aromatic protons at δ_H 6.80 (2H, d, J=8.4 Hz, H-3, -5) and 7.21 (2H, d, J=8.4 Hz, H-2, -6), indicated a 4-substituted phenol moiety for the C ring. The HMBC spectrum of **3** showed phenyl proton signals H-2, -6 (δ_H 7.21) coupled to C-7 (δ_C 87.8), which was also coupled to the H_2-9 signal (δ_H 3.93), the H-8 signal (δ_H 3.59) coupled to C-2′ (δ_C 130.7), the phenyl proton signal H-6′ (δ_H 7.41) coupled to C-7′ (δ_C 153.0), and the H-7′ signal (δ_H 7.40) coupled to the aldehyde carbonyl signal C-9′ (δ_C 193.3). All these correlations also suggested the main part of the structure of **3** is a neolignan aldehyde, as described above. The relative configuration of C-7 and C-8 was determined to be an *E* form, as evidenced from the observation of cross peaks between H-7 and H_2-9, and a lack of correlation between H-7 and H-8 in the NOESY experiment.[13] The positive $[\alpha]_D^{24}$ value [+6.7° (c=1.4, $CHCl_3$)] and chemical shifts of H-7 (δ_H 5.60) and H-8 (δ_H 3.59) in **3** suggested a 7*S*,8*R*-configuration, as in the case of (*E*)-3-[(2*S*,3*R*)-2,3-dihydro-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-7-methoxy-1-benzo[*b*]furan-5-yl]-2-propenal reported previously.[14] Further analysis of all the spectral data allowed the assignment of **3** as (*E*)-3-[(2*S*,3*R*)-2,3-dihydro-2-(4′-hydroxyphenyl)-3-hydroxymethyl-1-benzo[*b*]furan-5-yl]-2-propenal, and named as (+)-morrisonicolanin.

Experimental

General Experimental Procedures Melting points were collected using a Yanagimoto micromelting point apparatus. Optical Rotations were measured using a JASCO DIP-180 digital polarimeter at room temperature. IR spectra were recorded on a Perkin-Elmer 781 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AM-300 instrument using tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in δ values (ppm), and coupling constants (*J*) are given in hertz (Hz). Electron-impact mass spectra (EI-MS) were obtained on a Finnigan TSQ-U6C, and JEOL SX-102A mass spectrometer, respectively. Extracts were chromatographed on silica gel (Merck 3374, 70—230 mesh), and further purified with a semipreparative normal phase HPLC column (250×10 mm, 7 μm, Li Chrosorb Si 60).

Plant Material The heartwood of *P. morrisonicola* Hayata was collected from MountTaichung, Taiwan, and was identified by Prof. Shao-Shun Ying, Department of Forest, National Taiwan University. A voucher specimen (No. 226237) has been deposited at the Herbarium of the Department of Botany, National Taiwan University, Taipei, Taiwan.

Extraction and Isolation The air dried heartwood of *P. morrisonicola* (7.5 kg) was extracted with 90l Me_2CO three times (7 d each) at room temperature. The combined extracts were evaporated under a vacuum to give a black residue, which was suspended in 8 l water and then partitioned with

EtOAc (2 l×3). The ethyl acetate layer (85.5 g) was then chromatographed by a Si-column using mixtures of *n*-hexane and EtOAc as eluents. HPLC of low polar fractions on a normal-phase column with 30% and 60% EtOAc in *n*-hexane as eluents afforded **2** (10.5 mg) and **3** (9.0 mg), respectively. Relative high polar fractions were dissolved in pyridine and Ac_2O, and the mixture was left overnight at room temperature. Then, the reaction mixture was added dropwise to ice water under stirring, and the resultant suspension was extracted with EtOAc. After the usual work-up, the extract was further purified by normal-phase HPLC with 30% EtOAc in *n*-hexane to yield the pure diacetate derivative **1a** (5.2 mg).

trans-7,9-Diacetoxy-*p*-menthan-7-ol (**1a**): Colorless oil; $[\alpha]_D^{24}$ +3.2° (*c*=0.19, $CHCl_3$); IR ν_{max} (dry film): 3429, 1719, 1381, 1248, 1036 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 0.95 (2H, m, H-2α, -6α), 1.09 (3H, s, H-10), 1.15 (1H, m, H-3β), 1.24 (1H, m, H-5β), 1.39 (1H, m, H-4α), 1.55 (1H, m, H-1β), 1.85 (2H, m, H-2β, -6β), 1.87 (1H, m, H-5α), 1.92 (1H, m, H-3α), 2.02, 2.09 (3H, s, $OCOCH_3$×2), 3.86 (2H, d, *J*=6.4 Hz, H-7), 3.96 (1H, d, *J*=11.2 Hz, H-9), 4.03 (1H, d, *J*=11.2 Hz, H-9); ^{13}C-NMR ($CDCl_3$) δ: 20.8 (C-10), 20.8 ($OCO\underline{C}H_3$×2), 25.6 (C-3), 26.6 (C-5), 29.4 (C-6), 29.5 (C-2), 36.9 (C-1), 44.9 (C-4), 69.3 (C-7), 70.0 (C-9), 73.3 (C-8), 171.2, 171.3 ($O\underline{C}OCH_3$×2); EI-MS (70 eV) (rel. int. %) *m*/*z* 272 (M^+, 18), 229 (35), 165 (32), 131 (42), 119 (63), 105 (100); HR-EI-MS *m*/*z* 272.1620 (M^+, Calcd for $C_{14}H_{24}O_5$ 272.1624).

2,6-Dihydroxy-3,4-dimethylbenzoic acid methyl ester (**2**): Slight yellow solid; mp 107—109 °C; UV λ_{max} (MeOH) nm (log ε): 258 (3.8), 321 (3.2); IR ν_{max} (KBr): 3409, 1633, 1608, 1493 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.08 (3H, s, H_3-9), 2.43 (3H, s, H_3-10), 3.98 (3H, s, H_3-8), 5.51 (1H, s, OH-2), 6.19 (1H, s, H-5), 12.01 (1H, s, OH-6); ^{13}C-NMR ($CDCl_3$) δ: 108.5 (C-1), 158.0 (C-2), 105.2 (C-3), 140.1 (C-4), 110.5 (C-5), 163.1 (C-6), 172.5 (C-7), 51.7 (C-8), 7.6 (C-9), 24.0 (C-10); EI-MS *m*/*z* (rel. int. %) 196 (M^+, 58), 164 (100), 136 (95), 84 (42), 79 (38), 55 (25), 51 (30), 145 (42); HR-EI-MS *m*/*z* 196.0751 (M^+, Calcd for $C_{10}H_{12}O_4$ 196.0736).

(+)-Morrisonicolanin (**3**): Amorphous solid; $[\alpha]_D^{24}$ +6.7° (*c*=1.4, $CHCl_3$); UV λ_{max} (MeOH) nm (log ε): 230 (4.0), 330 (3.6); IR ν_{max} (dry film): 3387, 2760, 1660, 1594, 1515 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 3.59 (1H, m, H-8), 3.93 (2H, m, H-9), 5.60 (1H, d, *J*=6.3 Hz, H-7), 6.68 (1H, dd, *J*=15.8, 7.8 Hz, H-8′), 6.80 (2H, d, *J*=8.4 Hz, H-3, -5), 6.88 (1H, d, *J*=8.5 Hz, H-5′), 7.21 (2H, d, *J*=8.4 Hz, H-2, -6), 7.40 (1H, d, *J*=15.8 Hz, H-7′), 7.41 (1H, d, *J*=8.5 Hz, H-6′), 7.45 (1H, s, H-2′) 9.62 (1H, d, *J*=7.8 Hz, H-9′); ^{13}C-NMR ($CDCl_3$) δ: 52.3 (C-8), 63.5 (C-9), 87.8 (C-7), 109.6 (C-5′), 115.1 (C-3, -5), 124.7 (C-6′), 125.5 (C-8′), 126.7 (C-1′), 127.0 (C-2, -6), 129.0 (C-3′), 130.7 (C-2′), 131.7 (C-1), 153.0 (C-7′), 156.6 (C-4), 162.6 (C-4′), 193.3 (C-9′); EI-MS *m*/*z* (rel. int. %) 296 (M^+, 1), 165 (25), 149 (60), 105 (53), 95 (70), 81 (68), 69 (80), 55 (100), 46 (30); HR-EI-MS *m*/*z* 296.1121 (M^+, Calcd for $C_{18}H_{16}O_4$ 296.1094).

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