New Constituents from the Heartwood of Picea morrisonicola HAYATA

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Three new constituents, including a p-menthane type monoterpen e, 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; monoterpen e; lignan; aromatic

A total of six species of the genus Picea (Pinaceae), including five transplantation (P. abies, P. glauca, P. glehni, P. asperata, P. orientalis) and one endemic species (P. morrisonicola), were grown in Taiwan. Among them, cumulative phytochemical examinations have revealed that P. abies, P. glehni, and P. morrisonicola contained ligan s, 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. 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 and two norditerpenes, were isolated and identified from the low polar layer of acetic 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 monoterpen e 1, an aromatic 2, and a lignan 3. The following describes the structural assignments of these compounds.

The acetic 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 acetoxyethylene protons at δ_{H} 3.86 (2H, d, J=6.4 Hz, H-7), one pair of AB type acetoxyethylene 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-9, the other acetyl carbonyl with H-7, and one hydroxy-bearing carbon C-8 with H-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 (NOEY) correlations of 1a, suggested that 1a was a p-menthane type skeleton, except that both H-7 and H-9 were converted to CH_{2}OAc functionalities, and a hydroxyl group was attached to C-8. In the NOEY spectrum of 1a, mutual correlations were as follows: H-7/H-2, -6; H-7/H-10; H-7/H-3α; H-7/H-4α; H-2/H-4α; H-2/H-6; H-2/H-10; H-6/H-10; H-7/H-6; H-7/H-6; H-6/H-10; H-2/H-6; H-7/H-6; H-7/H-2.
4\(\alpha\)/H-2\(\alpha\), -6\(\alpha\); H-1/H-3\(\beta\), -5\(\beta\), which demonstrated that the acetoxy methylene and 1-acetoxy-2-hydroxisopropyl 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 \(\delta\). In contrast to the lower field shift of \(\delta(4.03)\) in axial-oriented CH\(_2\)OAc of a cis-(4-t-butylclohexyloxy)methyl acetate analogue,\(^{9,10}\) \(1\alpha\) with a relative higher field shift of \(\delta(3.86)\) closely compatible with that \((ca. \ \delta(3.81\text{--}3.88)\) of a trans-(4-t-butylclohexyloxy)methyl acetate analogue,\(^{9,10}\) suggested that its CH\(_2\)OAc should be equatorial-oriented, the same as its hydroxy isopropyl group. Accordingly, compound \(1\alpha\) was concluded to be trans-7,9-diacetoxy-7-oxabicyclo[4.2.2]decane.

The molecular formula for \(1\alpha\), a 10\(\alpha\)-methyl\(\beta\)-farnesyl acetate analogue, \(9,10\) sug- gested the presence of a hydroxyl group \((3409 \text{ cm}^{-1})\) and a hydroxymethylene group \([\delta(3.89-3.95)\text{ CH}_2OH]\) of the neoglin skeleton with two branches,\(^{12}\) and a hydroxymethylene and a trans propenyl group, attached at C-8 \((\delta(52.3)\text{ and C-1')\text{ and (c(126.7), respectively.}}\)

All the above assignments were also supported by mutual correlations of H\(_3\)-9 \((\delta 5.51\text{ (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 carboxyl functional groups were determined to be located at C-3, -4, C-2, -6, and C-7, respectively, since correlations between H\(_7\)-9 and C-2, -3, -4; H\(_1\)-10 and C-3, -4, -5; OH-6 and C-1, -5, -6; and H\(_8\) and C-7 were observed in the HMBC spectrum. Two aromatic carbon signals at \(\delta 153.0\) 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\(_1\)-9 \((\delta 2.08)\) and H\(_10\)-10 \((\delta 2.43, 8.0\%\) enhancement), and H-5 and H-10 \((8.3\%\) enhancement), and OH-6 \((\delta 12.01, 12.8\%\) 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-dimethoxybenzoic acid methyl ester. To our knowledge, \(2\) has ever been synthesized \(via\) the methylation of 2,6-dihydroxy-3,4-dimethoxybenzoic acid using diazomethane previously.\(^{11}\) However, \(2\) was found to be new from a natural source.

\((+)-\text{Morrisonicolin (3)}\) was isolated as an amorphous solid. Its molecular formula C\(_{10}\)H\(_{13}\)O\(_4\) was deduced from \(^{13}\)C-NMR data and an [M] \(^+\) ion at \(m/e 296.1121\) in the HR-ELMS. Analysis of the IR spectrum of 3 suggested that it contained a hydroxyl group \((3387 \text{ cm}^{-1})\), a conjugated aldehyde \((2760, 1660 \text{ cm}^{-1})\), and a benzene ring \((1594, 1515 \text{ cm}^{-1})\). The \(^1\)H-NMR spectrum of 3 showed a conjugated trans-propenyl group \([\delta(6.88)\text{ (1H, d, J}=8.5\text{ Hz, H-5'), 7.41 (1H, d, J}=8.5\text{ Hz, H-6'), 7.45 (1H, s, H-2)'), two methyne protons \([\delta(3.59)\text{ (1H, m, H-8), 5.60 (1H, d, J}=6.3\text{ Hz, H-7'), and a hydroxymethylene group }[\delta(3.93)\text{ (2H, m, H-9), similar to those of the A and B rings (phenylidyhydrobenzofuran) of the neooglin skeleton with two branches,}\text{ and a hydroxymethylene and a trans propenyl group, attached at C-8 (52.3) and C-1')\text{ and (c(126.7), respectively.}}\)

Further analysis of all the spectral data allowed the assignment of 3 as \(\text{E-3-[2(S,3R),2,3-dihydro-2-(4'-hydroxy-3'-methoxyphenyl)-3-hydroxymethyl-7-methoxy-1-benzo(b)furano-5-yl]-2-propen reported previously.}\)

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 \(\delta\) values (ppm), and coupling constants \((J\text{ in Hz})\).

**Plant Material** The heartwood of \(P. \text{ morrisonicola Hayata}\) was collected from Mount Taichung, 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 \(P. \text{ morrisonicola}\) (7.5 kg) was extracted with 901 Me\(_2\)CO 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 81 water and then partitioned with
EtOAc (2×3). 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 Ac₂O, 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-5-menthan-7-ol (1a): Colorless oil; [α]D 24 +3.2° (c = 0.19, CHCl₃); IR νmax (dry film): 3429, 1719, 1381, 1248, 1036 cm⁻¹; ¹H-NMR (CDCl₃): δ 0.95 (2H, m, H-2, -6), 1.12 (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, OCOCH₃), 3.86 (2H, d, J = 6.4 Hz, H-7), 3.96 (1H, d, J = 11.2 Hz, H-9), 4.05 (1H, d, J = 11.1 Hz, H-9); ¹³C-NMR (CDCl₃): δ 23.0 (rel. int. %) 258 (3.8), 321 (3.2); IR νmax (KBr): 3409, 1633, 1608, 1493 cm⁻¹; Slight yellow amorphous solid; [α]D 24 +37° (c = 1.4, CHCl₃); UV λmax (MeOH) nm (log e): 258 (3.8), 321 (3.2); IR νmax (dry film): 3387, 2760, 1660, 1594, 1515 cm⁻¹; ¹H-NMR (CDCl₃): 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-5′), 6.80 (2H, dd, J = 15.8, 8.4 Hz, H-3′, -5′), 6.88 (1H, d, J = 8.5 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′); ¹³C-NMR (CDCl₃): 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⁺, Caled for C₁₃H₁₄O₅ 296.1104).

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References