New Abietane-Type Diterpenes from the Heartwood of *Picea morrisonicola*

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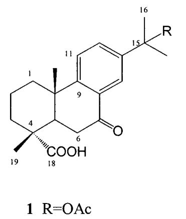
New abietane-type diterpenes, 15-acetoxy-7-oxodehydroabietic acid (1), picealactones A (2), B (3), and C (4), together with the known 7-oxodehydroabietic acid (5) were isolated and identified from the heartwood of *Picea morrisonicola*. The structures of 1—4 were determined on the basis of spectral data explanation. Compounds 2—4 possessed a rare 5-dehydro-18, 6-olide functionality. Compounds 1 and 2 were first isolated from natural source.

Key words *Picea morrisonicola*; 15-acetoxy-7-oxodehydroabietic acid; picealactone A; picealactone B; picealactone C

About 200 species of *Picea* (Pinaceae) were discovered in the world. Five transplantation (*P. abies*, *P. glauca*, *P. glehnii*, *P. asperata*, and *P. orientalis*) and one endemic species (*P. morrisonicola*) are grown in Taiwan. Chemical constituents of some species including *P. abies*,[1—3] *P. glauca*,[4] and *P. glehni*,[5] have been studied. These components contain lignans, flavonoids, and their glucosides, and diterpenoids. *P. morrisonicola* is a good building material. In our laboratory, we have investigated the chemical principles from many species of Cupressaceae and Taxodiaceae, and found many interesting structures of abietane-type diterpenes. In this time, we first studied the chemical components of a species of Pinaceae, *P. morrisonicola*. In a previous paper,[6] four norabietane derivatives were isolated and identified from its heartwood. On continuous studies of the same extracts, four abietane compounds, 15-acetoxy-7-oxodehydroabietic acid (**1**), piceanolactones A (**2**), B (**3**), and C (**4**) together with the known 7-oxodehydroabietic acid (**5**)[7] were isolated. In this paper, we report on the isolation and characterization of four new abietane-type diterpenes. Compounds **2**—**4** contained a rare 5-dehydro-18,6-olide functionality, which only presented in *ent*-kaurane derivative, stevionolide.[8]

Compound **1** had high resolution electron impact mass spectrum (HR-EI-MS) and ^{13}C-NMR data (Table 1) consistent with the molecular formula $C_{22}H_{28}O_5$. Absorptions in the IR spectrum were attributable to an acetoxyl (1732 cm^{-1}), a carboxylic acid (3200—2600, 1700, 930 cm^{-1}), a conjugated ketone (1679 cm^{-1}), and a benzene ring (1603, 1493 cm^{-1}). The UV spectrum (λ_{max} 249, 297 nm) revealed that a benzoyl functionality was similar to that of 7-oxodehydroabietic acid (**5**). The ^{1}H-NMR spectrum (Table 1) revealed that **1** has an acetoxy isopropyl group attached to a phenyl group [δ 1.72 (6H, s) and 2.01 (3H, s, CH_3COO–)], two singlet methyl groups [δ 1.21 (3H, s, H-20) and 1.32 (3H, s, H-19)], and three 1,2,4-trisubstituted phenyl protons [δ 7.30 (1H, d, J=8.4 Hz), 7.48 (1H, dd, J=8.4, 2.2 Hz), and 7.95 (1H, d, J=2.2 Hz)]. Other signals at δ 2.46 (1H, dd, J=14.1, 3.6 Hz, H-5), 2.69 (1H, dd, J=17.9, 14.1 Hz, H_β-6), and 2.74 (1H, dd, J=17.9, 3.6 Hz, H_α-6) exhibited heteronuclear multiple bond correlation (HMBC) to the conjugated carbonyl group (C-6, δ_C 198.4). They can be assigned to H-5 and H_2-6. The signal at δ 2.31 (br d, J=12.3 Hz) could be assigned to H_β-1 a typical of a dehydroabietane-type derivative.[9,10] Comparison of the ^{1}H- and ^{13}C-NMR (Table 1) data of **1** with those of the known 7-oxodehydroabietic acid (**5**) suggests that **1** possesses the same skeletal structure with an additional acetoxyl group at C-15. Correlations of H-11 (δ 7.30)/H_β-1 (δ 2.31), and H-19 (δ 1.32)/H-20 (δ 1.21) in the nuclear Overhauser enhancement exchange spectroscopy (NOESY) confirmed the assigned structure. Therefore compound **1** is 15-acetoxy-7-oxodehydroabietic acid. It was a synthetic intermediate from the oxidization of dehydroabietic acid.[11]

The IR absorption bands of picealactone A (**2**) indicated the presence of a conjugated carbonyl (1673 cm^{-1}), a benzene ring (1608, 1500 cm^{-1}), and geminal dimethyl group (1383, 1360 cm^{-1}). An absorption band at 1808 cm^{-1} gave hint of a functionality of β,γ-unsaturated γ-lactone. The HR-EI-MS and ^{13}C-NMR data (twenty signals) (Table 1) showed that **2** was a diterpene with the formula $C_{20}H_{22}O_3$. The ^{1}H-NMR signals suggested the presence of an isopropyl attached to a phenyl moiety [δ 1.26 (6H, d, J=6.8 Hz) and 2.98 (1H, sep, J=6.8 Hz)], three 1,2,4-trisubstituted phenyl protons [δ 7.42 (1H, d, J=8.1 Hz), 7.49 (1H, dd, J=8.1, 1.8 Hz), and 8.12 (1H, d, J=1.8 Hz)], two singlet of methyl groups (δ 1.62, 1.58), and a typical H_β-1 signal of a dehydroabietane at δ 2.34 (1H, br d, J=12.3 Hz). Based on the above evidence, compound **2** is a 7-oxodehydroabietane-type diterpene. Its UV maxima at 253 and 320 nm showed a bathochromic shift when compared with that of **1**. This evidence hinted the addition of a cross conjugation Δ^5 to the C-7 ketone. The chemical shift of the C-7 ketone at δ_C 173.9 [HMBC correlation with 8.12 (H-14); higher field than C-7 in compound **1**] is a further proof of an additional conjugation. Four ^{13}C-NMR signals at δ_C 47.1, 145.5, 142.5, and 179.5 were assigned to C-4, C-5, C-6, and C-18, respectively. The HMBC correlations of C-5/H-20, H-19 and C-18/H-19 confirmed the position of a β,γ-unsaturated γ-lactone. The NOESY correlations, H_β-1/H-11; H-19/H-20 were in accordance with the proposed structure of compound **2**, which was named as 7-



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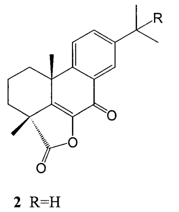
5 R=H



2 R=H
3 R=OH

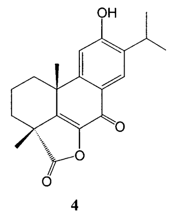


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Table 1. ^{1}H- and ^{13}C-NMR (δ Values) Data for **1**—**4** (300 and 75 Mz in CDCl$_3$)

No.	**1**		**2**		**3**		**4**[a]	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	36.8	1.44 m 2.31 br d (12.3)	42.4	1.35 m 2.34 br d (12.3)	42.3	1.34 m 2.48 br d (13.6)	40.7	1.34 m 2.29 br d (12.5)
2	18.0	1.65 m	19.0	1.83 m, 1.8 m	18.9	1.83 m, 1.85 m	20.4	1.83 m, 1.85 m
3	37.6	1.57 m	36.0	1.50 m, 2.15 m	35.9	1.47 m, 2.11 m	37.7	1.50 m, 2.11 m
4	46.3		47.1		47.1		48.4	
5	43.3	2.46 dd (14.1, 3.6)	145.5		145.6		147.6	
6	26.1	2.74 dd (17.9, 3.6) 2.69 dd (17.9, 14.1)	142.5		142.4		143.9	
7	198.4		173.9		173.7		175.7	
8	130.5		130.8		130.8		124.4	
9	153.7		148.4		149.1		153.8	
10	37.2		38.9		38.9		44.2	
11	123.5	7.30 d (8.4)	128.5	7.42 d (8.1)	125.9	7.50 d (8.4)	113.0	7.06 s
12	130.1	7.48 dd (8.4, 2.2)	131.6	7.49 dd (8.1, 1.8)	129.6	7.82 dd (8.4, 2.0)	162.0	
13	144.1		148.1		148.1		137.0	
14	123.2	7.95 d (2.2)	124.7	8.12 d (1.8)	122.7	8.32 d (2.0)	126.6	7.98 s
15	81.0		33.8	2.98 sep (6.8)	72.3		28.5	3.30 sep (6.9)
16	28.5	1.72 s	23.7	1.26 d (6.8)	31.7	1.61 s	23.2	1.24 d (6.9)
17	28.5	1.72 s	23.7	1.26 d (6.8)	31.7	1.61 s	23.2	1.24 d (6.9)
18	183.0		179.5		179.5		182.1	
19	16.1	1.32 s	21.1	1.62 s	21.0	1.62 s	22.0	1.59 s
20	23.5	1.21 s	24.4	1.58 s	24.4	1.59 s	25.4	1.56 s
$OCO\underline{CH_3}$	22.3	2.01 s						
$O\underline{C}OCH_3$	170.8							
$ArO\underline{H}$								8.60 s

a) In CD$_3$COCD$_3$.

oxo-5,8,11,13-abietatetraen-18,6-olide. Matsumoto *et al.* have oxidized methyl 7-oxodehydroabietate with perbenzoic acid in CHCl$_3$ to give rise to and compound **2** as one of the oxidative products.[12)]

Picealactone B (**3**) had the molecular formula $C_{20}H_{22}O_4$ on the basis of exact MS data. Hydroxyl (3445 cm^{-1}), conjugated ketone (1673 cm^{-1}), aromatic (3042, 1608, 1492 cm^{-1}), geminal dimethyl (1385, 1370 cm^{-1}), and β,γ-unsaturated γ-lactone (1802 cm^{-1}) functionalities were present in its IR spectrum. Four singlet methyl groups at δ 1.59, 1.61, 1.61, 1.62, and three 1,2,4-trisubstituted phenyl protons at δ 7.50 (d, J=8.4 Hz), 7.82 (dd, J=8.4, 2.0 Hz), and 8.32 (d, J=2.0 Hz) (Table 1) indicated that **3** is also a 5,8,11,13-abietatetraen-18,6-olide. The ^{1}H- and ^{13}C-NMR data as well as the UV maxima (258, 325 nm) of **3** are very similar to those of **2**. The only difference is an additional hydroxyl group at C-15 (δ 72.3, s). Together with the results of the HMBC (C-5/H-19, H-20; C-7/H-14; C-10/H-11; C-18/H-19) and NOESY (H-12,-14/H-16,H-17; H-20/H-11) spectra, compound **3** was elucidated as 15-hydroxy-7-oxo-5,8,11,13-abietatetraen-18,6-olide.

The fourth diterpene, picealactone C (**4**), crystalline needles, had the molecular formula $C_{20}H_{22}O_4$ based on the HR-EI-MS and ^{13}C-NMR data (Table 1). The absorptions in the IR spectrum were attributable to a hydroxyl group (3348 cm^{-1}), a benzene ring (1589, 1500 cm^{-1}), a conjugated ketone (1670 cm^{-1}), and a β,γ-unsaturated γ-lactone (1802 cm^{-1}). ^{1}H-NMR signals indicated the presence of a phenyl group [δ 7.06, 7.98 (each 1H, s)], a phenolic proton (δ 8.60, exchangeable with D$_2$O), an isopropyl group [δ 3.30 (1H, sep, J=6.9 Hz), 1.24 (6H, d, J=6.9 Hz)], and two singlet methyl groups (δ 1.59, 1.56). The typical H$_\beta$-1 signal of a dehydroabietane diterpene was observed at δ 2.19 (1H, br d, J=12.5 Hz). Comparison of the ^{1}H- and ^{13}C-NMR spectral data for **4** and **2** permitted the assignment of **4** as a derivative of **2** with an extra hydroxyl group at C-12. On the basis of additional confirmation by distortionless enhancement by polarization transfer (DEPT) and 2D NMR, the structure of **4** was elucidated as 12-hydroxy-7-oxo-5,8,11,13-abietatetraen-18,6-olide.

Experimental

General Experimental Procedures Melting point were determined with a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 781 spectrophtomer. ^{1}H- and ^{13}C-NMR spectra were obtained on a Bruker AM 300 spectrometer. EI-MS, HR-EI-MS, UV, and specific rotation were taken on a Finnigan TSQ-U6C, JEOL SX-102A MASS spectrometer, Hitachi S-3200, and a JASCO DIP-180 digital polarimeter, respectively. Extracts were chromatographed on silica gel (Merck 3374, 70—230 mesh) and 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 on Mount of Taichuang, Taiwan and was verified by Professor 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.

Extraction and Isolation Air-dried heartwood of *P. morrisonicola* was crushed into small pieces to give 7.5 kg, which was extracted with Me$_2$CO (90 l) three times (7 d each time) at room temperature. The combined extracts were evaporated *in vacuo* to give a black residue that was suspended with water (2 l) and then partitioned with EtOAc (2 l×3). The combined organic layers were purified by silica gel chromatography (using *n*-hexane–EtOAc mixture as solvent system). Five components, picealactones A (**2**) (11 mg), B (**3**) (9 mg), C (**4**) (9 mg), 7-oxodehydroabietic acid (**5**) (13 mg), and 15-acetoxy-7-oxodehydroabietic acid (**1**) (13 mg) were eluted with 30%, 30%, 40%, 40% and 50% EtOAc in hexane solvent system, respectively.

15-Acetoxy-7-oxodehydroabietic Acid (**1**): Amorphous solid; $[\alpha]_D^{25}$= +7.2° (c=0.34, CHCl$_3$); UV λ_{max}^{MeOH} nm (log ε) 249 (4.12), 297 (3.04); IR

(KBr) $\nu_{\max}$ 3200—2600, 1732, 1700, 1679, 1603, 1493, 1235, 1122, 1029, 930, 837, 731 cm^{-1}; ^{1}H- and ^{13}C-NMR (CDCl$_3$): Table 1; EI-MS (70 eV) (rel. int. %) *m/z* 372 (M$^+$, 3), 315 (28), 312 (39), 251 (100), 105 (32); HR-EI-MS *m/z* 372. 1946 (Calcd for $C_{22}H_{28}O_5$: 372.1937).

Picealactone A (**2**): mp 182—190 °C; $[\alpha]_D^{20}=+14.5°$ (c=0.45, CHCl$_3$); UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ε): 253 (4.07), 320 (3.05) nm; IR $\nu_{\max}$ (KBr) 3080, 1808, 1673, 1608, 1500, 1383, 1360, 1305, 1170, 1080, 996 cm^{-1}; ^{1}H- and ^{13}C-NMR (CDCl$_3$, 400 and 100 MHz): see Table 1; EI-MS (70 eV) (rel. int. %) *m/z* 310 (M$^+$, 18), 282 (10), 266 (25), 251 (20), 128 (42), 119 (38), 105 (100), 57 (40); HR-EI-MS *m/z* 310.1569 (M$^+$ Calcd for $C_{20}H_{22}O_3$: 310.1569).

Picealactone B (**3**): Amorphous solid; $[\alpha]_D^{19}=+23.5°$ (c=0.25, CHCl$_3$); UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ε): 257 (4.09), 325 (3.07); IR $\nu_{\max}$ (KBr) 3445, 3042, 1802, 1673, 1608, 1492, 1385, 1370, 1131, 986 cm^{-1}; ^{1}H- and ^{13}C-NMR (CDCl$_3$): Table 1; EI-MS (rel. int. %) *m/z* 326 (M$^+$, 100), 311 (85), 298 (70), 283 (96), 267 (90), 255 (55); HR-EI-MS *m/z* 326.1515 (M$^+$ Calcd for $C_{20}H_{22}O_4$, 326.1518).

Picealactone C (**4**): mp 251—253 °C; $[\alpha]_D^{26}=+20.1°$ (c=0.17, CHCl$_3$); UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ε) 257 (4.13), 323 (3.05); IR $\nu_{\max}$ (KBr) 3348, 1802, 1670, 1589, 1500, 1257, 1150, 1005 cm^{-1}; ^{1}H- and ^{13}C-NMR (CD$_3$COCD$_3$): Table 1; EI-MS (rel. int. %) *m/z* 326 (M$^+$, 95), 298 (55), 282 (100), 267 (65), 91 (45); HR-EI-MS *m/z* 326.1528 (M$^+$ Calcd for $C_{20}H_{22}O_4$, 326.1518).

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