

New Diterpenes from the Heartwood of *Chamaecyparis obtusa* var. *formosana*

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Received November 26, 1997

An abietane diterpene, 11,14-dihydroxy-8,11,13-abietatrien-7-one (**1**); a *seco*-abietane diterpene, obtuanhydride (**2**); and an isopimarane diterpene, 18,19-*O*-isopropylidene-18,19-dihydroxyisopimara-8(14),15-diene (**3**) were isolated from the heartwood of *Chamaecyparis obtusa* var. *formosana*. The structures of these new compounds were elucidated by spectroscopic methods.

The wood of *Chamaecyparis obtusa* S. et Z. var. *formosana* Rehd. (Taiwan hinoki; Cupressaceae) is an important building material in Formosa due to its strong resistance to wood-decaying fungi. Previous chemical studies of the wood reported only essential oil and acidic components.^{1–4} We have investigated compounds from the bark of this plant and have identified two carbamates, together with steroids, diterpenes, and a lignan.⁵ No further phytochemical studies on the wood of this plant have been reported.

The Me₂CO extract of the plant was partitioned between EtOAc and H₂O. After repeated SiO₂ column chromatography and HPLC, three diterpenes—11,14-dihydroxy-8,11,13-abietatrien-7-one (**1**), obtuanhydride (**2**), and 18,19-*O*-isopropylidene-18,19-dihydroxyisopimara-8(14),15-diene (**3**)—were isolated. Based on the following spectral evidence (COSY, HMQC, HMBC, and NOESY), the structures of these new diterpenes were elucidated as follows.

Diterpene **1** had the molecular formula C₂₀H₂₈O₃ on the basis of exact mass (HRMS) at *m/z* 316.2036. It showed hydroxyl (3375 cm⁻¹), aromatic (3040, 1608, and 1515 cm⁻¹), and conjugated carbonyl (1643 cm⁻¹) absorption in its IR spectrum. The UV spectrum indicated a benzoyl group (231 and 273 nm), and the ¹H NMR spectrum (Table 1) revealed an isopropyl group attached to a phenyl residue and three singlets of methyl groups. The ¹³C NMR (Table 2, assigned by HMQC) data are similar to sugiol⁶ except for an extra hydroxyl group attached to the phenyl group. Two phenolic protons present at δ 4.41 and 13.06, disappeared upon addition of D₂O. The singlet at δ 13.06 indicated a hydrogen bond between a hydroxyl (C-14) and a carbonyl (C-7).^{7,8} The C-11 hydroxyl group was correlated with the signal at δ 3.19. When there is a hydroxyl group at C-11, the H_β-1 signal shifts downfield to ca. δ 3.0–3.5 in this type of dehydroabietane diterpenoid.^{9,10} Other ABX signals at δ 1.80, 2.63, and 2.64 were assigned to H-5 and H-6, respectively. The only phenyl proton δ 6.75 was assigned to H-12 because of its correlation to C-9 in the HMBC spectrum. The NOESY correlations of **1** are shown in Figure 1. Therefore, the structure of **1** was assigned to be 11,14-dihydroxy-8,11,13-abietatrien-7-one.

Table 1. ¹H (δ values) Data for **1**, **2**, and **3** (300 MHz in CDCl₃)

H	1	2	3
1α	1.39 m	1.24 m	1.02 m
β	3.19 br d (13.5) ^a	1.98 br d (13.3)	1.74 m
2α	1.53 m	1.55 m	1.50 m
β	1.69 m	1.77 m	
3	1.26 m, 1.48 m	1.12 m, 1.48 m	0.89 m, 2.25 m
5	1.80 dd (9.8, 7.4)	2.72 s	0.95 dd (12.7, 6.5)
6α	2.63 dd (17.0, 7.4) ^b		1.84 m
β	2.64 dd (17.0, 9.8)		1.41 m
7α			2.00 m
β			2.27 m
9			1.69 t (7.9)
11		6.81 s	1.43–1.62 m
12	6.75 s		1.32–1.47 m
14		7.62 s	5.20 br s
15	3.28 sep (6.9)	3.13 sep (6.9)	5.74 dd (17.5, 10.4)
16	1.16 d (6.9)	1.21 d (6.9)	4.86 dd (10.4, 1.4)
			4.89 dd (17.5, 1.4)
17	1.18 d (6.9)	1.23 d (6.9)	1.01 s
18	0.95 s	1.06 s	3.18 d (11.3)
			3.73 d (11.3)
19	0.93 s	1.39 s	3.67 d (11.3)
			3.78 dd (11.3, 1.3)
20	1.36 s	1.50 s	0.67 s
2'			1.31 s
3'			1.34 s
OH	4.41 s, 13.06 s	5.79 br s	

^a 3.43 br d (13.6) in CD₃COCD₃. ^b 2.56 dd (16.9, 2.6), 2.74 (16.9, 14.5) in CD₃COCD₃.

The MS of **2** gave an exact mass at *m/z* 330.1827, indicating the molecular formula C₂₀H₂₆O₄. The UV maxima at 217, 233, and 287 nm indicated a conjugated aromatic carbonyl group, while the IR spectrum of **2** exhibited bands attributable to hydroxy, aromatic, and conjugated anhydride groups. The ¹H NMR (Table 1) spectrum was consistent with a dehydroabietane structure for this compound. Three methyl singlets at δ 1.06, 1.39, and 1.50; an isopropyl attached to an aromatic ring [δ 1.21 (3H, d, *J* = 6.9 Hz), 1.23 (3H, d, *J* = 6.9 Hz), and 3.13 (1H, sep, *J* = 6.9 Hz)], together with the H_β-1 signal at δ 1.98 (br d, *J* = 13.3 Hz) are common for the dehydroabietane skeleton. Other ¹H NMR peaks of **2** showed one aromatic proton signal at δ 7.62 (H-14, shielded by conjugated carbonyl group) and a phenolic proton at δ 5.79 (D₂O exchange). There was no conjugated ketone carbonyl ¹³C NMR signal between δ 195 and 205, but the shift of the two anhydride carbonyl signals (δ 165.8, 166.8) (Table 2) was observed. The shift that the H-5 signals exhibit at δ 2.72 indicated that

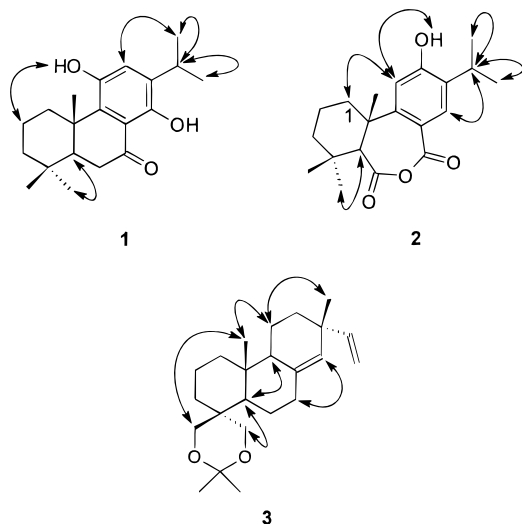
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Table 2. ^{13}C (δ values) Data for **1**, **2**, and **3** (75 MHz in CDCl_3)

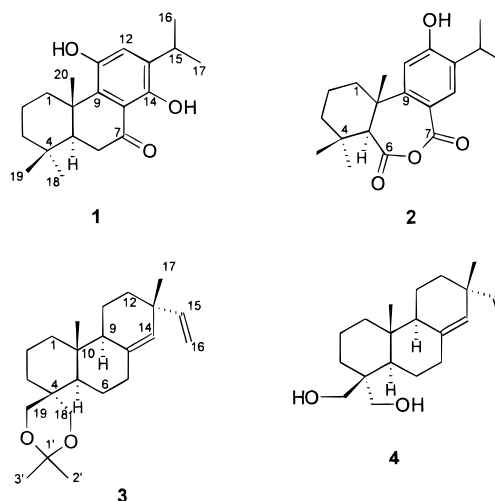
C	1	2	3
1	36.4	42.1	38.8
2	19.0	18.8	17.9
3	41.0	40.6	33.3
4	33.3	34.4	38.0
5	49.7	59.2	53.2
6	35.9	166.8	22.8
7	206.8	165.8	35.9
8	115.5	119.5	136.3
9	136.0	151.0	49.9
10	40.1	40.8	38.3
11	144.1	114.3	18.8
12	123.9	157.4	34.3
13	135.8	133.7	37.3
14	155.6	132.1	129.0
15	25.9	26.7	148.9
16	22.0	22.0	110.1
17	22.2	22.2	25.9
18	33.0	32.9	71.1
19	21.5	22.3	62.2
20	17.9	23.0	16.0
1'			98.5
2'			26.4
3'			21.0

**Figure 1.** Major NOESY correlations for compounds **1**, **2**, and **3**.

it was linked to the anhydride group. The above evidence suggested that the anhydride involves C-6 and C-7. Examination of Dreiding models suggests the H_3 -19 and H_3 -20 signals should be at lower field than normal due to deshielding by the O atom of the anhydride. The other ^1H and ^{13}C signals, together with NOESY (Figure 1) and HMBC correlations, confirmed the structure of obtuanhydride as formula **2**.

Compound **3** was given the molecular formula $\text{C}_{23}\text{H}_{26}\text{O}_2$ on the basis of HRMS (M^+ 344.2715 m/z). Its IR spectrum showed the presence of monosubstituted and trisubstituted olefinic groups (3082, 1680, 1628, 990, 912, and 835 cm^{-1}). The ^1H NMR spectrum showed the following: δ 0.67 and 1.01 (3H each, s), 3.18 and 3.78 (1H each, d, $J = 11.3$ Hz), 3.67 (1H, d, $J = 11.3$ Hz), 3.78 (1H, dd, $J = 11.3, 1.3$ Hz), 4.86 (1H, dd, $J = 10.4, 1.4$ Hz), 4.89 (1H, dd, $J = 17.5, 1.4$ Hz), and 5.74 (1H, dd, $J = 17.5, 10.4$ Hz). The ^1H NMR signals [δ 1.31 and 1.34 (3H each, s)] and ^{13}C NMR signals (δ 21.0, 26.4, and 98.5) indicated that compound **3** was a 2,2-dimethyldioxolane. Removal of a C_3 acetonide unit from the

formula $\text{C}_{23}\text{H}_{26}\text{O}_2$ of **3** would afford a parent diterpene with two methyl groups, one trisubstituted and one monosubstituted double bond, and two hydroxymethyl groups. The structure of **3** was suggested to be an acetonide of structure **4** from consideration of its spectral data and comparison of ^{13}C NMR data (Table 2) of **3** with 18-hydroxyisopimara-8(14),15-diene.¹¹ The signals at δ 3.67 and 3.78 were assigned as H_2 -19 because the former signal showed an NOE correlation with H_3 -20 (δ 0.67), and the latter signal exhibited W coupling with H_α -3. NOESY correlations are shown in Figure 1. Compound **3** may be an artificial product inasmuch as the original extraction of the wood involved Me_2CO . The naturally occurring compound may be 17-,19-dihydroxyisopimara-8(14),15-diene (**4**), which would also be a new isopimarane derivative.



Experimental Section

General Experimental Procedures. Melting points were determined with a Yanagimoto micro-melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 983G spectrophotometer. ^1H and ^{13}C spectra were run on a Bruker AM-300 spectrometer. EIMS, UV, and specific rotations were taken on a JEOL JMS-HX 300, a Hitachi S-3200 spectrometer, and a JASCO DIP-1000 digital polarimeter, respectively. Extracts were chromatographed on Si gel (Merck 70–230 mesh, 230–400 mesh, ASTM).

Plant Material. The *Chamaecyparis obtusa* var. *formosana* was collected in Taichung, Taiwan, in 1995. The plant material was identified by Mr. Muh-Tsuen Gun, and a voucher specimen has been deposited at the Herbarium of the Department of Botany of the National Taiwan University, Taipei, Taiwan.

Extraction and Isolation. The air-dried slices of heartwood of *Chamaecyparis obtusa* var. *formosana* (11 kg) were extracted with Me_2CO (120 L) at room temperature (3 days \times 2). To the evaporated Me_2CO extract was added H_2O to 1 L, and this phase was then partitioned with EtOAc (3 \times 1 L). The combined EtOAc layers were evaporated to leave a black syrup (680 g), which was chromatographed on Si gel and by HPLC repeatedly with hexane–EtOAc gradient solvent systems. 18,19-*O*-Isopropylidene-18,19-dihydroxyisopimara-8(14),15-diene (**3**) (3 mg); 11,14-dihydroxy-8,11,13-abietatrien-7-one (**1**) (18 mg); and obtuanhydride (**2**) (14

mg), were eluted with 7%, 10%, and 20% EtOAc in hexane solvent systems, respectively.

11,14-Dihydroxy-8,11,13-abietatrien-7-one (1): light yellow needles; mp 178–179 °C; $[\alpha]^{24}_{\text{D}} +65.8^{\circ}$ (*c* 0.37, CHCl₃); UV (MeOH) λ_{max} (log ϵ) 231 (3.94), 273 (3.80) nm; IR (dry film) ν_{max} 3375, 1643, 1608, 1515, 1485, 1384, 1170, 1040, 900 cm⁻¹; ¹H NMR, see Table 1, and ¹³C NMR, see Table 2; EIMS (70 eV) *m/z* 316 [M]⁺ (100), 301 (38), 231 (25), 219 (20), 205 (16), 177 (12), 69 (17), 55 (18); HREIMS *m/z* 316.2036 (calcd for C₂₀H₂₈O₃, 316.2038).

Obtuanhydride (2): yellow plates; mp 167–168 °C; $[\alpha]^{24}_{\text{D}} -34.0^{\circ}$ (*c* 0.35, CHCl₃); UV (MeOH) λ_{max} (log ϵ) 217 (4.08), 233 (4.17), 287 (3.95) nm; IR (dry film) ν_{max} 3383, 3020, 1774, 1731, 1606, 1575, 1506, 1244, 1230, 1107, 1038, 982, 907 cm⁻¹; ¹H NMR, see Table 1, and ¹³C NMR, see Table 2; EIMS (70 eV) *m/z* 330 [M]⁺ (45), 315 (7), 287 (57), 271 (46), 229 (28), 217 (28), 204 (100), 161 (35), 115 (20), 91 (17), 69 (23), 55 (38); HREIMS *m/z* 330.1827 (calcd for C₂₀H₂₆O₄, 330.1831).

18,19-O-Isopropylidene-18,19-dihydroxyisopimara-8(14),15-diene (3): colorless oil; $[\alpha]^{24}_{\text{D}} -7.1^{\circ}$ (*c* 0.13, CHCl₃); IR (dry film) ν_{max} 3082, 1680, 1628, 1370, 1191, 1157, 1060, 1022, 990, 912, 835 cm⁻¹; ¹H NMR, see

Table 1, and ¹³C NMR, see Table 2; EIMS (70 eV) *m/z* 344 [M]⁺ (100), 329 (88), 286 (22), 271 (18), 256 (48), 241(45), 188 (15), 173 (18), 145 (33), 133 (29), 119 (26), 91 (39), 79 (25), 55 (28); HREIMS *m/z* 344.2715 (calcd for C₂₃H₃₆O₂, 344.2715).

Acknowledgment. This research was supported by the National Science Council of the Republic of China.

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NP970531+