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

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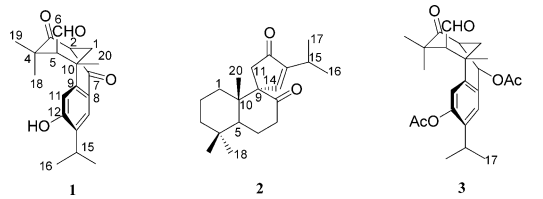
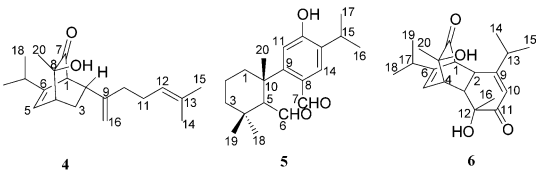
Two novel diterpenes, obtusanal B (1) and obtusadione (2), along with obtusanal A (3), obtunone (4), 12-hydroxy-6,7-secoabieta-8,11,13-triene-6,7-dial, 8,12-dihydroxydielmentha-5,9-diene-7,11-dione and myrcene, isolated from the heartwood of *Chamaecyparis obtusa* var. *formosana*, were characterized by spectroscopic means, including 2D-NMR techniques. Compounds 1 and 2 are 7(6→2)abeoabietane and 14(8→9)abeoabietane type diterpenes, respectively. Their biosyntheses were proposed.

Key words *Chamaecyparis obtusa* var. *formorsana*; Cupressaceae; 7(6→2)abeoabietane; 14(8→9)abeoabietane; obtusanal; obtusadione

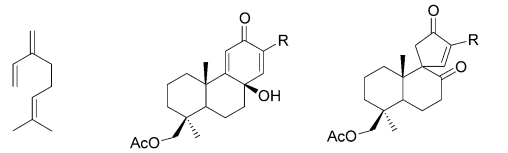
Only two endemic species, *Chamaecyparis formosana* and *C. obtusa* var. *formosana*, among seven species of genus *Chamaecyparis* are found in the central mountains of Taiwan. They are all huge trees and can live for over a thousand years. The heartwood of *C. obtusa* var. *formosana*, with distinguished purple-pink coloring and strong resistance against termites and fungi, caused the heartwood of this plant to have greater economic value than that of *C. formosana*. In previous papers on the chemical studies of the heartwood of *C. obtusa* var. *formosana*, we reported the structural elucidation of novel diterpenes, lignans, monoterpenes and sesquiterpenes.[1—7] Further detailed reinvestigation of the same extract yielded two novel diterpenes, obtusanal B (**1**), obtusadione (**2**), together with obtusanal A (**3**) (reported in the communication as obtusanal),[3] obtunone (**4**),[1] 12-hydroxy-6,7-secoabieta-8,11,13-triene-6,7-dial (**5**),[8] 8,12-dihydroxydielmentha-5,9-diene-7,11-dione (**6**),[9] and myrcene (**7**).[10] The structures of these novel diterpenes were elucidated on the basis of spectral evidence, and compounds **1** and **2** are 7(6→2)abeoabietane and 14(8→9)abeoabietane diterpenes.



1 **2** **3**



4 **5** **6**



7 **8** R=H, **9** R=Et **10** R=H, **11** R=Et

Obtusanal B (**1**) was isolated as colorless needles; its molecular formula, $C_{20}H_{24}O_4$, was established through ^{13}C-NMR (Table 1) and high-resolution electron impact mass spectroscopy (HR-EI-MS) data and represents nine indices of hydrogen deficiency (IHD). The IR spectrum of **1** showed absorptions for hydroxy (3356 cm^{-1}), aldehyde (1735 cm^{-1}), cyclohexanone (1711 cm^{-1}), conjugated carbonyl (1662 cm^{-1}), and aromatic groups (1601, 1576 cm^{-1}). The UV spectrum indicated a benzoyl functionality (λ_{max} 236, 290, and 316 nm). The ^{1}H-NMR spectrum exhibited signals for three singlet methyl groups [δ 0.59 (H-18), 1.06 (H-19), 1.41 (H-20)], one isopropyl group linked to phenyl [δ 1.24, 1.26 (3H each, d, J=6.9 Hz, H-16, -17), 3.14 (1H, sep, J=6.9 Hz, H-15)], an aldehyde [δ 9.81 (d, J=6.0 Hz, H-6)], two methine protons [δ 2.62 (dd, J=6.0, 2.3 Hz, H-5), 3.77 (dd, J=3.4, 2.3 Hz, H-2)], two methylene protons [δ 2.52 (ddd, J=14.0, 2.3, 2.3 Hz, H_α-1), 2.76 (dd, J=14.0, 3.4 Hz, H_β-1)], two singlet *para*-phenyl protons [δ 6.81 (H-11), 7.93, (H-14)], and an exchangeable phenolic proton (δ 5.91). The signal of H-14 appeared at a lower field (δ 7.93) due to deshielding by a carbonyl group. H_α-1 (2.52) exhibited a larger geminal coupling (J=14.0 Hz), a vicinal coupling (J=2.3 Hz), and a W-form coupling to H_α-5 (δ 2.62, dd, J=6.0, 2.3 Hz). Additionally, H_α-5 expressed W-form coupling. These spectral data suggested the presence of a cyclohexanone moiety. Three carbonyl ^{13}C-NMR signals (Table 1) at δ 189.6, 201.7, and 206.3 suggested the presence of conjugated ketone, cyclohexanone, and aldehyde groups, respectively. The correlated spectroscopy (COSY) coincided with the above mentioned structural units. Except for three carbonyl ^{13}C-NMR signals (Table 1), the remaining 17 carbon signals included three methyl carbons, three isopropyl carbons, six phenyl carbons with four substitutents including one oxygenated function (δ 159.3), and 5 carbons of cyclohexanone. Analysis of the heteronuclear multiple-bond correlation spectroscopy (HMBC) spectrum and comparison of the spectral data with those of compound **3** indicated that compound **1** possessed the same skeleton as **3**, having one carbonyl and one hydroxyl group in the place of two acetoxyl groups existing in compound **3**. The relative stereochemistry was described as structure **1** by nuclear Overhauser enhancement and exchange spectroscopy (NOESY) (Fig. 1).

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Table 1. ^{13}C-NMR Data ($CDCl_3$, 100 MHz) of Compounds **1**—**2**

No.	**1**	**2**
1	36.9 t	33.7 t
2	61.6 d	18.3 t
3	206.3 s	41.7 t
4	44.9 s	33.6 s
5	68.7 d	47.1 d
6	201.7 d	23.4 t
7	189.6 s	39.0 t
8	125.2 s	208.8 s
9	150.3 s	67.8 s
10	35.8 s	44.0 s
11	112.3 d	37.9 t
12	159.3 s	207.3 s
13	135.4 s	153.3 s
14	126.9 d	152.3 d
15	26.9 d	24.9 d
16	22.1 q	21.1 q
17	22.3 q	21.4 q
18	31.3 q	33.8 q
19	25.5 q	22.0 q
20	27.3 q	17.6 q

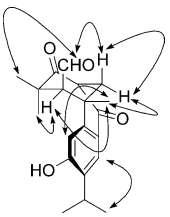

Fig. 1. NOESY of **1**

Obtusadione (**2**) had the molecular formula $C_{20}H_{30}O_2$, as determined by HR-EI-MS. No hydroxy absorption was observed in its IR spectrum, but one carbonyl (1712 cm^{-1}) and one olefinic (1610 cm^{-1}) absorption presented. The UV absorptions at λ_{max} 223, 245, 299 nm indicated the presence of a conjugated carbonyl system. Compound **2** had twenty ^{13}C-NMR signals (Table 1), including two carbonyl signals (δ 207.3, 208.8) and two olefinic signals [δ 153.3 (C-13), 152.3 (C-14)]. Two carbonyl ^{13}C-NMR signals, conjugated UV absorptions, and only one carbonyl absorption band (1712 cm^{-1}) in the IR spectrum indicated that it has one cyclohexanone and one conjugated cyclopentenone. A singlet of vinyl proton with a lowfield at δ 7.26 suggested that it was a β-olefinic proton of conjugated cyclopentenone, unambiguously. An isopropyl group attached at the α-position of conjugated cyclopentenone is attributable to its ^{1}H-NMR signals [δ 1.05, 1.09 (3H each, d, J=6.9 Hz, H-16, -17) and 2.60 (1H, sep, J=6.9 Hz, H-15)]. Based on three singlet of methyl groups [δ 0.87, 0.89, and 1.02 (H-20, H-19, and H-18 respectively)] together with an isopropyl group, we propose that the skeleton of **2** is similar to abietane-type diterpene. Two methylene protons at δ 2.57 (1H, ddd, J=14.6, 7.4, 6.4 Hz, H_α-7), and 2.49 (1H, ddd, J=14.6, 5.3, 2.1 Hz, H_β-7) were assigned as H_2-7 due to their chemical shifts and having HMBC correlation to C-8 carbonyl carbon (δ_C 208.8). The methylene appeared to be a pair of doublets at δ 3.21 (1H, d, J=18.7 Hz, H_α-11) and 2.13 (1H, d, J=18.7 Hz, H_β-11) in ^{1}H-NMR spectrum, and was considered to be in a position between a quaternary carbon (δ 67.8) and a carbonyl (δ 207.3, C-12) of cyclopentenone due to having HMBC correlation with δ 207.3 and 67.8. This quaternary carbon was assigned to C-9 (spirocarbon) due to showing HMBC with H-20, H-14, H-7. From the HMBC analysis (Fig. 2), the structure of **2** was judged to be 14(8→9)abeoabieta-13-ene-8,12-dione. Its relative stereochemistry was clear from NOESY (Fig. 3). The NOESY correlations, such as H-5/H-14 and H-5/H-18, confirmed the double bond positioned at an α-axial orientation, accounting for H_α-11 and H_β-11 having a larger different chemical shift due to H_α-11 receiving a deshielding effect from the carbonyl of cyclohexanone. 14(8→9)Abeoabietane is a novel skeleton in naturally occurring products, although in the photoirradiation of podocarpane, derivatives **8** and **9** yielded the two spirans **10** and **11**, respectively.[11)]

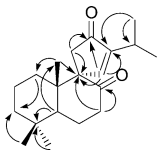

Fig. 2. HMBC of **2**

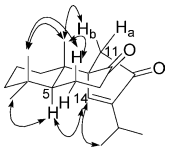

Fig. 3. NOESY of **2**

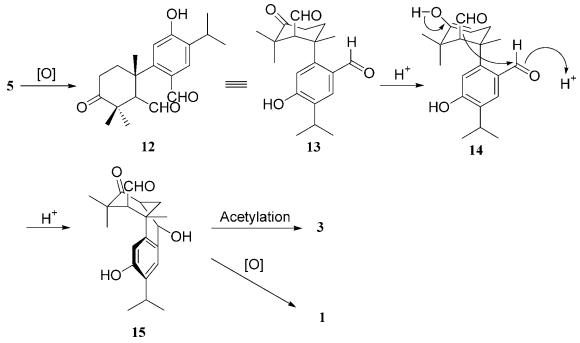

Chart 1. Proposed Mechanism for Biosynthesis of **1** and **3**

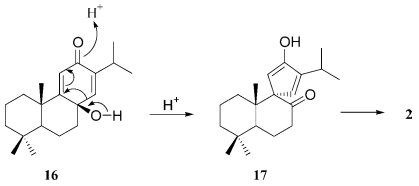

Chart 2. Proposed Mechanism for Biosynthesis of **2**

The proposed biosynthesis of **1** and **3** from **5** is sketched in Chart 1. Compound **12** is an oxidative product from **5**. The enol form **14** from **13** underwent bio-Aldol condensation to yield **15**, which was subsequently acetylated and partially oxidized to produce **3** and **1**, respectively. Compound **2** was proposed to derive from **16** (Chart 2), which is an unobserved compound occurring naturally but was prepared from ferruginol by oxidization with phenyl seleninic anhydride or

benzoyl peroxide.[12,13] Intermediate **17**, obtained from **16** by acidic treatment, tautomerized to **2**.

Experimental

General Experimental Procedures Extracts were chromatographed on silica gel (Merck 70—230 mesh, 230—400 mesh, ASTM) and purified with a semi-prep. normal phase HPLC column (250×10 mm, 7 μm, LiChrosorb Si 60) taken on LDC Analytical-III; mp: Yanagimoto micro-melting apparatus; uncorrected. Specific rotation: Jasco DIP-180 digital polarimeter. IR spectra: Perkin-Elmer 983 G spectrophotometer. ^{1}H- and ^{13}C-NMR spectra: Bruker DMX-400 spectrophotometer. EI-MS: JEOL JMS-HX 300 mass spectrometer.

Plant Material The dried heartwood of *C. obtusa* var. *formosana* was collected from Taichung, Taiwan, in 1996. Mr. Muh-Tsuen Kao, formerly a technician with the Department of Botany, National Taiwan University, identified the plant. A voucher specimen has been deposited at the Herbarium of the Department of Botany, National Taiwan University, Taipei, Taiwan.

Extraction and Isolation The dried heartwood of *C. obtusa* var. *formosana* (11 kg) was extracted with Me_2CO (120 l) at r.t. (7d×2). To the evaporated Me_2CO extract, H_2O was added to bring the total volume to 1 l. This phase was extracted with AcOEt (1 l×3). The combined AcOEt layers afforded, after evaporation, a black syrup (680 g), which was purified by silica-gel chromatography and HPLC (normal phase on Lichrosorb Si 60), repeatedly, with hexane/AcOEt. Compound **2** (6.3 mg), **4** (8.8 mg), **5** (8.2 mg), **3** (6.7 mg), **1** (8.0 mg) and **6** (10.5 mg) were eluted with 10, 20, 20, 30, 30, and 30% AcOEt in hexane, respectively. The essential oil from steam distillation of the heartwood was subjected to analysis with GC-MS; myrcene (**7**) was identified by GC-MS computer base data (it was unpublished data).

Obtusanal B (**1**): mp 192—194 °C. ^{1}H-NMR: see text. ^{13}C-NMR: Table 1. IR (KBr) cm^{-1}: 3356, 1735, 1711, 1662, 1601, 1576, 1319, 1265. UV λ_{max} (MeOH) nm (log ε): 236 (3.78), 290 (3.72), 316 (3.61). HR-EI-MS *m*/*z*: 328.1665 (M^+, Calcd for $C_{20}H_{24}O_4$: 328.1674). EI-MS (70 eV) (rel. int. %) *m*/*z*: 328 (38, M^+), 299 (9), 244 (10), 216 (99), 201 (100), 149 (22), 83 (32). $[\alpha]_D^{28}$ −195.5° (*c*=0.41, $CHCl_3$).

Obtusadione (**2**): mp 178—179 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.87, 0.88, 1.02 (3H each, s, H-20, -19, -18), 1.05, 1.09 (3H each, d, *J*=6.9 Hz, H-16, -17), 1.16, 1.32 (1H each, m, H-1), 1.23, 1.45 (1H each, m, H-3), 1.46—1.53 (2H, m, H-2), 1.75, 2.10 (1H each, m, H-6), 1.85 (1H, dd, *J*=13.0, 2.7 Hz, H-5), 2.13, 3.21 (1H each, d, *J*=18.7 Hz, H-11), 2.60 (1H, Sep, *J*=6.9 Hz, H-15), 2.49 (1H, ddd, *J*=14.6, 5.3, 2.1 Hz, H_β-7), 2.57 (1H, ddd, *J*=14.6, 7.4, 6.8 Hz, H_α-7), 7.26 (1H, s, H-14). ^{13}C-NMR: Table 1. IR (KBr) cm^{-1}: 1712, 1610, 1390, 1368, 1257, 1195, 1106. UV λ_{max} (MeOH) nm (log ε): 223 (3.89), 245 (3.93), 299 (3.33). HR-EI-MS *m*/*z*: 302.2243 (M^+, Calcd for $C_{20}H_{30}O_2$: 302.2247). EI-MS (70 eV) (rel. int. %) *m*/*z*: 302 (42, M^+), 241 (13), 166 (31), 137 (18), 88 (32), 73 (36), 70 (76), 61 (100). $[\alpha]_D^{19}$ −79.1° (*c*=0.30, $CHCl_3$).

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