Two New Compounds from the Leaves of *Calocedrus microlepic* var. *formosana*

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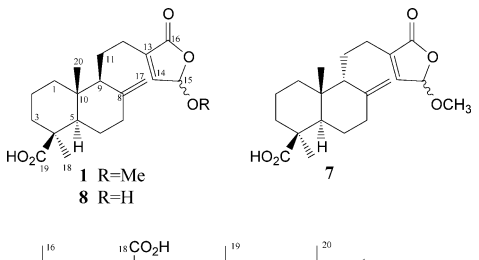
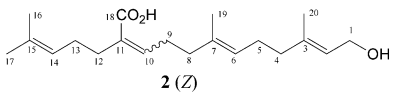
Two new compounds, 15-methoxypinusolidic acid (1) and isonerylgeraniol-18-oic acid (2) together with four knowns taiwaniaflavone (3), nerylgeraniol-18-oic acid (4), 3-(3,4-dihydroxyphenyl)-1-propanol (5), and amentoflavone (6) are isolated from the leaves of *Calocedrus microlepic* var. *formosana*. Compounds 1 and 2 were elucidated as labdane diterpene and linear diterpene, respectively, through spectral studies.

Key words *Calocedrus microlepic* var. *formosana*; diterpene; biflavone; 15-methoxypinusolidic acid; isonerylgeraniol-18-oic acid; amentoflavone

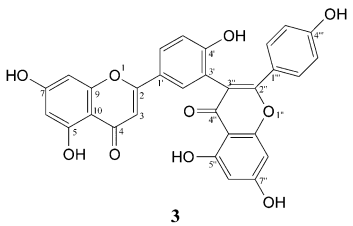
Calocedrus microlepic var. *formosana* (=*C. formosana*), a member of the Cupressaceae, is an economically important tree indigenous to Taiwan.[1] It is an endemic conifer commonly called 'shonan'. Its heartwood[2—4] and leaves[5,6] are rich in terpenoids and lignans. Due to the cytotoxic activity, we are encouraged to reinvestigate the chemical constituents of the leaves of this plant. The previous paper[7] reported twenty-seven known components, from which agathadiol, agatholal, and ferruginol exhibited significant activity to two cell lines, NUGC-3 and HONE-1. The further studies on the same extract, two new components 15-methoxypinusolidic acid (**1**) and isonerylgeraniol-18-oic acid (**2**) together with four knowns taiwaniaflavone (**3**),[8] nerylgeraniol-18-oic acid (**4**),[9] 3-(3,4-dihydroxyphenyl)-1-propanol (**5**),[10] and amentoflavone (**6**)[11] are isolated and identified. The latter four known compounds are the first time observed from the leaves of this plant. This paper deals with the structural elucidation of **1** and **2**.

High-resolution electron impact mass spectroscopy (HR-EI-MS) of compound **1** showed a molecular ion at *m*/*z* 362.2099 corresponding to the molecular formula $C_{21}H_{30}O_5$. The IR spectrum indicated the presence of a carboxylic acid (3200—2600, 1695 cm^{-1}), terminal methylene (3084, 1648, 893 cm^{-1}), and an α,β-unsaturated γ-lactone (1750 cm^{-1}). From the IR, ^{13}C- and distortionless enhancement by polarization transfer (DEPT) NMR spectra suggested that **1** was a diterpene containing a carboxylic acid, an α,β-unsaturated γ-lactone, and a methyoxyl functionalities. The ^1H-NMR spectral data showed that **1** had the characteristic pattern of a labdane-type diterpene with terminal olefinic protons (δ 4.54, 4.87) and two tertiary methyl protons (δ 0.58, 1.22). The IR absorption (1750 cm^{-1}), ^1H-NMR signals [δ 5.70 (H-15), 6.74 (H-14), and 3.55 (3H, s, –OC$\underline{H}_3$)], and ^{13}C-NMR signals [δ 102.4 (C-15), 139.2 (C-13), 141.5 (C-14), and 171.3 (C-16)] together with HMBC (heteronuclear multiple-bond correlation) (C-15/OC$\underline{H}_3$, H-14) demonstrated the presence of an α,β-unsaturated γ-lactone with a methoxyl group attached on γ-carbon (C-15). The carboxyl carbon at δ 182.8 (C-19) showed HMBC correlations with Me-18, H-3, and H-5, and a quaternary carbon at δ 44.2 was assigned as C-4 due to showing correlations with Me-18, H-2, and H-3. The following correlations δ_C 147.2/H-6, H-7, H-9; δ_C 106.8/H-7, H-9 clarified the two carbons as C-8 and C-17, respectively. The Me-18 (δ 1.22) had correlation with H-5 (δ 1.30) in NOESY (nuclear Overhauser enhancement and exchange spectroscopy) spectrum in addition to H-20 at higher field (δ 0.58),[12] which confirmed carboxylic acid at β-axial orientation. The H-11 (δ 1.59, 1.76) had correlation with H-20 (δ 0.58) determined the configuration of methine proton H-9 to be at α-axial orientation. A compound (elucidated as **7**)[13] was isolated from the leaves of *Biota orientalis* by Koo. The ^1H-, ^{13}C-NMR and other physical data of that compound and **1** are almost similar. Therefore, two compounds were proposed as same compound. Koo[13] assigned compound as **7** due to the NOESY correlation between H_3-20 and H-9. But





2 (*Z*)
4 (*E*)



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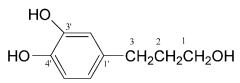

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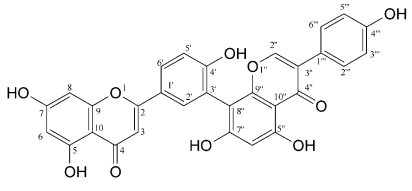

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we found chemical shift of H_a-11 (δ 1.59) is almost as H-9 (δ 1.58). This is an only reason causing Koo gave the uncorrected result. Up to now, the all derivatives of labdane-type have not been found C-9 epimer (except **7**). Therefore compound **1** was assigned as 15-methoxypinusolide, unambiguously. The previous report[12] we also have isolated 15-hydroxypinusolidic acid (**8**) from same plant as Koo's report.

Compound **2** has been isolated as an amorphous solid and has the molecular formula of $C_{20}H_{32}O_3$ based on its exact mass and ^{13}C-NMR spectrum. The IR spectrum of **2** showed bonds attributable to hydroxyl (3409 cm^{-1}), olefinic (3045, 1665 cm^{-1}), and carboxylic acid groups (3200—2600, 1691, 925 cm^{-1}). The ^{1}H-NMR spectrum showed signals for an allyl primary alcohol [δ 4.15 (2H, d, J=7.2 Hz)], where four singlet methyl groups (δ 1.57, 1.58, 1.64, 1.65) linked on the olefinic groups, four three substituted olefinic protons [δ 5.07 (2H, m), 5.39 (1H, t, J=7.2 Hz), 5.96 (1H, t, J=7.6 Hz)], and six allyl methylene groups [δ 2.10—2.55, 12H]. Twenty ^{13}C-NMR signals appeared for four CH_3, seven allyl CH_2 (including one oxygenated CH_2 at δ_C 59.4), eight olefinic carbons (four CH and four C), and one C of carbonyl (δ_C 171.6). Five indices of hydrogen deficiency (IHD) were determined from DEPT experiment and HR-EI-MS. Therefore compound **2** is a derivative of 2,6,10,14-phytatetraene with one hydroxyl at head C-1 and a carboxylic acid instead of a methyl group. The signals at δ 1.65 (H-16) and 1.57 (H-17) had HMBC with C-14 (δ_C 123.5) and C-15 (δ_C 132.2) were assigned as terminal gem-dimethyl groups. The signal at δ 1.64 was assigned as H-20 due to NOESY correlation with H-1. The NOESY spectrum, H-2 (δ 5.39)/H-4 (δ 2.10); H-6 (δ 5.07)/H-8 (δ 2.10); H-10 (δ 5.96)/H-12 (δ 2.24) established the stereochemistry of Δ^2-, Δ^6- and Δ^{10}-double bonds. The comparison of ^{1}H-NMR data between **2** and **4**, the only difference is H-10. H-10 (δ 6.83) positioned at lower field confirmed the 10*E* in compound **4**. Therefore, the structure of **2** was determined as (2*E*,6*E*,10*Z*)-1-hydroxy-2,6,10,14-phytatetraen-18-oic acid.

Experimental

General Experimental Procedures Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer 781 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were obtained on a Bruker DMX-500 at 500 and 125 MHz in $CDCl_3$ or acetone-d_6, with tetramethylsilane (TMS) as an internal standard. EI-MS, FAB-MS, UV, and specific rotations were recorded on a JEOL JMS-HX 300, a JOEL JMS-HX 110, a Hitachi S-3200 spectrometer, and a JASCO DIP-180 digital polarimeter, respectively. Extracts were chromatographed on silica gel (230—400 mesh, ASTM).

Plant Material The leaves of *C. microlepic* var. *formosana* were collected in Nan-Tou, Taiwan. The plant was identified by Mr. Muh-Tsuen Gun, formerly a technician of the Department of Botany, National Taiwan University.

Extraction and Isolation The air dried leaves of *C. microlepic* var. *formosana* were crushed to give 17 kg of raw material, which was extracted with MeOH (140 l) at room temperature (7 d×2). The extract was evaporated *in vacuo* to yield a residue which was suspended in H_2O (1 l), and this was then partitioned with hexane, ethyl acetate and *n*-BuOH (each 1 l×3), successively. The combined ethyl acetate layer provided a black syrup (200 g), which was subsequently chromatographed over silica gel with a hexane/EtOAc gradient solvent system. Crude compounds was further purification by HPLC [Merck LichroCART 250-10 Cat. 1.50179 Lichrosorb Si 60 (7 μm)] gave **1** (28 mg), **2** (16 mg), **3** (17 mg), **4** (14 mg), **5** (20 mg), **6** (16 mg).

15-Methoxypinusolidic Acid (**1**): Yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 6.74 (1H, br s, H-14), 5.70 (1H, br s, H-15), 4.87, 4.54 (1H each, br s, H-17), 3.55 (3H, s, –OC<u>H</u>$_3$), 2.45, 2.12 (1H each, m, H-12), 2.38, 1.85 (1H each, m, H-7), 2.13, 1.03 (1H each, m, H-3), 1.96, 1.86 (1H each, m, H-6), 1.83, 1.50 (1H each, m, H-2), 1.82, 1.05 (1H each, m, H-1), 1.76, 1.59 (1H each, m, H-11), 1.58 (1H, m, H-9), 1.30 (1H, br d, J=11.5 Hz, H-5), 1.22 (3H, s, H-18), 0.58 (3H, s, H-20). ^{13}C-NMR ($CDCl_3$) δ: 182.8 (C-19), 171.3 (C-16), 147.2 (C-8), 141.5 (C-14), 139.2 (C-13), 106.8 (C-17), 102.4 (C-15), 56.9 (OCH3), 56.2 (C-5), 55.7 (C-9), 44.2 (C-4), 40.5 (C-10), 39.2 (C-1), 38.6 (C-7), 37.9 (C-3), 29.0 (C-18), 26.0 (C-6), 24.5 (C-12), 21.7 (C-11), 19.8 (C-2), 12.8 (C-20). IR (KBr) cm^{-1}: 3200—2600, 3084, 1750, 1695, 1648, 1660, 1110, 893. UV λ_{max} (MeCN) nm (log ε): 201.0 (3.08), 286.0 (1.78). HR-EI-MS *m/z*: 362.2099 (M^+, Calcd for $C_{21}H_{30}O_5$: 362.2093). EI-MS (70 eV) (rel. int. %) *m/z*: 362 (5, M^+), 344 (9), 317 (12), 285 (10), 235 (25), 189 (41), 128 (100), 81 (24). $[\alpha]_D^{24}$ +39.0° (c=0.4, $CHCl_3$).

(2*E*,6*E*,10*Z*)-1-Hydroxy-2,6,10,14-phytatetraen-18-oic Acid (**2**): Amorphous solid. ^{1}H-NMR ($CDCl_3$) δ: 5.96 (1H, t, J=7.6 Hz, H-10), 5.39 (1H, t, J=7.2 Hz, H-2), 5.07* (2H, m, H-6, -14), 4.15 (2H, d, J=7.2 Hz, H-1), 2.55 (2H, m, H-9), 2.24 (2H, m, H-12), 2.10* (8H, m, H-4, -5, -8, -13), 1.65 (3H, s, H-16), 1.64 (3H, s, H-20), 1.58 (3H, s, H-19), 1.57 (3H, s, H-17). ^{13}C-NMR ($CDCl_3$) δ: 171.6 (C-18), 144.7 (C-10), 139.4 (C-3), 134.6 (C-7), 132.2 (C-15), 130.8 (C-11), 124.2 (C-6), 123.5 (C-14), 123.4 (C-2), 59.4 (C-1), 34.6 (C-12), 27.8 (C-9), 25.7 (C-4, -5, -8, -13), 17.7 (C-17), 16.1 (C-16), 16.0 (C-19, -20). IR (KBr) cm^{-1}: 3409, 3200—2600, 3045, 1691, 1665, 1451, 925. UV λ_{max} (MeCN) nm (log ε): 193.0 (4.06). HR-EI-MS *m/z*: 320.2340 (M^+, Calcd for $C_{20}H_{32}O_3$: 320.2343). EI-MS (70 eV) (rel. int. %) *m/z*: 320 (1, M^+), 287 (8), 187 (27), 121 (69), 107 (80), 93 (100). * Obscured by another signals.

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