Labdane-Type Diterpenoids from the Wood of *Cunninghamia konishii*

Yen-Cheng Li and Yueh-Hsiung Kuo*

Department of Chemistry, National Taiwan University, Taipei, Taiwan, Republic of China.

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Five new labdane-type diterpenes, 12β,19-dihydroxymanoyl oxide (1), 8(17),13-labdadien-12,15-olid-19-oic acid (2), 12,15-epoxy-8(17),13-labdadien-18-oic acid (3), 8α-hydroxy-11*E*,13*Z*-labdadien-15-al (4), and (13*R*)-13-hydroxy-8(17), 11*E*,14-labdatrien-18-oic acid (5) were isolated from the wood of *Cunninghamia konishii*. Their structures were elucidated by two-dimensional NMR spectroscopy.

Key words *Cunninghamia konishii*; Taxodiaceae; diterpene; labdane

Cunninghamia (Taxodiaceae) has two species in eastern Asia. One, *Cunninghamia konishii* Hayata, is endemic to Taiwan and distributed in the northern and central parts of Taiwan at altitudes of 1300—2700 m. The wood of this tree is one of the best building material in Taiwan. In previous investigations of the chemical composition, the essential oils of the wood[1,2] and the milled bark[3] were reported. We have investigated the chemical components of the wood and found five new diterpenes from the ethyl acetate-soluble fraction of its methanol extract including abietane, manoyl oxide, and norabietane.[4] In connection with our interest in the components of this plant, a further chemical study on the same ethyl acetate-soluble fraction was undertaken. Five new labdane-type diterpenoids, 12β,19-dihydroxymanoyl oxide (**1**), 8(17),13-labdadien-12,15-olid-19-oic acid (**2**), 12,15-epoxy-8(17),13-labdadien-18-oic acid (**3**), 8α-hydroxy-11*E*,13*Z*-labdadien-15-al (**4**), and (13*R*)-13-hydroxy-8(17),11*E*,14-labdatrien-18-oic acid (**5**), were purified by repeated chromatography on silica gel and HPLC. In this paper, we report the structural elucidation of the five new abietane- and labdane-type diterpenes.

The new compound **1** obtained in this study exhibited a molecular formula of $C_{20}H_{34}O_3$, based on high-resolution electron-impact mass spectroscopy (HR-EI-MS). Its IR spectrum indicated hydroxyl (3385 cm^{-1}) and vinyl (3072, 1646, 994, 923 cm^{-1}) functional groups. The ^{1}H- and ^{13}C-NMR (Table 1) spectra showed that **1** contained four singlet methyl groups, a carbinyl proton [δ_H 3.92 (dd, J=8.0, 4.0 Hz); δ_C 81.6], a hydroxymethyl group [δ_H 3.67, 3.48 (1H each, d, J=10.8 Hz); δ_C 65.4], and a vinylic ABX system [δ 5.93 (dd, J=17.6, 10.8 Hz), 5.28 (dd, J=17.6, 1.6 Hz), and 5.11 (dd, J=10.8, 1.6 Hz); δ_C 143.1, 113.0], similar to jabugodiol (**6**),[5] except that the hydroxyl group at C-16 in **6** is moved to C-12 in **1**. Regarding the stereochemistry of **1**, pronounced nuclear Overhauser enhancement and exchange spectroscopy (NOESY) correlations between the hydroxymethyl protons (H$_2$-19) and H$_3$-20, H$_3$-20 and H$_3$-17, and H$_3$-17 and H$_3$-16 illustrated that three fused rings were all the *trans* and chair form. The evidence showing the protons of H$_2$-19, H$_3$-20, H$_3$-17, and H$_3$-16 were all in the axial orientation. The signal at δ_C 74.5 was assigned to C-13 due to its heteronuclear multiple-bond (HMBC) correlation to H-16, H-15, and H-14. The carbinyl proton at δ 3.92 with a doublet of a doublet (J=8.0, 4.0 Hz) indicated that the axial proton must be located at C-1, C-3, C-7, or C-12. This proton has a NOESY correlation with H-14 (δ 5.93) and an HMBC with C-13 (δ_C 74.5), thereby locating it at the 12-α-axial position. The evidence above confirmed that the structure of **1** as 12β,19-dihydroxymanoyl oxide.

The new labdane compound **2** was found to have the molecular formula $C_{20}H_{28}O_4$ based on HR-EI-MS. The infrared spectrum showed bands due to a vinyl group (3078 cm^{-1}), an acid (3300—2700, 1692 cm^{-1}), and a conjugated double bond (1641 cm^{-1}). The IR band at 1757 cm^{-1} is assignable to the conjugated γ-lactone, and its presence was further substantiated by the UV absorption maximum at 210 nm. Its ^{1}H-NMR spectrum confirmed the presence of three tertiary methyl groups, two on saturated carbons (δ 0.59, 1.24, each s) and one on olefine (δ 2.08, s). Other functionalities that were apparent from the ^{1}H- and ^{13}C-NMR spectral data of **2** included an α,β-unsaturated γ-lactone group [δ_H 4.85 d (H-12), 5.75 br s (H-14); δ_C 83.4 d, 116.6 d, 169.3 s, 173.1 s] (Table 1), an exocylic double bond (δ_H 4.42, 4.93, br s; δ_C 106.0 d, 148.0 s), and a carboxylic acid (δ_C 182.0 s). The above features were in agreement with a labdane skeleton containing a β,γ-disubstituted butenolide ring, one carboxylic acid, and one terminal double bond. On the basis of HMBC interactions from H-12 to C-9/C-11/C-13/C-14/C-16, H-14 to C-12/C-15, and H$_3$-16 to C-12/C-13/C-14, it was possible to locate the β-methyl butenolide ring in the side chain. The carboxylic acid group of **2** was placed at C-4β because the H$_3$-20 methyl signal resonated at a higher field (δ 0.59) and no NOE correlation was seen between H$_3$-20 and H$_3$-18 (δ 1.24). A geminal relation between H$_3$-18 and the carboxylic group was revealed from the HMBC correlation between δ 1.24 and δ_C 182.0 (C-19). The olefinic methyl group positioned on C-13 was further established by a two-dimensional (2D) NOESY experiment (NOE correlation H$_3$-16/H-12 and H$_3$-16/H-14). The coupling pattern of H-5 (δ 1.39, dd, J=10.3, 3.5 Hz) as well as Overhauser effect (NOE) correlation to H$_3$-18 but not to H$_3$-20 supported the *trans*-fusion and chair-chair configuration. Based on the above results, the structure of compound **2** was elucidated as 8(17),13-labdadien-12,15-olid-19-oic acid.

Compound **3** was obtained an amorphous substance, and the molecular formula was determined to be $C_{20}H_{30}O_3$ by HR-EI-MS. It showed absorption bands due to an exocylic double bond (3085, 1641, 895 cm^{-1}), and an acid (3300—2700, 1694 cm^{-1}) in the IR spectrum. The ^{1}H-NMR spectrum showed two tertiary methyl groups [δ 0.70, 1.13 (3H each, s)], an olefinic methyl group (δ 1.77, s), a methine proton attached to an oxygen atom [δ 4.11 (1H, dd, J=7.6,

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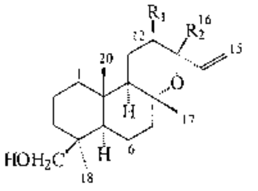
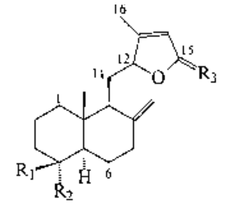
* To whom correspondence should be addressed. e-mail: yhkuo@ccms.ntu.edu.tw

Table 1. ^{13}C-NMR Spectral Data of Compounds **1**—**5** (100 MHz, in $CDCl_3$)

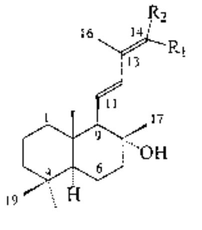
Position	1	2	3	4	5
1	39.5	38.7	37.6	41.0	39.4
2	18.0	19.7	18.3	18.4	18.2
3	36.2	37.8	36.9	41.8	36.2
4	38.4	44.1	47.5	33.3	47.2
5	57.8	55.9	49.2	55.7	48.8
6	19.8	26.0	26.7	20.1	25.6
7	43.5	38.4	37.6	42.7	37.2
8	81.3	148.0	148.5	72.4	149.3
9	60.4	51.7	51.8	66.6	60.5
10	36.2	40.1	38.6	38.4	38.1
11	24.1	28.0	25.0	135.6	125.9
12	81.6	83.4	80.3	138.3	138.8
13	74.5	169.3	135.0	153.6	73.4
14	143.1	116.6	118.3	129.3	144.1
15	113.0	173.1	69.8	191.5	112.2
16	23.5	13.9	19.1	13.3	28.1
17	21.1	106.0	106.7	25.1	108.5
18	27.1	29.0	184.1	33.4	184.1
19	65.4	182.0	16.3	21.6	16.6
20	15.2	12.9	14.9	16.1	15.2



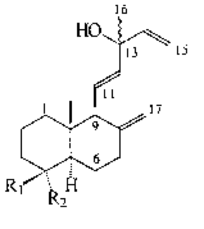
1 R1 = OH, R2 = CH3
6 R1= H, R2 = CH2OH (*ent*-)



R1 R2 R3
2 COOH 18CH3 O
3 CH3 COOH H2



4 R1 = H, R2 = 15CHO
7 R1= 15CH3, R2 = H



5 R1 = CH3, R2 = COOH
8 R1= COOCH3, R2 = CH3

4.0 Hz)], a methylene group located between an oxygen atom and a double bond [δ_H 4.27 (1H, dd, J=16.0, 1.2 Hz), 4.65 (1H, dd, J=16.0, 4.0 Hz)], a trisubstituted double bond [δ_H 5.57 (m)], and a labdane-type exocyclic double bond [δ_H 4.50, 4.84 (each 1H, br s)]. It is apparent from its ^{1}H- and ^{13}C-NMR spectra (Table 1) that compound **3** is closely related to **2**. The ^{13}C-NMR spectrum of **3** lacked the lactone carbonyl signal as in **2** but instead had an oxygenated methylene (δ_C 69.8). The H_3-20 methyl signal of **3** resonating at δ 0.70 (lower field than that in **2**) supported placement of the the carboxylic acid group at C-4α, and the signal at δ 1.13 (3H, s) was assigned to C-4β, attributable to the NOE correlation with H_3-20 and HMBC correlation with the carbonyl (δ 184.1) of the carboxylic group. The pronounced NOESY correlations between H-12 and H-16, H-16 and H-14, and H-14 and H-15 were consistent with a 1,5-dihydrofuran moiety. Therefore the structure of **3** was proposed to be 12,15-epoxy-8(17),13-labdadien-18-oic acid.

HR-EI-MS of **4** indicated the molecular formula $C_{20}H_{32}O_2$, and the IR spectrum showed a conjugated carbonyl group (ν_{max} 1659 cm^{-1}) and a hydroxyl group (3416 cm^{-1}). The existence of 20 carbons with 31 directly attached protons was confirmed from ^{13}C/DEPT experiments, and was consistent with one hydroxyl group. The chemical shifts (C-1 to C-10 and C-17 to C-20) in the ^{13}C-NMR spectrum of **4** showed similarities to those neoabionol (**7**).[6] Therefore the labdane skeleton and the relative stereochemistry around the rigid decalin ring system could be established. The UV absorption band at λ_{max} 268 nm and ^{13}C-NMR data [191.5 (d), 129.3 (d), 153.6 (s), 138.3 (d), 135.6 (d)] indicated an extended a double bond conjugated to α,β-unsaturated aldehyde with β,δ-dialkyl substituents.[7] The ^{1}H-NMR spectrum of **4** indicated the appearance of five singlets of methyl groups (δ 0.80, 0.87, 0.95, 1.23, 2.27), three olefinic protons [δ 5.90 (d, J=8.4 Hz), 6.25—6.29 (2H, overlapping) in $CDCl_3$; δ 5.81 (d, J=8.4 Hz), 6.29 (d, J=15.6 Hz), 6.45 (dd, J=15.6, 10.0 Hz) in acetone-d_6], and one formyl proton (δ 10.09, d, J=8.4 Hz). Comparison of the ^{1}H-NMR spectrum between **4** and **7** concluded that the terminal olefinic methyl group (C-15) of **7** was oxidized to a formyl group. The doublet formyl proton of **4** supported the existence of the formyl group at C-15 and *cis* to H_3-16 from the NOE correlation between them. The lack of a NOE correlation between H-11 and H-12 as well as the coupling constant (J=15.6 Hz) indicated they were *trans*. The structure of **4** was proposed to be 8α-hydroxy-11*E*,13*Z*-labdadien-15-al.

The new labdatriene-type derivative compound **5** was found to have the molecular formula $C_{20}H_{30}O_3$, and its IR spectrum showed hydroxyl (3430 cm^{-1}) and acid carbonyl (1694 cm^{-1}) absorption bands. Its ^{1}H-NMR spectrum contained an ABX system [δ 5.96 (1H, dd, J=17.2, 10.4 Hz, H-14), 5.22 (1H, d, J=17.2 Hz, H-15a), 5.05 (1H, d, J=10.4 Hz, H-15b)], an AB system [δ 5.70 (1H, dd, J=15.6, 9.6 Hz, H-11), 5.58 (1H, d, J=15.6 Hz, H-12)], and a terminal double bond [δ 4.73, 4.47 (1H each, br s, H-17a, H-17b)], which was similar to that of the cryptotrienolic acid methyl ester (**8**).[8] The signals attributed to the side chain portion of **5** in the ^{1}H- and ^{13}C-NMR spectra demonstrated that **5** has both vinylic and 1,2-disubstituted double bonds. The J values of proton signals due to the disubstituted double bond indicated the presence of the C-11, -12 double bond with an *E*-configuration, which is adjacent to the quaternary carbon (C-13) in **5**, and was very similar to those of (11*E*,13*S*, and 11*E*,13*R*)-11,14-labdadiene-8,13-diol.[8] The configuration of the C-13 position was considered to be *R* based on a comparison of the chemical shifts of H-16 and C-16 in **5** with those in (11*E*,13*S*)- and (11*E*,13*R*)-11,14-labdadiene-8,13-diol[9] [δ_H 1.37, δ_C 28.1 for **5**; δ_H 1.41, δ_C 27.2 for (11*E*,13*S*); δ_H 1.39, δ_C 28.5 for (11*E*,13*R*)]. Comparison of the chemical shift of the three methyl singlets of **5** and **8** [δ 1.37 (H_3-16), 1.16 (H_3-19), 0.81 (H_3-20) for **5**; δ 1.39 (H_3-16), 1.20 (H_3-18), 0.62 (H_3-20) for **8**] suggested that the carboxylic acid group is located in an α-equatorial orientation. The relative stereochemistry was also determined by the NOESY method; the H_3-19 exhibited a NOE correlation with H_3-20. HMBC and NOESY methods also confirmed the assigned structure of **5**. Hence compound **5** was assigned to be 13-hydroxy-8(17),11*E*,14-labdatrien-18-oic acid.

Experimental

General Experimental Procedures ^{1}H-, ^{13}C-, and 2D-NMR spectra were obtained on a Varian Unity 400 spectrometer. EI-MS, HR-EI-MS, UV, and specific rotations were recorded on a Finnigan TSQ-46C, JEOL SX-102A, a Hitachi S-3200 spectrometer, and a JASCO DIP-180 digital polarimeter, respectively. Extracts were chromatographed on silica gel (Merck 3374, 70—230 mesh) and purified by a semipreparative normal-phase HPLC column (250×10 mm, 7 μm, LiChrosorb Si 60).

Plant Material The wood of *C. konishii* HAYATA was collected at Luantashan, Nantau Hsien, Taiwan, in December 1996 and was identified by Professor Shao-Shun Ying, Department of Forestry, National Taiwan University. A voucher specimen (013492) has been deposited at the Herbarium of the Department of Botany, National Taiwan University, Taipei, Taiwan.

Extraction and Isolation The wood of *C. konishii* was crushed into pieces to give 6.5 kg (air-dried) of raw material, which was extracted with MeOH (60 l) three times (7 d×3) at room temperature. The combined extracts were evaporated *in vacuo* to give a black residue (60.2 g) that was suspended in water (500 ml) and extracted successively with *n*-hexane (500 ml×3), EtOAc (500 ml×4), and *n*-BuOH (500 ml×3). The EtOAc fraction (15.6 g) was chromatographed on a silica-gel column (450 g) (using hexane–EtOAc and EtOAc–MeOH mixtures as solvent systems). Elution with hexane–EtOAc (3 : 2), (1 : 1), and (2 : 3) gave crude **2**, **3**, and **4**, respectively, while crude compounds **1** and **5** were eluted with hexane–EtOAc (3 : 7). Further purification on HPLC gave **1** (5.5 mg), **2** (6.3 mg), **3** (7.5 mg), **4** (6.1 mg), and **5** (8.2 mg) using hexane–EtOAc–CH_2Cl_2–*i*-PrOH (3 : 1 : 1 : 0.2), acetone–CH_2Cl_2–*i*-PrOH (1 : 10 : 0.2), acetone–CH_2Cl_2–*i*-PrOH (1 : 6 : 0.2), hexane–EtOAc–CH_2Cl_2–*i*-PrOH (3 : 1 : 2 : 0.2), and acetone–CH_2Cl_2–*i*-PrOH (1 : 5 : 0.2), respectively.

12β,19-Dihydroxymanoyl Oxide (**1**): Amorphous; $[\alpha]_D^{26}$ +3.1° (c=0.21, $CHCl_3$), IR ν_{max} cm^{-1}: 3385 (OH), 3072 (C–H, vinyl), 1646 (C=C), 1452, 994, 923. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 5.93 (1H, dd, J=17.6, 10.8 Hz, H-14), 5.28 (1H, dd, J=17.6, 1.6 Hz, H-15a), 5.11 (1H, dd, J=10.8, 1.6 Hz, H-15b), 3.92 (1H, dd, J=8.0, 4.0 Hz, H-12), 3.67 (1H, d, J=10.8 Hz, H-19a), 3.48 (1H, d, J=10.8 Hz, H-19b), 1.20 (3H, s, H-16), 1.11 (3H, s, H-17), 0.96 (3H, s, H-18), 0.80 (3H, s, H-20); ^{13}C-NMR, see Table 1; EI-MS (70 eV) (rel. int. %) m/z: 322 (M^+, 2), 307 (5), 304 (7), 298 (10), 279 (13), 251 (100); HR-EI-MS m/z 322.2511 (Calcd for $C_{20}H_{34}O_3$, 322.2508).

8(17),13-Labdadien-12,15-olid-19-oic Acid (**2**): Amorphous; $[\alpha]_D^{27}$ +8.1° (c=0.32, $CHCl_3$); UV λ_{max}^{MeOH} (log ε): 210 nm (4.01); IR ν_{max} cm^{-1}: 3300—2700 (OH), 3078 (C–H, vinyl), 1757 (C=O, conjugated γ-lactone), 1692 (C=O, acid), 1641 (C=C), 1383, 1177, 1035. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 5.75 (1H, br s, H-14), 4.93 (1H, br s, H-17a), 4.85 (1H, d, J=11.6 Hz, H-12), 4.42 (1H, br s, H-17b), 2.08 (3H, s, H-16), 1.24 (3H, s, H-18), 0.59 (3H, s, H-20); ^{13}C-NMR, see Table 1; EI-MS (70 eV) (rel. int. %) m/z: 332 (M^+, 100), 316 (37), 300 (12), 288 (38), 286 (47), 273 (27), 271 (22), 264 (12); HR-EI-MS m/z 332.1980 (Calcd for $C_{20}H_{28}O_4$, 332.1988).

12,15-Epoxy-8(17),13-labdadien-18-oic Acid (**3**): Amorphous; $[\alpha]_D^{27}$ +37.6° (c=0.19, $CHCl_3$); IR ν_{max} cm^{-1}: 3300—2700 (OH), 3085, 1641 (C–H, olefine), 1694 (C=O, acid), 1447, 1383, 1286. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 5.57 (1H, m, H-14), 4.84 (1H, br s, H-17a), 4.65 (1H, dd, J=16.0, 4.0 Hz, H-15a), 4.50 (1H, br s, H-17b), 4.27 (1H, dd, J=16.0, 1.2 Hz, H-15b), 4.11 (1H, dd, J=7.6, 4.0 Hz, H-12), 1.77 (3H, s, H-16), 1.13 (3H, s, H-19), 0.70 (3H, s, H-20); ^{13}C-NMR, see Table 1; EI-MS (70 eV) (rel. int. %) m/z: 318 (M^+, 18), 316 (100), 314 (15), 300 (19), 290 (16), 288 (19), 285 (17), 275 (14), 272 (29), 270 (14), 252 (14); HR-EI-MS m/z 318.2199 (Calcd for $C_{20}H_{30}O_3$, 318.2196).

8α-Hydroxy-11*E*,13*Z*-labdadiene-15-al (**4**): Pale yellow oil; $[\alpha]_D^{26}$ +87.5° (c=0.20, $CHCl_3$); UV λ_{max}^{MeOH} (log ε) nm: 268 (4.35); IR ν_{max} cm^{-1}: 3416 (OH), 3085 (C–H, vinyl), 1659 (C=O, conjugated), 1620 (C=C), 1460, 1387, 976. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 10.09 (1H, d, J=8.4 Hz, H-15), 6.25—6.29 (2H, overlapping, H-11, -12), 5.90 (1H, d, J=8.4 Hz, H-14), 2.27 (3H, s, H-16), 1.96 (1H, d, J=10.2 Hz, H-9), 1.23 (3H, s, H-17), 0.95 (3H, s, H-20), 0.87 (3H, s, H-18), 0.80 (3H, s, H-19); ^{1}H-NMR (acetone-d_6, 400 MHz) δ: 10.12 (1H, d, J=8.0 Hz, H-15), 6.45 (1H, dd, J=15.6, 10.0 Hz, H-11), 6.29 (1H, d, J=15.6 Hz, H-12), 5.81 (1H, d, J=8.0 Hz, H-14), 2.33 (3H, s, H-16), 1.95 (1H, d, J=10.0 Hz, H-9), 1.23 (3H, s, H-17), 1.00 (3H, s, H-20), 0.88 (3H, s, H-18), 0.83 (3H, s, H-19); ^{13}C-NMR, see Table 1; EI-MS (70 eV) (rel. int. %) m/z: 304 (M^+, 100), 287 (57), 273 (54), 259 (52), 245 (43), 231 (49); HR-EI-MS m/z 304.2401 (Calcd for $C_{20}H_{32}O_2$, 304.2404).

(13*R*)-13-Hydroxy-8(17),11*E*,14-labdatrien-18-oic Acid (**5**): Amorphous. $[\alpha]^{26}{}_D$ +51.7° (c=0.17, $CHCl_3$); IR (dry film) ν_{max} 3430 (OH), 3078 (C–H, vinyl), 1694 (C=O, acid), 1643 (C=C), 1461, 1385, 973, 892 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 5.96 (1H, dd, J=17.2, 10.4 Hz, H-14), 5.70 (1H, dd, J=15.6, 9.6 Hz, H-11), 5.58 (1H, d, J=15.6 Hz, H-12), 5.22 (1H, d, J=17.2 Hz, H-15a), 5.05 (1H, d, J=10.4 Hz, H-15b), 4.73 (1H, br s, H-17a), 4.47 (1H, br s, H-17b), 2.37 (1H, d, J=9.6 Hz, H-9), 1.37 (3H, s, H-16), 1.16 (3H, s, H-19), 0.81 (3H, s, H-20); ^{13}C-NMR, see Table 1; EI-MS (70 eV) (rel. int. %) m/z: 318 (M^+, 20), 303 (18), 300 (100), 290 (40), 284 (36), 272 (38); HR-EI-MS m/z 318.2203 (Calcd for $C_{20}H_{30}O_3$, 318.2196).

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