

## Diterpenoids from the Wood of *Cunninghamia konishii*

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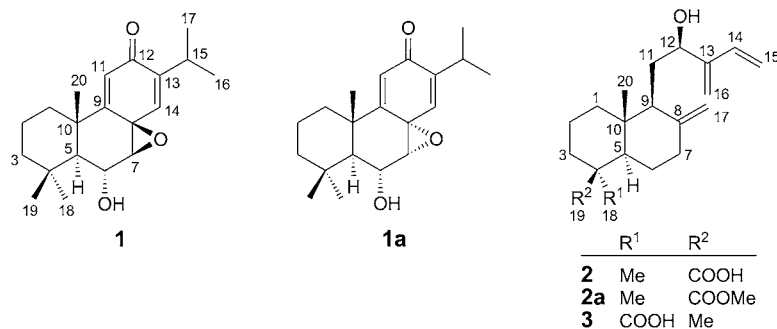
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Investigation of the wood of *Cunninghamia konishii* resulted in the isolation and characterization of one new abietane diterpenoid, (6 $\alpha$ ,7 $\beta$ )-7,8-epoxy-6-hydroxyabieta-9(11),13-dien-12-one (**1**), and two new labdane diterpenoids, (12*R*)-12-hydroxylabda-8(17),13(16),14-trien-19-oic acid (**2**) and (12*R*)-12-hydroxylabda-8(17),13(16),14-trien-18-oic acid (**3**). The structures of these new compounds were elucidated by analysis of their spectroscopic data.

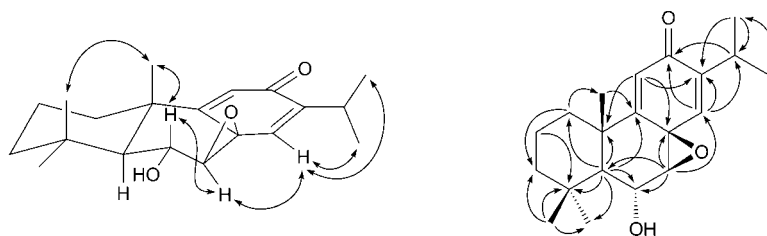
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**Introduction.** – *Cunninghamia konishii* HAYATA (Taxodiaceae), one of the two *Cunninghamia* species occurring in eastern Asia, is an endemic coniferous tree distributed in the northern and central part of Taiwan at altitudes of 1300–2700 m [1]. The wood of this tree is one of the best building materials available in Taiwan. The chemical constituents of wood [2–10], bark [11], leaf [8], and whole plant [12] of *C. konishii* have been reported. Some isolates of this plant exhibited antifungal [8][9], anti-inflammatory [10], and cytotoxic activities [12]. In connection with our interest in the chemical composition of this plant, a further chemical study on the same AcOEt-soluble fraction led to the isolation of three new diterpenoids, (6 $\alpha$ ,7 $\beta$ )-7,8-epoxy-6-hydroxyabieta-9(11),13-dien-12-one (**1**), (12*R*)-12-hydroxylabda-8(17),13(16),14-trien-19-oic acid (**2**), and (12*R*)-12-hydroxylabda-8(17),13(16),14-trien-18-oic acid (**3**; Fig. 1). The isolation and structure elucidation of these new compounds are described herein.

**Results and Discussion.** – Compound **1** was isolated as a yellowish oil. Its molecular formula, C<sub>20</sub>H<sub>28</sub>O<sub>3</sub>, was determined by HR-EI-MS (*M*<sup>+</sup>, 316.2031). The presence of a cross-conjugated dienone was evidenced by the UV maxima at 268 and 234 nm, and through IR (3098, 1660, 1628, and 1601 cm<sup>-1</sup>), and <sup>1</sup>H- ( $\delta$ (H) 5.97 (s), 6.30 (s)), and

Fig. 1. Structures of compounds **1**–**3**

<sup>13</sup>C-NMR data (Table;  $\delta$ (C) 186.9 (C=O), 161.3, 150.0, 136.8, 128.7). In addition, the <sup>1</sup>H-NMR spectrum (Table) exhibited three Me *singlets* ( $\delta$ (H) 1.07 (*s*, Me(18)), 1.09 (*s*, Me(19)), 1.18 (*s*, Me(20))), and signals of an <sup>i</sup>Pr group ( $\delta$ (H) 1.04 (*d*,  $J = 6.8$ , Me(16)), 1.07 (*d*,  $J = 6.8$ , Me(17)), 2.93 (*sept.*,  $J = 6.8$ , H–C(15))) attached to a C=C bond. Further data from the COSY, NOESY (Fig. 2), DEPT, HMQC, and HMBC (Fig. 2) experiments indicated that the structure of **1** was similar to that of 7 $\alpha$ ,8 $\alpha$ -epoxy-6 $\alpha$ -hydroxyabieta-9(11),13-dien-12-one (**1a**) [13]. The NOESY (Fig. 2) correlations Me(20)/H–C(6), H–C(6)/H–C(7), and H–C(7)/H–C(14) indicated H–C(6) in  $\beta$ -orientation for H–C(6). By using MM2 theoretical calculations, with epoxy ring in  $\beta$ -orientation, the most stable conformation has the angles H–C(5)–C(6)–H and H–C(6)–C(7)–H of 174.5° and 88.0°, respectively. The results agreed with the coupling constant of H–C(5) and H–C(6) ( $J = 10.4$ ), and no coupling was observed between H–C(6) and H–C(7). Comparing the optical rotations of **1** and **1a** ( $[\alpha]_D^{25}$  of +58.6 and –42.5, resp.) and on the basis of the above evidence, the structure of **1** was elucidated as (6 $\alpha$ ,7 $\beta$ )-7,8-epoxy-6-hydroxyabieta-9(11),13-dien-12-one, which was further confirmed by COSY, NOESY (Fig. 2), <sup>13</sup>C-NMR, DEPT, HMQC, and HMBC (Fig. 2) experiments.

Fig. 2. Key NOESY (H ↔ H) and HMB correlations (H → C) of **1**

Compound **2** was isolated as a yellowish oil. Its molecular formula, C<sub>20</sub>H<sub>30</sub>O<sub>3</sub>, was determined by HR-EI-MS ( $M^+$ , 318.2192). The absorption bands (3401, 3300–2500, 1692, 1647, 988, and 892 cm<sup>–1</sup>) in the IR spectrum indicated the presence of OH,

Table.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR Data ( $\text{CDCl}_3$ ) of Compounds **1**–**3**.  $\delta$  in ppm,  $J$  in Hz.

Position	<b>1</b>		<b>1a</b> [13]		<b>2</b>		<b>3</b>	
	$\delta(\text{H})$	$\delta(\text{C})$	$\delta(\text{H})$	$\delta(\text{C})$	$\delta(\text{H})$	$\delta(\text{C})$	$\delta(\text{H})$	$\delta(\text{C})$
1	1.35–1.43 (m), 1.90–1.94 (m)	36.8	1.38–1.45 (m), 1.68–1.73 (m)	38.1	1.16–1.20 (m), 1.73–1.81 (m)	37.9	1.21–1.25 (m), 2.02–2.30 (m)	37.0
2	1.46–1.50 (m)	18.5	1.55–1.59 (m), 1.68–1.73 (m)	18.1	1.50–1.54 (m), 1.73–1.81 (m)	19.8	1.52–1.74 (m)	18.4
3	1.18–1.22 (m), 1.35–1.43 (m)	43.0	1.21–1.25 (m), 1.38–1.45 (m)	43.2	1.02–1.06 (m), 2.00–2.07 (m)	39.0	1.52–1.74 (m)	37.9
4	–	33.0	–	34.7	–	44.2	–	47.5
5	1.74 (d, $J=10.4$ )	45.2	1.21 (d, $J=9.0$ )	58.2	1.36–1.40 (m)	56.2	2.02–2.30 (m)	52.4
6	4.20 (d, $J=10.4$ )	69.4	4.40 (d, $J=9.0$ )	66.4	1.73–1.81 (m), 1.93–1.97 (m)	26.1	1.38–1.51 (m)	26.9
7	3.81 (s)	65.9	3.72 (s)	70.9	1.87–1.91 (m), 2.39–2.43 (m)	38.7	1.78–1.82 (m), 2.36–2.40 (m)	37.8
8	–	57.5	–	56.2	–	148.7	–	148.5
9	–	161.3	–	162.5	2.00–2.07 (m)	51.8	2.02–2.30 (m)	49.4
10	–	41.3	–	38.0	–	40.1	–	38.6
11	6.30 (s)	128.7	6.30 (s)	126.2	1.63–1.67 (m)	31.3	1.52–1.74 (m)	30.9
12	–	186.9	–	187.1	4.38 (t, $J=6.0$ )	69.9	4.39 (t, $J=6.0$ )	69.9
13	–	150.0	–	149.5	–	150.5	–	150.4
14	5.97 (s)	136.8	6.03 (s)	138.6	6.30 (dd, $J=17.8, 11.2$ )	136.0	6.31 (dd, $J=17.8, 11.2$ )	136.0
15	2.93 (sept., $J=6.8$ )	26.4	2.96 (sept., $J=7.0$ )	26.3	5.09 (d, $J=11.2$ ), 5.39 (d, $J=17.8$ )	114.7	5.09 (d, $J=11.2$ ), 5.40 (d, $J=17.8$ )	114.8
16 <sup>a)</sup>	1.04 (d, $J=6.8$ )	21.4	1.06 (d, $J=7.0$ )	21.3	5.11, 5.19 (2 br. s)	113.3	5.12, 5.20 (2 br. s)	113.4
17 <sup>a)</sup>	1.07 (d, $J=6.8$ )	21.6	1.08 (d, $J=7.0$ )	21.3	4.51, 4.86 (2 br. s)	106.6	4.52, 4.85 (2 br. s)	107.1
18	1.07 (s)	25.9	1.07 (s)	35.3	1.22 (s)	29.0	–	184.4
19	1.09 (s)	22.9	1.07 (s)	22.4	–	183.3	1.13 (s)	16.3
20	1.18 (s)	36.6	1.19 (s)	21.6	0.57 (s)	12.9	0.68 (s)	14.8

<sup>a)</sup> Exchangeable.

COOH and vinyl groups and, terminal C=C bonds. The  $^{13}\text{C}$ -NMR spectrum (*Table*) of **2** was similar to that of (12*R*)-12-hydroxylabda-8(17),13(16),14-trien-19-oic acid methyl ester (**2a**) [14], except that the signal of a COOMe group ( $\delta(\text{C})$  51.1, 177.7) in **2a** was replaced with that of a COOH group ( $\delta(\text{C})$  183.3) in **2**. The  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra (*Table*) revealed the presence of two tertiary Me and, six  $\text{CH}_2$  groups, a HOCH group ( $\delta(\text{H})$  4.38 (*t*,  $J = 6.0$ );  $\delta(\text{C})$  69.9), three terminal  $\text{sp}^2$ - $\text{CH}_2$  groups ( $\delta(\text{H})$  4.51 (*br. s*), 4.86 (*br. s*);  $\delta(\text{C})$  106.6, 148.7;  $\delta(\text{H})$  5.11 (*br. s*), 5.19 (*br. s*);  $\delta(\text{C})$  113.3, 150.5;  $\delta(\text{H})$  5.09 (*d*,  $J = 11.2$ ), 5.39 (*d*,  $J = 17.8$ ), 6.30 (*dd*,  $J = 17.8, 11.2$ );  $\delta(\text{C})$  114.7, 136.0), and two  $\text{sp}^3$  quaternary C-atoms. The NOESY (*Fig. 3*) correlations Me(18)/H-C(5) and H-C(5)/H-C(9) supported  $\alpha$ -orientation for Me(18) and H-C(9), and  $\beta$ -orientation for COOH(19). The secondary OH group was attached at C(12) based on the cross-peaks between C(12) and H-C(9),  $\text{CH}_2(11)$ , H-C(14), and  $\text{CH}_2(16)$  in the HMBC spectrum (*Fig. 3*) and the COSY correlation  $\text{CH}_2(11)/\text{H-C}(12)$ . Based on the chemical shifts of C(12) of (12*R*)-12-hydroxylabda-8(17),13(16),14-trien-19-oic acid methyl ester ( $\delta(\text{C})$  69.8) and (12*S*)-12-hydroxylabda-8(17),13(16),14-trien-19-oic acid methyl ester ( $\delta(\text{C})$  72.2) [14], and of C(12) of **2** ( $\delta(\text{C})$  69.9) indicated also (*R*)-configuration. Therefore, compound **2** was characterized as (12*R*)-12-hydroxylabda-8(17),13(16),14-trien-19-oic acid, which was further confirmed by COSY, NOESY (*Fig. 3*), DEPT, HMQC, and HMBC (*Fig. 3*) experiments.

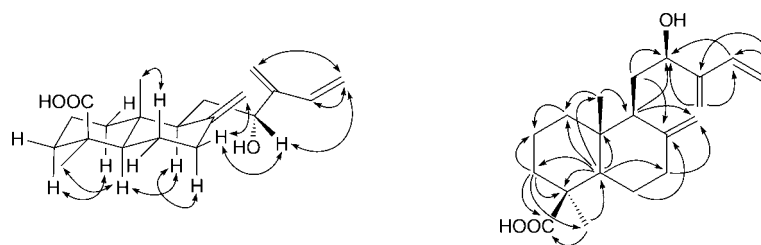


Fig. 3. Key NOESY ( $\text{H} \leftrightarrow \text{H}$ ) and HMB correlations ( $\text{H} \rightarrow \text{C}$ ) of **2**

Compound **3** was isolated as a yellowish oil. Its molecular formula,  $\text{C}_{20}\text{H}_{30}\text{O}_3$ , was determined by HR-EI-MS ( $M^+$ , 318.2205). The spectra of **3** were similar to those of **2**, and **3** was found to be a diastereoisomer of **2**. The complete  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR signal assignments are compiled in the *Table*. The NOESY (*Fig. 4*) correlations Me(19)/Me(20), Me(20)/ $\text{CH}_2(11)$ , and H-C(5)/H-C(9) evidenced  $\beta$ -orientation for Me(19) and  $\alpha$ -orientation H-C(9). The chemical shift of H-C(5) ( $\delta(\text{H})$  2.02–2.30) at lower field than that of the corresponding H-C(5) in **2** indicated that it was deshielded by C(18)OOH and further supported the  $\alpha$ -orientation for C(18)OOH. The chemical shift of C(12) at  $\delta(\text{C})$  69.9 indicated (*R*)-configuration at C(12) of **3** [14]. Based on the above evidence, compound **3** was identified as (12*R*)-12-hydroxylabda-8(17),13(16),14-trien-18-oic acid, which was further confirmed by COSY, NOESY (*Fig. 4*), DEPT, HMQC, and HMBC (*Fig. 4*) experiments.

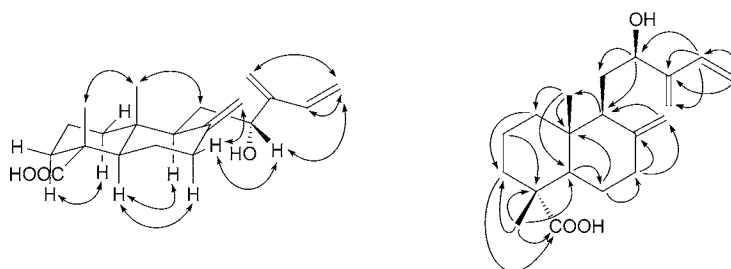


Fig. 4. Key NOESY ( $H \leftrightarrow H$ ) and HMB correlations ( $H \rightarrow C$ ) of **3**

### Experimental Part

**General.** TLC: Silica gel 60  $F_{254}$  precoated plates ( $\text{SiO}_2$ ; Merck). Column chromatography (CC):  $\text{SiO}_2$ , 60 (Merck 70–230 mesh, ASTM). HPLC: LDC Analytical-III system; column: LiChrosorb Si 60, 7  $\mu\text{m}$ , 250  $\times$  10 mm. Optical rotation: Jasco-DIP-180 polarimeter; in  $\text{CHCl}_3$ . UV Spectra: Hitachi-S-3200 spectrophotometer;  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) in nm. IR Spectra: Perkin-Elmer-983G FT-IR spectrophotometer;  $\tilde{\nu}$  in  $\text{cm}^{-1}$ .  $^1\text{H}$ -,  $^{13}\text{C}$ -, and 2D-NMR Spectra: Varian-Unity-Plus-400 spectrometers;  $\delta$  in ppm rel. to  $\text{Me}_4\text{Si}$  as internal standard,  $J$  in Hz. EI- and HR-EI-MS: Jeol-JMS-HX300 mass spectrometer;  $m/z$  (rel. %).

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

**Extraction and Isolation.** Dried wood (6.5 kg) of *C. konishii* was crushed into pieces and extracted with MeOH (60 l) three times (7 d each time) at r.t. After evaporation, the extract (60.2 g) was suspended in  $\text{H}_2\text{O}$  (500 ml) and partitioned into hexane (500 ml  $\times$  3), AcOEt (500 ml  $\times$  4), and BuOH (500 ml  $\times$  3), successively. The AcOEt fraction (15.6 g) was submitted to CC ( $\text{SiO}_2$ , hexane/AcOEt and AcOEt/MeOH). Elution with hexane/AcOEt 9:1, 3:2, and 2:3 gave crude **1**, **2**, and **3**, resp. Further purification by HPLC (hexane/ $\text{CH}_2\text{Cl}_2$ /AcOEt/ $i$ PrOH 7:3:1:0.2, 10:3:1:0.2, and 4:2:1:0.2) gave **1** (2.7 mg), **2** (3.7 mg), and **3** (3.5 mg), resp.

(6 $\alpha$ ,7 $\beta$ )-7,8-Epoxy-6-hydroxyabieta-9(11),13-dien-12-one (= (4 $\alpha$ R,5 $\alpha$ S,6R,6 $\alpha$ S,10 $\alpha$ S)-5 $\alpha$ ,6,6 $\alpha$ ,7,8,9,10,10 $\alpha$ -Octahydro-6-hydroxy-7,7,10 $\alpha$ -trimethyl-3-(1-methylethyl)-2H-phenanthro[8 $\alpha$ ,9-b]oxiren-2-one; **1**). Yellowish oil.  $[\alpha]_{\text{D}}^{25} = +58.6$  ( $c = 0.25$ ,  $\text{CHCl}_3$ ). UV (MeOH): 268 (3.95), 234 (sh, 4.15). IR (neat): 3418 (OH), 3098, 1660 (conjugated C=O), 1628, 1601 (C=C), 1256, 1048.  $^1\text{H}$ - ( $\text{CDCl}_3$ , 400 MHz) and  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ , 100 MHz): see the Table. EI-MS: 316 (100,  $M^+$ ), 301 (45), 287 (68), 271 (22), 255 (13), 245 (11). HR-EI-MS: 316.2031 ( $M^+$ ,  $\text{C}_{20}\text{H}_{28}\text{O}_3^+$ ; calc. 316.2039).

(12R)-12-Hydroxyabda-8(17),13(16),14-trien-19-oic Acid (= (1S,4 $\alpha$ R,5S,8 $\alpha$ R)-Decahydro-5-[(2R)-2-hydroxy-3-methylidenepent-4-en-1-yl]-1,4a-dimethyl-6-methylidenenaphthalene-1-carboxylic Acid; **2**). Yellowish oil.  $[\alpha]_{\text{D}}^{27} = +61.3$  ( $c = 0.34$ ,  $\text{CHCl}_3$ ). UV (MeOH): 228 (4.34). IR (neat): 3401 (OH), 3300–2500 and 1692 (COOH), 3079, 1647 (C=C), 1170, 1067, and 988 and 892 ( $-\text{CH}=\text{CH}_2$  and  $>\text{C}=\text{CH}_2$ ).  $^1\text{H}$ - ( $\text{CDCl}_3$ , 400 MHz) and  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ , 100 MHz): see the Table. EI-MS: 318 (62,  $M^+$ ), 304 (60), 300 (79), 290 (9), 285 (39), 279 (100), 271 (28), 255 (23), 246 (62). HR-EI-MS: 318.2192 ( $M^+$ ,  $\text{C}_{20}\text{H}_{30}\text{O}_3^+$ ; calc. 318.2196).

(12R)-12-Hydroxyabda-8(17),13(16),14-trien-18-oic Acid (= (1R,4 $\alpha$ R,5S,8 $\alpha$ R)-Decahydro-5-[(2R)-2-hydroxy-3-methylidenepent-4-en-1-yl]-1,4a-dimethyl-6-methylidenenaphthalene-1-carboxylic Acid; **3**). Yellowish oil.  $[\alpha]_{\text{D}}^{27} = +43.9$  ( $c = 0.32$ ,  $\text{CHCl}_3$ ). UV (MeOH): 221 (4.36). IR (neat): 3413 (OH), 3300–2500 and 1692 (COOH), 3084, 1640 (C=C), 1383, 1170, 1034, and 990 and 893 ( $-\text{CH}=\text{CH}_2$  and  $>\text{C}=\text{CH}_2$ ).  $^1\text{H}$ - ( $\text{CDCl}_3$ , 400 MHz) and  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ , 100 MHz): see the Table. EI-MS: 318 (96,  $M^+$ ), 304 (39), 300 (100), 290 (39), 285 (51), 272 (62), 257 (17), 252 (52). HR-EI-MS: 318.2205 ( $M^+$ ,  $\text{C}_{20}\text{H}_{30}\text{O}_3^+$ ; calc. 318.2196).

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