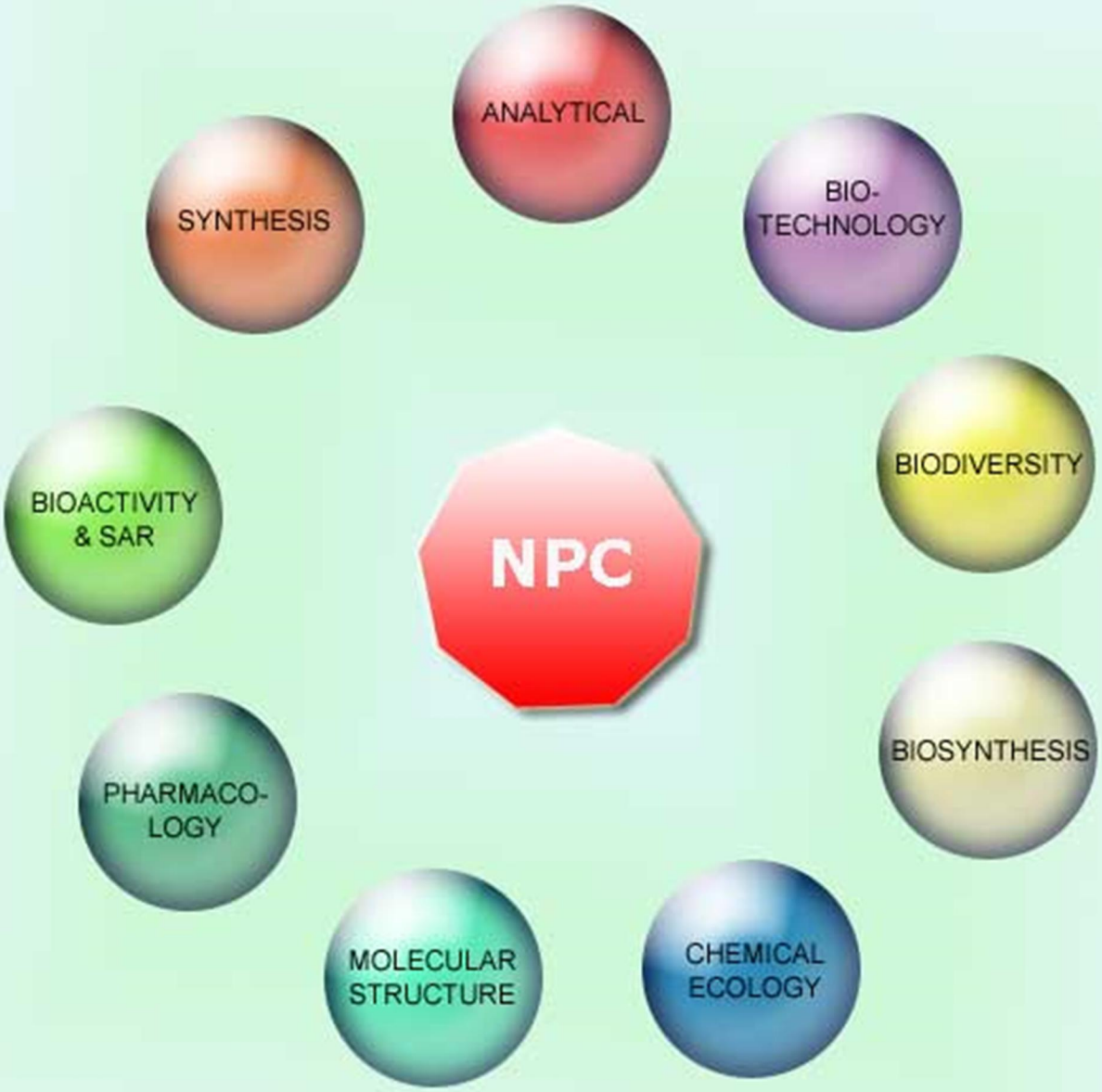
NATURAL PRODUCT COMMUNICATIONS

An International Journal for Communications and Reviews Covering all Aspects of Natural Products Research



This Issue is Dedicated to
Professor Josep Coll Toledano
On the Occasion of his 70th Birthday

Volume 9. Issue 8. Pages 1059-1222. 2014
ISSN 1934-578X (printed); ISSN 1555-9475 (online)
www.naturalproduct.us

NPC Natural Product Communications

INFORMATION FOR AUTHORS

Full details of how to submit a manuscript for publication in Natural Product Communications are given in Information for Authors on our Web site http://www.naturalproduct.us.

To Subscribe: Natural Product Communications is a journal published monthly. 2014 subscription price: US$2,395 (Print, ISSN# 1934-578X); US$2,395 (Web edition, ISSN# 1555-9475); US$2,795 (Print + single site online); US$595 (Personal online). Orders should be addressed to Subscription Department, Natural Product Communications, Natural Product Inc., 7963 Anderson Park Lane, Westerville, Ohio 43081, USA. Subscriptions are renewed on an annual basis. Claims for nonreceipt of issues will be honored if made within three months of publication of the issue. All issues are dispatched by airmail throughout the world, excluding the USA and Canada.

NPC Natural Product Communications

2014
Vol. 9
No. 8
1127 - 1128

Two New Labdane-type Diterpenes from the Wood of *Cunninghamia konishii*

Chi-I Chang[a], Yen-Cheng Li[b], Che-Yi Chao[c], Sheng-Yang Wang[d], Hsun-Shuo Chang[e], Louis Kuoping Chao[f], Chang Syun Yang[g] and Yueh-Hsiung Kuo[g,h,*]

[a]*Department of Biological Science and Technology, National Pingtung University of Science and Technology, Pingtung 912, Taiwan*
[b]*Department of Chemistry, National Taiwan University, Taipei 106, Taiwan*
[c]*Department of Health and Nutrition Biotechnology, Asia University, Taichung 413, Taiwan*
[d]*Department of Forestry, National Chung-Hsing University, Taichung 402, Taiwan*
[e]*Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung 807, Taiwan*
[f]*Department of Cosmeceutics, China Medical University, Taichung 404, Taiwan*
[g]*Department of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, College of Pharmacy, China Medical University, Taichung 404, Taiwan*
[h]*Department of Biotechnology, Asia University, Taichung 413, Taiwan*

[*]*kuoyh@mail.cmu.edu.tw*

Received: May 31st, 2014; Accepted: June 19th, 2014

Phytochemical investigation of the methanol extract of the wood of *Cunninghamia konishii* resulted in the isolation of two new acidic labdane-type diterpenoids, 12(*S*)-hydroxy-15,16-epoxylabda-8(17),13-dien-19-oic acid (**1**) and 12(*S*)-hydroxy-15,16-epoxylabda-8(17),13-dien-18-oic acid (**2**), along with one known labdane-type diterpene, 7,13*E*-labdadien-15-ol (**3**). Their structures were determined by analysis of spectroscopic data and comparison with the data of known analogues.

Keywords: Chinese herb, Taxodiaceae, *Cunninghamia konishii*, Labdane, Diterpenoid.

Two *Cunninghamia* species (Taxodiaceae) grow in eastern Asia, one of which is *C. konishii* Hayata, an endemic coniferous tree distributed in the northern and central part of Taiwan [1]. The wood of this plant exhibits soft, lightweight, aromatic, and rot-resistant properties and is thus one of the best building materials. In earlier investigations, monoterpenes, sesquiterpenes, diterpenes, and lignans were isolated from the wood, bark, leaf, and whole plant of *C. konishii* [2a-m]. Several isolates of this plant have been proven to possess anti-inflammatory [2i] and antifungal activity [2g-i], and cytotoxicity [2k]. In the continuing phytochemical investigation [2e,f,i,l,m], we further identified two new acidic labdane-type diterpenoids (**1** and **2**) (Figure 1) and one known labdane-type diterpene, 7,13*E*-labdadien-15-ol (**3**) [3a] from the wood of *C. konishii*.

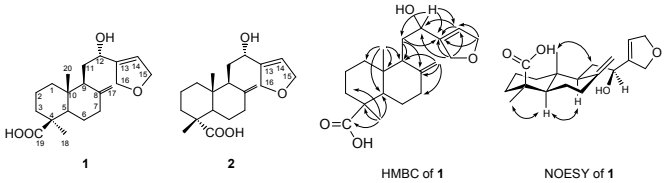


Figure 1: Structures of compounds **1** and **2** and selected NOE correlations of **1**.

Table 1: ^{1}H and ^{13}C NMR data for 1 and 2 (400 and 100 MHz in $CDCl_3$).

	1		**2**	
No.	δ_C	δ_H	δ_C	δ_H
1	39.1 (t)	1.81 (m), 1.15 (m)	37.9 (t)	1.71 (m), 1.16 (m)
2	19.8 (t)	1.72 (m), 1.56 (m)	18.4 (t)	1.63 (m)
3	37.8 (t)	2.10 (m), 1.13 (m)	36.9 (t)	2.02 (m), 1.63 (m)
4	44.1 (s)		47.4 (s)	
5	56.1 (d)	1.34 (dd, 10.4, 4.8)	49.5 (d))	1.93 (dd, 10.2, 3.5)
6	26.0 (t)	2.01 (m), 1.37 (m)	26.7 (t)	1.50 (m), 1.39 (m)
7	38.6 (t)	2.38 (m), 2.15 (m)	37.8 (t)	2.38 (m), 2.05 (m)
8	148.2 (s)		148.0 (s)	
9	52.4 (d)	1.56 (t,7.6)	52.9 (d)	1.76 (m)
10	40.3 (s)		38.8 (s)	
11	29.1 (t)	1.78 (m)	28.7 (t)	1.72 (m)
12	73.1 (d)	4.28 (dd, 9.2, 5.6)	73.0 (d)	4.27 (dd, 9.2, 4.6)
13	137.8 (s)		137.7 (s)	
14	120.6 (d)	5.80 (brs)	120.8 (d)	5.80 (brs)
15	69.7 (t)	4.63 (d, 13.4), 4.51 (d, 13.4)	69.7 (t)	4.63 (d, 13.6), 4.52 (d, 13.6)
16	69.0 (t)	4.65 (d, 13.6) 4.63 (d, 13.6)	68.9 (t)	4.65 (d, 13.6) 4.63 (d, 13.6)
17	107.0 (t)	4.90 (brs), 4.65 (brs)	107.6 (t)	4.88 (brs), 4.67 (brs)
18	28.9 (q)	1.22 (s)	183.4 (s)	
19	181.5 (s)		16.4 (q)	1.14 (s)
20	12.9 (q)	0.61 (s)	14.8 (q)	0.71 (s)

[a)] Coupling constants are presented in Hz.

Compound **1**, yellowish oil, showed IR absorption bands for a carboxylic acid, hydroxy and vinyl groups, and terminal double bonds at 3425−2520, 3394, 3078, 1692, 1647 and 895 cm^{-1}. The resonances for a trisubstituted double bond (δ_H 5.80 (1H, brs); δ_C 120.6, 137.8), a carbinol (HOCH) group (δ_H 4.28 (1H, dd, J = 9.2, 5.6 Hz); δ_C 73.1), two oxymethylenes (δ_H 4.51, 4.63 (each 1H, d, J = 13.4 Hz), 4.63, 4.65 (each 1H, d, J = 13.6 Hz); δ_C 69.7, 69.0), one terminal double bond (δ_H 4.65 (1H, brs), 4.90 (1H, brs)), and two tertiary methyls (δ_H 0.61, 1.22 (each 3H, s)) were observed in the ^{1}H and ^{13}C NMR spectra (Table 1). A DEPT experiment was used to differentiate 20 carbon signals as two methyl, six aliphatic methylene, two aliphatic methine, two aliphatic quaternary, two oxygenated methylene, one oxygenated methine, one olefinic methylene, one olefinic methine, two quaternary olefinic, and one carbonyl. From the above spectroscopic characteristics, compound **1** was tentatively proposed to be a labdane diterpenoid with a C_6-side chain moiety on C-9. The molecular formula was deduced to be $C_{20}H_{30}O_4$ from a molecular ion at *m/z* 334.2152 in the HR-EI-MS, which indicated six degrees of unsaturation. Five of these were attributed to the presence of a bicyclic structure, two olefins, and one C=O group, and the remaining degree of unsaturation hinted that the C_6-side chain contains one additional cyclic structure. The

HMBC correlations (Figure 1) between H-14 (δ_H 5.80)/C-12 (δ_C 73.1) and C-13 (δ_C 137.8); H-15 (δ_H 4.51)/C-13 and C-14; H-16 (δ_H 4.65)/C-13 and C-14 aided the construction of the structure of a 3-substituted 2,5-dihydrofuran in the side chain. An α-orientated hydroxy group was positioned at C-12, which was confirmed by the HMBC correlations (Figure 1) between H-12 (δ_H 4.28)/C-9 (δ_C 52.4), C-11 (δ_C 29.1), C-13 (δ_C 137.8), C-14 (δ_C 120.6), and C-16 (δ_C 69.0) and the comparison of the chemical shifts of C-12 of (12*R*)-12-hydroxylabda-8(17),13(16),14-trien-19-oic acid methyl ester (δ_C 69.8) and (12*S*)-12-hydroxylabda-8(17),13(16),14-trien-19-oic acid methyl ester (δ_C 72.2) [3b]. The significant NOE correlations between H-5 (δ_H 1.34)/H-9 (δ_H 1.56); H-5/Me-18 (δ_H 1.22); and H-11 (δ_H 1.78)/Me-20 (δ_H 0.61) in the NOESY spectrum (Figure 1) indicated an α-orientation for H-5, H-9, and Me-18 and a β-orientation for H-11 and Me-20. Compound **1** showed a positive specific rotation, +21.7, consistent with that of (12*S*)-12-hydroxylabda-8(17),13(16),14-trien-19-oic acid methyl ester ([α] = +41) and was thus identified as a labdane derivative with the 12*S* configuration [4]. Accordingly, compound **1** was elucidated as (12*S*)-12-hydroxy-15,16-epoxylabda-8(17),13-dien-19-oic acid (**1**).

Compound **2** was also obtained as yellowish oil. Its HR-EI-MS showed a molecular ion peak at *m/z* 334.2154, which corresponded to the molecular formula, $C_{20}H_{30}O_4$, indicating six degrees of unsaturation. The IR spectrum displayed absorption bands for carboxylic acid and hydroxy groups, and one terminal double bond at 3420−2510, 3381, 3072, 1692, 1647 and 890 cm^{-1}. The ^{1}H and ^{13}C NMR data of the side chain of **2** were found to be close to those of **1** and compound **2** was thus proposed as the 4-epimer of **1**. The COOH group was attached on C-4 in a β orientation, which was assured by the comparison of the chemical shifts of **2** with those of (12*S*)-12-hydroxylabda-8(17),13(16),14-trien-19-oic acid methyl ester [3b] and the significant NOE correlation between Me-19 (δ_H 1.14) and Me-20 (δ_H 0.71) in the NOESY spectrum of **2**. Thus, compound **2** was elucidated as (12*S*)-12-hydroxy-15,16-epoxylabda-8(17),13-dien-18-oic acid (**2**).

Experimental

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, NTU. A voucher specimen (013492) has been deposited at the Herbarium of the 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) 3 times (7 days each time) at room temperature. Following removal of the solvent, the extract (60.2 g) was suspended in water (500 mL), and then partitioned into *n*-hexane (500 ml ×3), EtOAc (500 ml ×4), and BuOH (500 ml ×3), successively. The EtOAc fraction (15.6 g) was subjected to a silica gel (450 g) column using *n*-hexane–EtOAc and EtOAc–MeOH mixtures as solvent systems to obtain 11 fractions. HPLC of fr. 6 from *n*-hexane/EtOAc (1/1) elution on a Merck LiChrosorb Si 60 column with CH_2Cl_2–acetone–*i*-PrOH (10:1:0.2) as eluent yielded **1** (2.2 mg). Fr. 7 from *n*-hexane–EtOAc (3:2) elution was further purified by HPLC to give **2** (3.1 mg) using *n*-hexane–CH_2Cl_2–EtOAc–*i*-PrOH (6:3:1:0.2). Fr. 4 from *n*-hexane–EtOAc (7:3) elution was further purified by HPLC to give **3** (2.5 mg) using *n*-hexane–CH_2Cl_2–EtOAc–*i*-PrOH (8:2:1:0.2).

12(*S*)-Hydroxy-15,16-epoxylabda-8(17),13-diene-19-oic acid (**1**)
Yellowish oil.
[α] $^{27}_D$: +21.7 (*c* 0.20, $CHCl_3$).
IR: 3425−2520, 3394, 3078, 1692, 1647, 1383, 1034, 895 cm^{-1}.
^{1}H and ^{13}C NMR: Table 1.
EI-MS *m/z* (rel. int.): 334 $[M]^+$ (2), 333 (22), 332 (100), 316 (32), 314 (81), 288 (21), 286 (19), 266 (17).
HR- EI-MS: *m/z* 334.2152 (calcd for $C_{20}H_{30}O_4$ 334.2145, $[M]^+$).

12(*S*)-Hydroxy-15,16-epoxylabda-8(17),13-diene-18-oic acid (**2**)
Yellowish oil.
[α] $^{27}_D$: +10.3 (*c* 0.28, $CHCl_3$).
IR: 3420−2510, 3381, 3072, 1692, 1647, 1383, 1047, 890 cm^{-1}.
^{1}H and ^{13}C NMR: Table 1.
EI-MS *m/z* (rel. int.): 334 $[M]^+$ (2), 332 (9), 314 (21), 301 (22), 288 (17), 175 (38), 121 (100).
HR- EI-MS: *m/z* 334.2154 (calcd for $C_{20}H_{30}O_4$ 334.2145, $[M]^+$).

Acknowledgments - This work was kindly supported by a grant from the China Medical University (CMU100-S-10), in part by CMU under the Aim for Top University Plan of the Ministry of Education, Taiwan, and Health Clinical Trial and Research Center of Excellent (DOH 102-TD-B-111-004). We thank Ms Shu-Yun Sun for the MS measurements in the Instrumentation Center of the College of Science, National Taiwan University. We are also grateful to the National Center for high-performance computing for computer time and facilities.

References

[1] Li HL, Keng H. (**1994**) *Flora of Taiwan*, 2nd ed. Editorial Committee of the Flora of Taiwan, Taipei, 582–585.
[2] (a) Ikeda T, Fujita Y. (**1929**) Essential oils of *Cunninghamia konishii* Hayata. *Bulletin of the Chemical Society of Japan*, ***50***, 32–45; (b) Ikeda T, Fujita Y. (**1929**) The pinene in *Cunninghamia konishii* Hayata. *Bulletin of the Chemical Society of Japan*, ***50***, 66–70; (c) Cheng YS, Lin CS. (**1979**) Study of the extractive constituents from the wood of *Cunninghamia konishii* Hayata. *Journal of the Chinese Chemical Society*, ***26***, 169–172; (d) Chang ST, Yin HW. (**1991**) Identification of the needle crystal appeared on the wood surface of *Cunninghamia konishii* Hayata. *Bulletin of Taiwan Forest Research Institute New Series*, ***6***, 57–63; (e) Li YC, Kuo YH. (**1998**) Five new diterpenoids from the wood of *Cunninghamia konishii*. *Journal of Natural Products*, ***61***, 997–1000; (f) Li YC, Kuo YH. (**2002**) Labdane-type diterpenoids from the wood of *Cunninghamia konishii*. *Chemical & Pharmaceutical Bulletin*, ***50***, 498–500; (g) Cheng SS, Lin CY, Gu HJ, Chang ST. (**2011**) Antifungal activities and chemical composition of wood and leaf essential oils from *Cunninghamia konishii*. Journal *of* Wood *Chemistry and* Technology, ***31***, 204–217; (h) Cheng SS, Chung MJ, Lin CY, Wang YN, Chang ST. (**2012**) Phytochemicals from *Cunninghamia konishii* Hayata act as antifungal agents. *Journal of Agricultural and Food Chemistry*, ***60***, 124–128; (i) Chen YC, Li YC, You BJ, Chang WT, Chao LK, Lo LC, Wang SY, Huang GJ, Kuo YH. (**2013**) Diterpenoids with anti-inflammatory activity from the wood of *Cunninghamia konishii*. *Molecules*, ***18***, 682–689; (j) Cheng YS, Tsai MD. (**1972**) Terpenes and sterols of *Cunninghamia konishii*. *Phytochemistry*, ***11***, 2108–2109; (k) He K, Shi G, Zeng L, Ye Q, McLaughlin JL. (**1997**) Konishiol, a new sesquiterpene, and bioactive components from *Cunninghamia konishii*. *Planta Medica*, ***63***, 158–160; (l) Chen YC, Li YC, Chiu HL, Cheng WY, Hong YH, Sung PJ, Kuo CC, Wu BT, Kuo YH. (**2013**) Diterpenoids from the wood of *Cunninghamia konishii*, *Helvetica Chimica Acta*, ***96***, 2282–2287; (m) Chang CI, Li YC, Kuo CC, Chao CY, Chang HS, Wu JH, Wang SY, Kuo YH. (**2013**) Two new lignans from the wood of *Cunninghamia konishii*, *Natural Product Communications*, ***8***, 805–806.
[3] (a) De Pascual TJ, Urones JG, Marcos IS, Martin DD, Alvarez VM. (**1986**) Labdane diterpenoids from *Halimium viscosum*. *Phytochemistry*, ***25***, 711–713; (b) Barrero AF, Altarejos J. (**1993**) ^{13}C NMR data for labdane diterpenoids. *Magnetic Resonance in Chemistry*, ***31***, 299–308.
[4] Iwamoto M, Ohtsu H, Tokuda H, Nishino H, Matsunaga S, Tanaka R. (**2001**) Anti-tumor promoting diterpenes from the stem bark of *Thuja standishii* (Cupressaceae). *Bioorganic and Medicinal Chemistry*, ***9***, 1911–1921.

Accounts/Reviews

Natural Product Communications
2014
Volume 9, Number 8

Contents

Articles dedicated to Prof. Josep Coll Toledano on the Occasion of his 70th Birthday

Continued inside backcover