Napalolides A−D, Four New Sesquiterpene Lactones from *Carpesium nepalense*

Yun-Lian Lin* and Jun-Chih Ou

National Research Institute of Chinese Medicine, Taipei, 112 Taiwan

Yueh-Hsiung Kuo

Department of Chemistry, National Taiwan University, Taipei, Taiwan

Jung-Kuei Lin

Chia-Nan Junior College of Pharmacy, Taiwan

Kuo-Hsiung Lee

School of Pharmacy, The University of North Carolina at Chapel Hill, North Carolina 27599-7360

Received November 27, 1995[⊗]

Four new germacranolide sesquiterpenes, nepalolides A (**2**), B (**3**), C (**4**), and D (**5a**), and one known germacranolide, ineupatorolide A (**1**), were isolated from *Carpesium nepalense*. The structures of these new compounds were elucidated from spectral evidence and chemical transformation.

The genus *Carpesium* (Compositae) has been reported as a rich source of antifungal and antibacterial sesquiterpene lactones,[1−5] and *C. nepalense* Nees. has been used in Chinese traditional medicine as a substitute for *C. abrotanolides* Linn. in treating hepatitis.[6] As a part of our interest in biologically active compounds from natural sources, we have investigated the constituents of *C. nepalense*. This note reports the isolation and structure elucidation of a known sesquiterpene, ineupatorolide A (**1**),[7] along with four new germacranolides, nepalolides A (**2**), B (**3**), C (**4**), and D (**5a**), from the aerial parts of this plant.

The EtOH extract of the aerial parts of *C. nepalense* was fractionated with EtOAc, *n*-BuOH, and H_2O. The EtOAc-soluble fraction was repeatedly chromatographed using Si gel column chromatography to afford five germacranolide lactones.

^{1}H- and ^{13}C-NMR spectral data (Tables 1 and 2) suggested that compounds **1**, **2**, **3**, and **4** have the same basic carbon skeleton but different ester residues. Compound **1** was identified as the known germacranolide sesquiterpene, ineupatorolide A. Nepalolide A (**2**), mp 163−164 °C, has a molecular formula of $C_{20}H_{28}O_6$ (as deduced by ^{13}C-NMR and MS analysis). It contains one tertiary and one secondary methyl group [δ_H 1.12 and 1.10 (d, J = 6.9 Hz)], a hydroxy group (ν_{max} 3520 cm^{-1}), one carbonyl group (ν_{max} 1705 cm^{-1}; δ_C 214.7), an α-methylene γ-lactone [ν_{max} 1770 cm^{-1}; λ_{max} (log ϵ) 213 nm (3.71); δ_H 5.70 and 6.36 (each 1H, d, J = 2.0 Hz)], and a senecioyl ester side chain [ν_{max} 1720 and 1210 cm^{-1}; δ_H 1.90 and 2.12 (each 3H, s), 5.75 (1H, s), and δ_C 166.1, 160.1, 114.5, 27.5, and 20.5]. Long-range coupling cross peaks (as shown in Figure 1) were observed between C-1′ and H-5, C-11 and H-8, C-7 and H-5 and H-13, C-4 and H-15, C-9 and H-8, and between C-1, C-10, and C-9 and H-14. The chemical shift of C-4 (δ 73.3) suggested the presence of a hydroxy group on this quaternary carbon. These data resulted in the establishment of a planar structure for nepalolide A (**2**). The relative stereoconfigurational structure for nepalolide A (**2**) was revealed from the following chemical correlation between ineupatorolide A (**1**) and nepalolide A (**2**). Exposure of ineupatorolide A (**1**) to K_2CO_3−MeOH resulted in the formation of compound **6**.[7] Similar treatment of nepalolide A (**2**) also formed product **6**. Baruah *et al.* reported the isolation of an inseparable mixture of **2** and **7** from *Inula eupatorioides*[8] (no physical data for either compound separately was shown, but only ^{1}H-NMR data of the mixture). Basic methanolysis of this mixture of **2** and **7** also yielded product **6**. Therefore, the structure and the stereoconfiguration of nepalolide A is shown unambiguously in structure **2**.

Nepalolide B (**3**) was obtained as colorless needles, mp 139−140 °C. It had a molecular formula of $C_{20}H_{28}O_6$ from MS analysis and ^{13}C-NMR data, and also showed ^{1}H- and ^{13}C-NMR spectra similar to those of **2** except for the signals arising from the ester moiety [δ_H 1.79 (3H, d, J = 7.2 Hz), 1.83 (3H, s), 6.88 (1H, q, J = 7.2 Hz) and δ_C 167.8 (C=O), 127.6 (C-2′), 139.1 (C-3′), 14.5 and 12.2 (C-4′ and C-5′)]. Spin decoupling, ^{1}H−^{1}H COSY, and HMBC spectra also established the presence of the same basic carbon skeleton as **2**, but the ester side chain was replaced by a tigloyl group in **3**. Compound **6** also was obtained from nepalolide B (**3**) by basic methanolysis.

The spectral characteristics of nepalolide C (**4**) are very similar to those of **2** and **3**. Comparison of the ^{1}H- and ^{13}C-NMR data (Tables 1 and 2) of the three compounds indicated that the ester residue in **4** is an angeloyl group [δ_H 6.12 (1H, q, J = 7.2 Hz), 1.98 (3H, d, J = 7.2 Hz), and 1.92 (3H, s)]. This structure was further supported by an HMBC experiment. Treatment of nepalolide C (**4**) with K_2CO_3−MeOH gave compound **6**. Therefore, nepalolide C can be assigned as structure **4**. This compound previously was isolated as an inseparable mixture with ineupatorolide A (**1**) from *Inula eupatorioides* by Baruah *et al.*[7] and designated ineupatorolide B (**4**). Epoxidation of the mixture gave ineupa-

* To whom correspondence should be addressed. Phone: (919) 962-0066. FAX: (919) 966-3893.

⊗ Abstract published in *Advance ACS Abstracts,* October 1, 1996.

S0163-3864(96)00563-0 CCC: $12.00

Table 1. ^{1}H NMR Spectral Data of Compounds 1, 2, 3, 4, and 5a (300 MHz, in $CDCl_3$)

proton	1	2	3	4	5a
H-5	4.57 d (6.8)	4.68 d (6.6)	4.72 d (6.3)	4.74 d (6.6)	3.5 d (6.6)
H-6	4.47 dd (6.8,2.8)	4.53 dd (6.6,3.0)	4.54 dd (6.3,3.0)	4.54 dd (6.6,3.0)	4.56 dd (6.6,2.7)
H-7	3.58 m	3.60 m	3.60 m	3.63 m	2.49 m
H-8	2.70 dd (14.0,3.2)	2.60 dd (14.1,3.5)	2.64 dd (14.1,3.9)	2.64 dd (14.5,3.0)	2.60 dd (14.0,4.0)
	2.72 dd (14.0,11.5)	2.68 dd (14.1,10.0)	2.66 d (14.1,11.5)	2.66 dd (14.5,11.5)	2.77 dd (14.0,10.8)
H-10	2.94 ddq (11.0,3.0,7.0)	2.84 ddq (11.0,3.0,7.0)	2.84 ddq (11.0,3.0,7.0)	2.84 m	2.93 ddq (11.0,3.0,7.0)
H-11					2.28 m
H-13	5.55 d (3.0)	5.70 d (2.0)	5.74 d (2.1)	5.75 d (2.1)	1.27 d (6.9)
	6.28 d (3.0)	6.36 d (2.0)	6.37 d (2.1)	6.37 d (2.1)	
H-14	1.14 d (7.0)	1.10 d (7.0)	1.10 d (7.0)	1.10 d (6.9)	1.09 d (6.8)
H-15	1.18 s	1.12 s	1.14 s	1.12 s	1.19 s
H-2′	2.45 m	5.75 s			
H-3′	2.05 m, 1.69 m		6.88 q (7.2)	6.12 q (7.2)	
H-4′	0.92 t (7.5)	1.90 s	1.79 d (7.2)	1.98 d (7.2)	
H-5′	1.11 d (6.9)	2.12 s	1.83 s	1.92 s	

Table 2. ^{13}C NMR Spectral Data of Compounds 1, 2, 3, 4, and 5a (75 MHz, in $CDCl_3$)

carbon	1	2	3	4	5a
C-1	19.1 t	22.9 t	22.8 t	22.9 t	19.0 tt
C-2	33.5 t	33.8 t	33.7 t	33.7 t	31.4 t
C-3	34.9 t	35.2 t	35.2 t	35.2 t	34.6 t
C-4	73.3 s	73.3 s	73.5 s	73.1 s	74.6 s
C-5	77.2 d	76.7 d	77.9 d	77.0 d	78.3 d
C-6	76.5 d	76.5 d	77.0 d	76.6 d	75.2 d
C-7	41.6 d	41.1 d	41.1 d	41.1 d	41.5 d
C-8	44.9 t	50.8 t	50.6 t	50.8 t	45.3 t
C-9	213.0 s	214.7 s	214.7 s	214.7 s	214.3 s
C-10	46.2 d	45.1 d	45.2 d	45.0 d	46.3 d
C-11	138.1 s	137.9 s	137.8 s	137.8 s	41.5 d
C-12	168.9 s	168.8 s	168.8 s	168.8 s	177.7 s
C-13	121.5 t	123.5 t	123.5 t	123.6 t	13.4 q
C-14	19.7 q	19.7 q	19.7 q	19.8 q	17.4 q
C-15	24.7 q	24.7 q	24.8 q	24.8 q	23.7 q
C-1′	177.8 s	166.1 s	167.8 s	167.5 s	
C-2′	41.7 d	114.5 d	127.6 s	126.7 s	
C-3′	26.6 t	160.1 s	139.1 d	140.1 d	
C-4′	11.6 q	27.5 q	14.5 q	20.4 q	
C-5′	16.7 q	20.5 q	12.2 q	15.8 q	

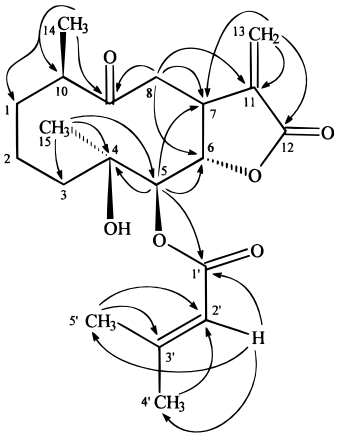
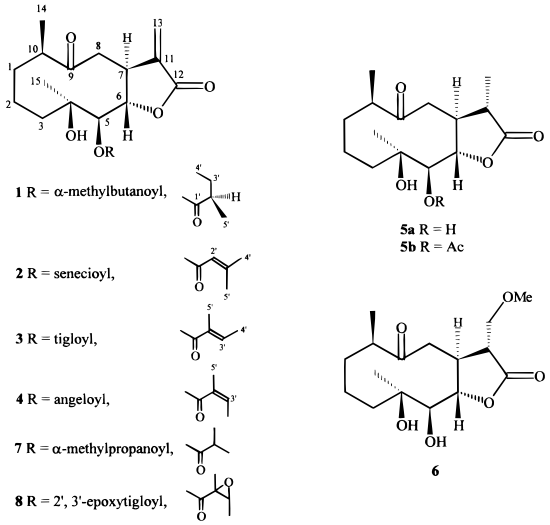


Figure 1. Correlation observed in the HMBC spectrum of **2**.

torolide A epoxide and ineupatorolide B expoxide (**8**). Only the mixture ^{13}C-NMR data were reported; no other physical data for ineupatorolide B were described in the paper.[7]

The molecular formula of nepalolide D (**5a**) was assigned as $C_{15}H_{24}O_5$ according to ^{13}C-NMR and MS analysis. Its ^{1}H-NMR (Table 1) spectrum showed no ester side chain, and the exocyclic methylene group was saturated [δ 1.27 (d, J = 6.9 Hz)]. Acetylation of **5a** (Ac_2O−pyridine, room temperature overnight) afforded a monoacetate (**5b**) [ν_{max} 1770 and 1240 cm^{-1}, ^{1}H NMR ($CDCl_3$) δ 2.14 (3H, s)]. The presence of a 11β,13-dihydro derivative of a methylene lactone was deduced from the methyl doublet at δ 1.27 (H-13) and a double quartet at δ 2.28 (H-11). The coupling value of $J_{7,11}$ (10.3 Hz), obtained by decoupling experiment, indicated an 11β-proton.[9−12] Based on the above discussion, nepalolide D (**5a**) was determined to be 4β,5β-dihydroxy-9-oxo-11βH-germacran-6α,12-olide.



Experimental Section

General Experimental Procedures. Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 781 spectrophotometer. NMR were run on a Bruker AC-300 spectrometer. Optical rotations were run in $CHCl_3$ on a JASCO DIP-370 polarimeter. UV spectra were taken on a Hitachi U-3200 spectrophotometer.

Plant Material. The aerial parts of *Carpesium nepalense* Nees. were collected in Tsiufeng, Nantou, Taiwan, in September 1993. Plant material was identified by comparison with a voucher specimen, which is deposited at the Herbarium of the Department of Botany of National Taiwan University.

Extraction and Isolation. The aerial parts of *C. nepalense* Nees. (5 kg) were extracted twice with EtOH (30 L) at 50 °C. The EtOH extract was evaporated *in vacuo* to yield a black residue (356 g), which was taken up in H_2O and partitioned successively with EtOAc and *n*-BuOH (each 1 L). The EtOAc fraction (85g) was chromatographed on a Si gel column, eluting with hexane−EtOAc (2:1−1:2). The eluents were combined

to give fractions A−F according to TLC analysis (hexane−EtOAc = 3:2 and MeOH−$CHCl_3$ = 1:20). Fractions C and D were combined, then chromatographed on Si gel (230−400 mesh), eluting with 15−30% EtOAc in hexane or MeOH (1−5%) in $CHCl_3$, to yield **1** (1.68g), **2** (0.86g), **3** (1.19g), **4** (1.75g), and **5a** (26 mg).

Ineupatorolide A (1): colorless needles from EtOH; mp 156−157 °C; $[\alpha]^{25}_D$ + 15° (*c* 0.5, $CHCl_3$); EIMS (70eV) *m/z* [M^+] 366 (4), 346 (15), 193 (45), 141 (90), 83 (100); IR (Kbr) ν_{max} 3400 (OH), 3020, 1645, 865 (terminal methylene), 1770 and 1645 (α-methylene γ-lactone), 1710 and 1210 (ester), 1700 (ketone) cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) see Tables 1 and 2.

Nepalolide A (2): colorless needles from EtOH; mp 163−164 °C; $[\alpha]^{25}_D$ +28° (*c* 1.0, $CHCl_3$); EIMS (70eV) *m/z* [M^+] 364 (3), 346 (6), 83 (100), 55 (52); UV (MeOH) λ_{max} (log ϵ) 213 (3.71) nm; IR ν max 3520 (OH), 3020, 1640, 880 (terminal methylene), 1770 and 1640 (α-methylene γ-lactone), 1720 and 1210 (ester), 1705 (ketone) cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) see Tables 1 and 2.

Nepalolide B (3): colorless needles from EtOH; mp 139−140 °C; $[\alpha]^{25}_D$ +26.6° (*c* 1.0, $CHCl_3$); UV (MeOH) λ (log ϵ) 213 nm (3.71); IR (KBr) ν_{max} 3500 (OH), 1770 and 1640 (α-methylene γ-lactone), 1710 and 1210 (ester), 1690 (ketone) cm^{-1}; EIMS (70 eV) *m/z* [M^+] 364 (3), 346 (5), 83 (100); ^1H and ^{13}C NMR ($CDCl_3$) see Tables 1 and 2.

Nepalolide C (4): colorless needles from EtOH; mp 148−150 °C; $[\alpha]^{25}_D$ +28.0° (*c* 1.0, $CHCl_3$); UV (MeOH) λ (log ϵ) 214 (3.73) nm; IR (KBr) ν_{max} 3520 (OH), 1780 and 1660 (α-methylene γ-lactone), 1720 and 1205 (ester), 1700 (ketone), 3020, 1640, 885 (terminal methylene) cm^{-1}; EIMS (70eV) *m/z* [M^+] 364 (3), 346 (10), 83 (90), 55 (100); ^1H and ^{13}C NMR ($CDCl_3$) see Tables 1 and 2.

Nepalolide D (5a): colorless needles from EtOH; mp 212−213 °C; $[\alpha]^{25}_D$ −30° (*c* 0.3, $CHCl_3$); IR (KBr) ν_{max} 3520, 3500 (OH), 1745 (γ-lactone), 1695 (ketone) cm^{-1}; EIMS (70eV) *m/z* [M^+] 282 (3), 193 (38), 153 (35), 113 (30), 95 (30), 83 (31), 71 (46), 55 (98), 53 (100); ^1H and ^{13}C NMR ($CDCl_3$) see Tables 1 and 2.

Methanolysis of 1, 2, 3, and 4. A solution of 75 mg of compound **1**, **2**, **3**, or **4** and 230 mg of anhydrous K_2CO_3 in 5 mL of MeOH was stirred under Ar for 3 h until TLC indicated disappearance of the starting material. The reaction mixture was diluted with H_2O, acidified with HOAc, and extracted with $CHCl_3$. The washed and dried extract was evaporated, and the residue purified by Si gel column chromatography to yield product **6** (35 mg) (mp 143−145 °C, lit 145 °C[7]) in each reaction.

Acetylation of 5a. Compound **5a** (5 mg) in pyridine (1 mL) was treated with Ac_2O (0.5 mL). The reaction mixture stood at room temperature overnight and then was worked up by the usual method. The residue was purified on Si gel to obtain **5b** (5 mg).

Compound 5b: colorless needles; mp 181−182 °C; IR (KBr) ν_{max} 3440, 2935, 2860, 1770, 1740, 1710, 1455, 1360, 1240 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.64 (1H, d, J = 6.9 Hz, H-5), 4.52 (1H, dd, J = 6.9, 3.3 Hz, H-6), 3.05 (1H, m, H-10), 2.84 (1H, dd, J = 14.1, 11.0 Hz, H_a-8), 2.62 (1H, dd, J = 14.1, 3.5 Hz, H_b-8), 2.48 (1H, m, H-7), 2.30 (1H, m, H-11), 2.14 (3H, s, Ac), 1.29 (3H, d, J = 7.2 Hz, H-13), 1.16 (3H, s, H-15), 1.12 (3H, d, J = 6.9 Hz, H-14).

Acknowledgment. This work was supported by the National Science Council of Republic of China.

References and Notes

(1) Maruyama, M. *Phytochemistry* **1990**, *29*, 547−550.
(2) Maruyama, M.; Shibata, F. *Phytochemistry* **1975**, *14*, 2247−2248.
(3) Maruyama, M.; Omura, S. *Phytochemistry* **1977**, *16*, 782−783.
(4) Omura, M.; Omura, A.; Sato, K. *Phytochemistry* **1983**, *22*, 2773−2274.
(5) Dong, Y.; Ding, Y. *Zhiwu Xuebao* **1988**, *30*, 71−75.
(6) Chiu, N. Y.; Chang, K. H. *The Illustrated Medicinal Plants of Taiwan*; SMC Publishing: Taipei, 1995; Vol. 4, p 229.
(7) Baruah, R. N.; Sharma, R. P.; Thyagarajan, G.; Hern, W.; Govindan, S. V.; Blount, J. F. *J. Org. Chem*. **1980**, *45*, 4838−4843.
(8) Baruah, N. C.; Baruah, R. N.; Sharma, R. P.; Baruah, J. N.; Herz, W.; Watanabe, K.; Blount, J. F. *J Org. Chem*. **1982**, *47*, 137−140.
(9) Zdero, C.; Bohlmann, F. *Phytochemistry* **1990**, *29*, 189−194.
(10) Mahmoud, Z. F.; Kassem, F. F.; Abdel-Salam, N. A.; Zkero, C. *Phytochemistry* **1986**, *25*, 747−748.
(11) Ruangrungsi, N.; Rivepiboon, A.; Lange, G. L.; Decicco, C. P. *J. Nat. Prod*. **1988**, *51*, 163−167.
(12) Nishimura, K.; Miyase, T.; Ueno, A.; Noro, T.; Kuroyanagi, M.; Fukushima, S. *Phytochemistry* **1986**, *25*, 2375−2379.

NP960563H