Prenylbicyclogermacrane Diterpenoids from the Formosan Soft Coral *Nephthea elongata*

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Seven new prenylbicyclogermacrane diterpenoids, pacificins K—Q (1—7), were isolated from the methylene chloride solubles of the Formosan soft coral *Nephthea elongata*. Their structures were elucidated by extensive spectroscopic analysis and their cytotoxicity against selected cancer cells was measured *in vitro*.

Key words *Nephthea elongata*; prenylbicyclogermacrane diterpenoid; cytotoxicity

Soft corals of the genus *Nephthea* are rich in terpenoids and steroids.[1—15] As part of our search for bioactive substances from marine organisms, the Formosan soft coral *Nephthea elongata* KÜKENTHAL (Nephtheidae) was studied because the CH_2Cl_2 extracts showed significant cytotoxicity to P-388 (mouse lymphocytic leukemia) cell culture as determined by standard procedures.[16,17] Bioassay-guided fractionation resulted in the isolation and chraracterization of seven new prenylbicyclogermacrane diterpenoids, pacificins K—Q (**1**—**7**).

Pacificin K (**1**) proved to have molecular formula $C_{22}H_{36}O_3$ from its HR-FAB-MS, ^{13}C-NMR, and DEPT spectroscopic data. ^{13}C-NMR and DEPT spectrum of **1** exhibited the presence of six methyls, six sp^3 methylenes, three sp^3 methines, two sp^2 methine, two sp^3 quaternary carbon and one sp^2 quaternary carbon. The presence of two trisubstituted olefins in **1** was shown by the NMR data (δ_H 5.09 d, 5.25 d; δ_C 131.2 qC, 125.2 CH, 140.0 qC, 125.2 CH) (Tables 1, 2). The NMR data (δ_H 0.26 m, 0.40 m, 0.55 m, 0.98 m; δ_C 4.5 CH_2, 27.4 CH, 30.3 CH) (Tables 1, 2) pointed to a 1,2-disubstituted cyclopropane ring in **1**. The ^{1}H-NMR also spectrum contained signals for six tertiary methyl groups, (δ_H 0.60, 0.81, 1.61, 1.69, 1.86, 2.03). The presence of an ambiguous carbon bearing an oxygen (δ_C 71.6 qC) was shown in the ^{13}C-NMR spectrum. The spectral data of **1** exhibited some similarity to those of a prenylbicyclogermacrane diterpenoid, palmatol (**8**), isolated from *Alcyonium palmatum*,[18] except for the presence of an acetoxyl at C-11. COSY correlations from H-11 to H-10/H-12 and HMBC correlations (Fig. 1) from H-11 to C-12/C-10/O<u>C</u>OCH$_3$ confirmed the position of the acetoxyl at C-11. The relative stereochemistry of **1** was deduced from a 2D NOESY experiment (Fig. 2), which indicated that Me-19, Me-20, H-11, and H-16 were on one side of the molecule, while Me-18, H-10, and H-14 were on the opposite side of the molecule. From these data, pacificin K can be formulated as **1**.

Pacificin L (**2**) was shown to have a molecular formula of $C_{20}H_{34}O_3$ by its HR-FAB-MS, ^{13}C-NMR, and DEPT spectroscopic data. The ^{1}H- and ^{13}C-NMR spectral data of **2** exhibited some similarity to those of a prenylbicyclogermacrane diterpenoid, pacifin A (**9**), isolated from *Nephthea pacifica*,[14] except for the replacement of the olefin at C-2/C-3 by an

Table 1. ^{1}H-NMR Data (300 MHz) of **1**—**7**

H	**1**[a]	**2**[a]	**3**[b]	**4**[b]	**5**[b]	**6**[a]	**7**[b]
1	1.69 s	1.30 s	4.92 s, 5.10 s	4.87 s, 5.06 s	1.61 s	1.33 s	1.19 s
3	5.09 t (6.9)[c]	2.69 t (6.0)	5.41 t (6.4)	5.37 t (6.0)	5.28 t (6.9)	5.63 d (15.6)	2.54 t (6.3)
4	2.05 m	1.53 m, 1.70 m	1.69 m	1.73 m	2.16 m	5.69 m	1.58 m
5	1.20 m	1.53 m, 1.39 m	1.18 m, 1.28 m	1.18 m	1.36 m	2.02 m	1.23 m
7	1.45 m, 1.81 m	1.51 m, 1.76 m	1.48 m	1.87 m, 2.20 m	1.38 m, 1.67 m	1.57 m, 1.68 m	1.85 m, 2.22 m
8	2.15 m	1.28 m, 2.05 m	1.17 m, 1.94 m	1.15 m, 1.67 m	1.39 m, 2.23 m	1.31 m, 2.06 m	1.17 m, 1.68 m
	2.37 dt (2.0, 13.2)						
10	5.25 d (9.9)	2.85 d (10.2)	2.66 d (9.3)	5.11 m	2.75 d (10.8)	2.97 d (10.5)	5.10 d (10.5)
11	5.46 tt (11.1, 4.5)	1.40 m, 2.03 m	1.25 m, 1.93 m	1.95 m, 2.10 m	1.38 m, 2.15 m	1.32 m, 2.20 m	1.95 m, 2.12 m
12	1.98 m	1.97 m	1.80 m	1.76 m, 1.91 m	2.08 m, 2.14 m	2.25 m, 2.48 m	1.77 m, 1.88 m
	2.17 dd (12.3, 4.2)						
14	0.98 dt (9.3, 5.4)	1.16 m	0.84 dt (9.3, 5.4)	0.78 dt (9.3, 5.1)	0.88 dt (9.0, 5.1)	1.14 dt (9.3, 5.4)	0.79 dt (9.0, 5.4)
15α	0.40 dt (9.3, 5.4)	0.42 dt (9.0, 5.1)	0.16 dt (9.3, 5.4)	0.20 m	0.72 dt (9.0, 5.1)	0.69 m	0.14 dt (9.0, 5.4)
15β	0.26 dt (9.3, 5.4)	0.26 dt (9.0, 5.1)	−0.08 dt (9.3, 5.4)	0.02 dt (9.3, 5.1)	0.60 dt (9.0, 5.1)		−0.03 dt (9.0, 5.4)
16	0.55 m	0.72 m	0.48 m	0.39 m	0.73 dt (9.0, 5.1)	0.71 m	0.34 dt (9.0, 5.4)
17	1.61 s	1.33 s	1.71 s	1.67 s	1.72 s	1.33 s	1.18 s
18	0.60 s	0.53 s	0.39 s	0.41 s	0.27 s	0.51 s	0.42 s
19	1.86 s	1.33 s	1.15 s	1.49 s	1.75 s	1.21 s	1.50 s
20	0.81 s	0.77 s	0.52 s	0.59 s	4.19 s, 4.45 s	4.27 s, 4.57 s	0.60 s
OAc	2.03 s		1.76 s	1.73 s			

a) Recorded in $CDCl_3$ (assigned by COSY, HSQC, and HMBC experiments). *b*) Recorded in C_6D_6 (assigned by COSY, HSQC, and HMBC experiments). *c*) *J* values (in Hz) in parentheses.

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Table 2. ^{13}C-NMR Spectral Data (75 MHz) of **1**—**7**

	1[a)]	**2**[a)]	**3**[b)]	**4**[b)]	**5**[b)]	**6**[a)]	**7**[b)]
1	25.8	18.7	113.1	113.0	25.9	30.1	23.8
2	131.2	58.7	143.4	143.3	130.8	70.9	57.4
3	125.2	64.8	77.8	72.7	125.8	141.1	64.4
4	22.3	23.3	26.7	26.9	22.8	123.0	23.8
5	45.1	42.9	41.9	41.8	46.1	48.1	42.9
6	35.4	34.4	34.2	37.2	35.0	35.7	37.0
7	36.8	36.8	36.8	35.3	37.0	37.1	35.0
8	35.4	35.3	35.8	36.0	35.5	35.0	36.0
9	140.0	60.8	60.7	132.3	61.0	62.1	132.3
10	125.2	65.6	65.6	127.6	65.8	66.5	127.7
11	69.1	24.7	24.8	25.1	31.3	30.8	23.8
12	49.0	41.9	42.1	44.3	36.6	36.4	44.3
13	71.6	72.8	72.1	72.7	152.6	152.2	72.7
14	30.3	29.1	29.3	30.2	20.9	30.8	31.2
15	4.5	3.9	3.8	5.3	12.6	12.7	5.3
16	27.4	27.0	26.9	27.4	35.5	35.7	27.4
17	17.6	25.0	18.2	18.3	17.6	30.1	18.8
18	19.2	19.3	19.4	19.4	18.2	17.6	19.3
19	16.6	17.1	17.2	16.1	17.6	17.1	16.3
20	21.3	20.7	20.8	20.8	103.4	103.8	20.5
OAc	21.5		20.7	20.5			
	170.4		169.4	170.1			

a) Recorded in $CDCl_3$ (assigned by DEPT, COSY, HSQC, and HMBC experiments). *b*) Recorded in C_6D_6 (assigned by DEPT, COSY, HSQC, and HMBC experiments).

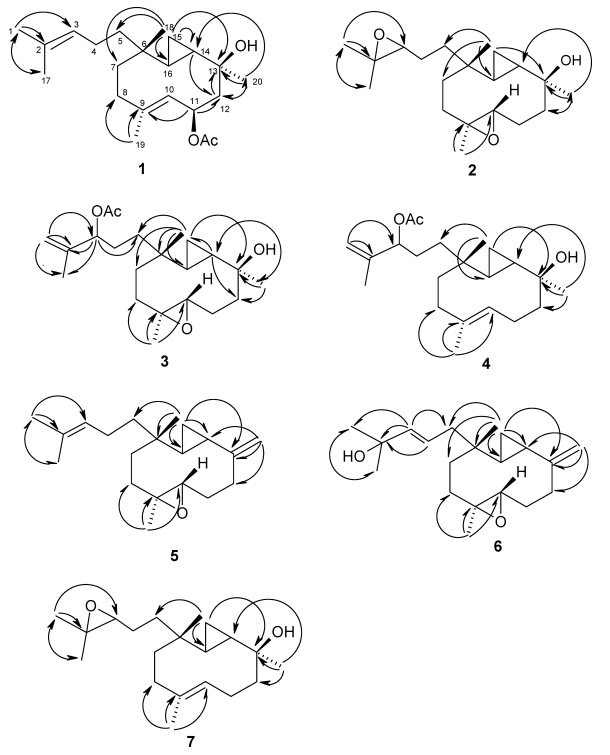

Fig. 1. Selected HMBC Correlations of **1**—**7**

epoxy. COSY from H-3 to H-4 and HMBC correlations (Fig. 1) H-17 to C-2/C-3 correlations from confirmed the position of the epoxyl at C-1/C-2. The relative stereochemistry of **2** was deduced from a 2D NOESY experiment (Fig. 2), which indicated that Me-19, Me-20, and H-16 were on one side of the molecule, while Me-18, H-10, and H-14 were on the opposite side of the molecule.

Pacificin M (**3**) analyzed for $C_{22}H_{36}O_4$ from its HR-FAB-MS, ^{13}C-NMR, and DEPT spectroscopic data. The ^{1}H- and

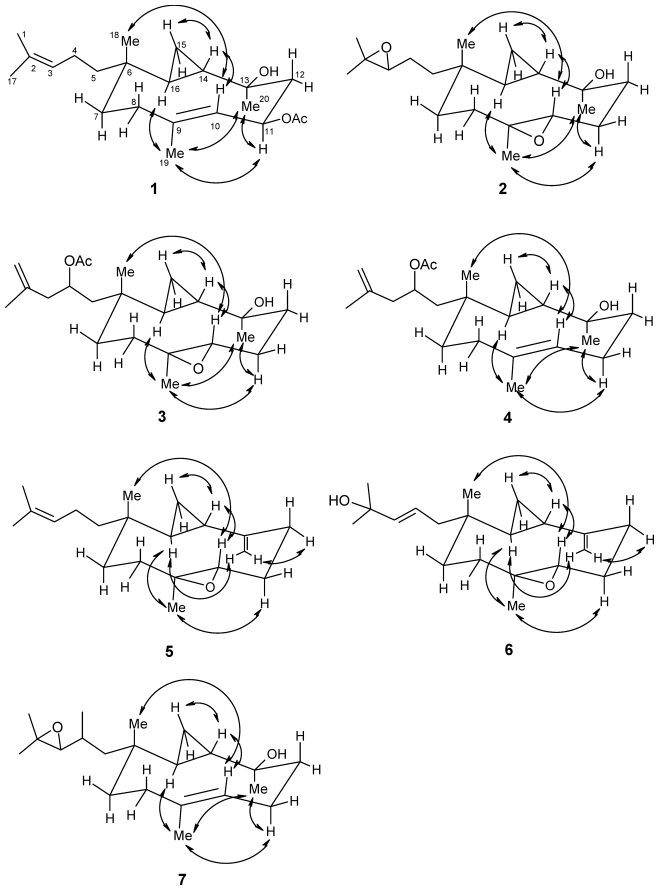

Fig. 2. Selected NOESY Correlations of **1**—**7**

^{13}C-NMR spectral data of **3** were analogous to those of a prenylbicyclogermacrane diterpenoid, pacifin D (**10**), isolated from *Nephthea pacifica*,[14] except that the hydroxyl at C-3 was replaced by an acetoxyl. COSY from H-3 to H-4 and HMBC correlations (Fig. 1) from H-3 to C-1/C-2/C-4/C-5/C-17/O<u>C</u>OCH$_3$ correlations from clearly positioned the acetoxyl at C-3. The relative stereochemistry of **3** was determined by a 2D NOESY experiment (Fig. 2), which showed similar results as described for **2**.

HR-FAB-MS and NMR spectroscopic data revealed pacificin N (**4**) to have a molecular formula of $C_{22}H_{36}O_3$. The ^{1}H- and ^{13}C-NMR spectral data (Tables 1, 2) resembled those of **3** except that the trisubstituted epoxide was replaced by a *E*-trisubstituted double bond. HMBC correlations (Fig. 1) from H-11 to C-9/C-10/C-12/C-13 help ascertain the position of the *E*-trisubstituted double bond. The relative stereochemistry of **4** was deduced from a 2D NOESY experiment (Fig. 2), which indicated that Me-19, Me-20, and H-16 were on one side of the molecule, while Me-18, H-10, and H-14 were on the opposite side of the molecule. Therefore, pacificin N was established as **4**.

Pacificin O (**5**) was proved to have the molecular formula of $C_{20}H_{32}O$ by HR-FAB-MS and NMR spectroscopic data. The ^{1}H- and ^{13}C-NMR spectral data (Tables 1, 2) were similar to those of pacificin I (**11**) isolated from *Nephthea pacifica*,[14] except that the trisubstituted epoxide was replaced by a *E*-trisubstituted double bond. HMBC correlations (Fig. 1) from H-11 to C-9/C-10/C-12/C-13 enabled the correct positioning of the *E*-trisubstituted double bond. The relative

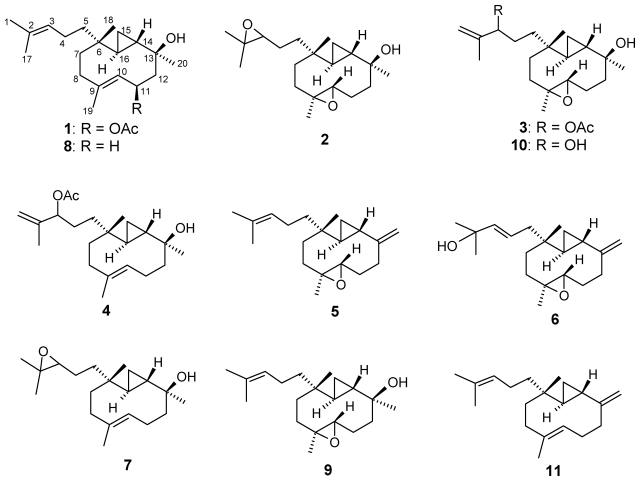

Chart 1. Structures of **1**—**11**

stereochemistry of **5** was determined based on a 2D NOESY experiment, which indicated that Me-19, H_2-20, and H-16 were on one side of the molecule, while Me-18, H-10, and H-14 were on the opposite side of the molecule (Fig. 2). From these data, pacificin O can be determined as **5**.

The molecular formula of pacificin O (**6**) proved to be $C_{20}H_{32}O_2$ by HR-FAB-MS and ^{13}C-NMR data. Detailed comparison of ^{1}H- and ^{13}C-NMR spectral data (Tables 1, 2) of **6** and **5** revealed that **6** differed from **5** in the side chain. COSY correlations between H-3/H-4 and H-4/H-5, HMBC correlations (Fig. 1) from H-1/H-17 to C-2/C-3, H-5 to C-3/C-6/C-7, as well as a $J_{3,4}$ of 15.6 Hz placed an *E* double bond between C-3 and C-4. HMBC correlations from H-3 to C-2/C-5/C-1/C-17 and from H-4 to C-2/C-5/C-6 confirmed the position of the tertiary hydroxyl at C-2 (Fig. 1). The relative stereochemistry of **6** was determined by a 2D NOESY experiment (Fig. 2), which showed similar results as described for **5**. From the aforementioned data, pacificin P can be formulated as **6**.

Pacificin Q (**7**) was shown to have the molecular formula of $C_{20}H_{34}O_2$ by its HR-FAB-MS and NMR spectra. The ^{1}H- and ^{13}C-NMR spectral data (Tables 1, 2) closely resembled those of **2** except that the trisubstituted epoxide was replaced by a *E*-trisubstituted double bond. HMBC correlations from H-11 to C-9/C-10/C-12/C-13 confirmed the position of the *E*-trisubstituted double bond (Fig. 1). The relative stereochemistry of **7** was determined by a 2D NOESY experiment (Fig. 2), which showed similar results as described for **4**. From these data, pacificin Q can be formulated as **7**.

Compounds **1** and **2** exhibited cytotoxicity against P-388 cell line with ED_{50} of 3.2 and 2.6 μg/ml, respectively. Compounds **3**—**7** were not cytotoxic against P-388 cell line.

Experimental

General Experimental Procedures Optical rotations were determined on a JASCO DIP-181 polarimeter. IR spectra were recorded on a Hitachi 26-30 spectrophotometer. The NMR spectra were recorded on Bruker Avance 300 NMR spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C, respectively, using TMS as internal standard. HR-MS were obtained with a JEOL JMS-SX/SX 102A mass spectrometer at 70 eV. Silica gel 60 (Merck, 230—400 mesh) was used for column chromatography; precoated silica gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) were used for TLC analysis.

Animal Material The soft coral *N. elongata* was collected at Green Island, off Taiwan, in September 2004, at a depth of 3 m and was stored for 2 weeks in a freezer until extraction. A voucher specimen, NSUGN-071, was deposited in the Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Taiwan.

Extraction and Isolation The bodies of the soft coral *N. elongata* were freeze dried to give 1.2 kg of a solid, which was extracted with CH_2Cl_2 (2 l×3, overnight for each cycle) at room temperature. After removal of solvent *in vacuo*, the residue (40 g) was chromatographed over a column containing silica gel 60 using *n*-hexane–EtOAc and MeOH–EtOAc mixtures as eluting solvents. Elution by *n*-hexane–EtOAc (1 : 8) afforded fractions containing **5**. Elution by *n*-hexane–EtOAc (55 : 45) afforded fractions containing **4**. Elution by *n*-hexane–EtOAc (1 : 1) afforded fractions containing **6** and **7**. Elution by *n*-hexane–EtOAc (2 : 1) afforded fractions containing **1**. Elution by *n*-hexane–EtOAc (1 : 3) afforded fractions containing **3**. Elution by EtOAc afforded fractions containing **2**. Compound **1** (2 mg) was further purified by HPLC (LiChrosorb RP-18, 7 μ, 25×250 mm), by eluting with MeOH–H_2O (85 : 15). Compound **2** (2 mg) was further purified by HPLC (LiChrosorb RP-18, 7 μ, 25×250 mm), by eluting with MeOH–H_2O (68 : 32). Compound **3** (4 mg) was further purified by HPLC (LiChrosorb RP-18, 7 μ, 25×250 mm), by eluting with MeOH–H_2O (67 : 33). Compound **4** (3 mg) was further purified by HPLC (LiChrosorb RP-18, 7 μ, 25×250 mm), by eluting with MeOH–H_2O (72 : 28). Compound **5** (3 mg) was further purified by HPLC (LiChrosorb RP-18, 7 μ, 25×250 mm), by eluting with MeOH–H_2O (77 : 23). Finally, compounds **6** (2 mg) and **7** (3 mg) were further purified by HPLC (LiChrosorb RP-18, 7 μ, 25×250 mm), by eluting with MeOH–H_2O (73 : 27).

Pacificin K (**1**): $[\alpha]_D^{25}$ −56° (*c*=0.3, $CHCl_3$). IR (neat) cm^{-1}: 3560, 1732. ^{1}H-NMR: see Table 1. ^{13}C-NMR: see Table 2. HR-FAB-MS *m/z*=371.2563 (Calcd for $C_{22}H_{36}O_3Na$=371.2562).

Pacificin L (**2**): $[\alpha]_D^{25}$ −67° (*c*=0.2, $CHCl_3$). IR (neat) cm^{-1}: 3590. ^{1}H-NMR: see Table 1. ^{13}C-NMR: see Table 2. HR-FAB-MS *m/z*=345.2408 (Calcd for $C_{20}H_{34}O_3Na$=345.2406).

Pacificin M (**3**): $[\alpha]_D^{25}$ −46° (*c*=0.1, $CHCl_3$). IR (neat) cm^{-1}: 3470, 1740. ^{1}H-NMR: see Table 1. ^{13}C-NMR: see Table 2. HR-FAB-MS *m/z*=387.2512 (Calcd for $C_{22}H_{36}O_4Na$=387.2511).

Pacificin N (**4**): $[\alpha]_D^{25}$ −39° (*c*=0.1, $CHCl_3$). IR (neat) cm^{-1}: 3465, 1736. ^{1}H-NMR: see Table 1. ^{13}C-NMR: see Table 2. HR-FAB-MS *m/z*=371.2565 (Calcd for $C_{22}H_{36}O_3Na$=371.2562).

Pacificin O (**5**): $[\alpha]_D^{25}$ −58° (*c*=0.1, $CHCl_3$). IR (neat) cm^{-1}: 1620. ^{1}H-NMR: see Table 1. ^{13}C-NMR: see Table 2. HR-FAB-MS *m/z*=311.2353 (Calcd for $C_{20}H_{32}ONa$=311.2351).

Pacificin P (**6**): $[\alpha]_D^{25}$ −45° (*c*=0.1, $CHCl_3$). IR (neat) cm^{-1}: 3485, 1610. ^{1}H-NMR: see Table 1. ^{13}C-NMR: see Table 2. HR-FAB-MS *m/z*=327.2302 (Calcd for $C_{20}H_{32}O_2Na$=327.2300).

Pacificin Q (**7**): $[\alpha]_D^{25}$ −31° (*c*=0.2, $CHCl_3$). IR (neat) cm^{-1}: 3495. ^{1}H-NMR: see Table 1. ^{13}C-NMR: see Table 2. HR-FAB-MS *m/z*=329.2455 (Calcd for $C_{20}H_{34}O_2Na$=329.2456).

Cytotoxicity Testing P-388 cells were kindly supplied by Dr. J. M. Pezzuto, formerly of the Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago; Cytotoxic assays were carried out according to the procedure described previously.[17] Three concentrations (50, 5, 0.5 μg/ml) of the tested compounds were used in the cytotoxicity assays.

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