

Prenylbicyclogermacrene Diterpenoids from the Formosan Soft Coral *Nephthea pacifica*

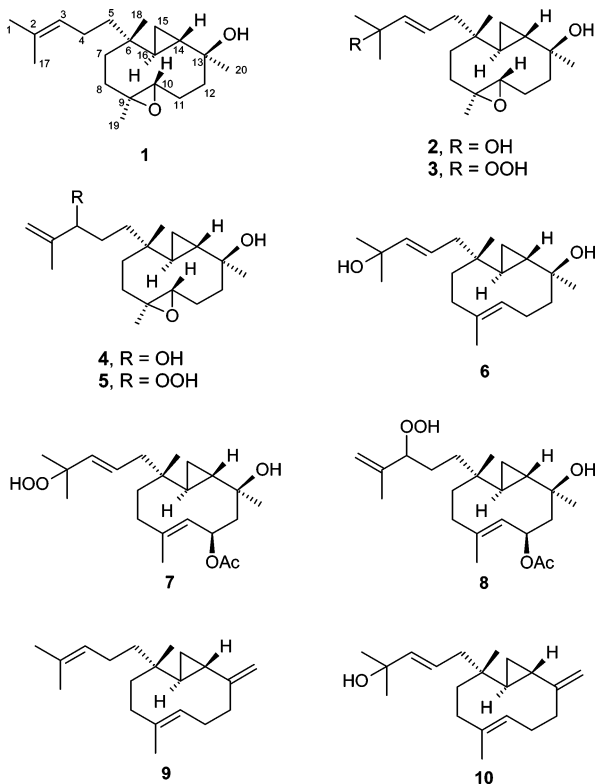
Ali A. H. El-Gamal,^{†,‡} Shang-Kwei Wang,[§] Chang-Feng Dai,[⊥] I-Gin Chen,[†] and Chang-Yih Duh^{*,†}

Department of Marine Resources, National Sun Yat-sen University, Kaohsiung, Taiwan, Republic of China, Department of Microbiology, Kaohsiung Medical University, Kaohsiung, Taiwan, Republic of China, and Institute of Oceanography, National Taiwan University, Taipei, Taiwan, Republic of China

Received July 23, 2004

Ten new prenylbicyclogermacrene diterpenoids, pacificins A–J (**1–10**), were isolated from the methylene chloride solubles of the Formosan soft coral *Nephthea pacifica*. The structures were elucidated by 1D and 2D NMR spectral analysis, and their cytotoxicity against selected cancer cells was measured in vitro.

Soft corals of the genus *Nephthea* are rich in terpenoids^{1–11} and steroids.¹² As part of our search for bioactive substances from marine organisms, the Formosan soft coral *Nephthea pacifica* Kükenthal (family Nephtheidae) was studied as CH₂Cl₂ extracts and showed significant cytotoxicity to A549 (human lung adenocarcinoma), HT-29 (human colon adenocarcinoma), and P-388 (mouse lymphocytic leukemia) cell cultures as determined by standard procedures.^{13,14} Bioassay-guided fractionation resulted in the isolation of 10 new prenylbicyclogermacrene diterpenoids, pacificins A–J (**1–10**).



Results and Discussion

Pacificin A (**1**) was isolated as a colorless amorphous solid. HREIMS, ¹³C NMR, and DEPT spectra established

the molecular formula of **1** as C₂₀H₃₄O₂, with four degrees of unsaturation. ¹³C NMR and DEPT spectra of **1** exhibited the presence of four methyls, seven sp³ methylenes, three sp³ methines, one sp² methine, three sp³ quaternary carbons, and one sp² quaternary carbon. The presence of two sp²-hybridized carbon atoms in the molecule, as deduced from the ¹³C and DEPT NMR spectra (Table 2), corresponding to one carbon–carbon double bond as the only double bond, indicated compound **1** to be tricyclic. The presence of a trisubstituted epoxy group in **1** was shown by the NMR data (δ_H 2.81 d; δ_C 61.9 qC, 66.2 CH) (Tables 1 and 2). The NMR data (δ_H 0.20 m, 0.38 m, 0.73 m, 1.10 m; δ_C 3.9 CH₂, 27.1 CH, 28.8 CH) (Tables 1 and 2) pointed to a cyclopropane ring in **1**. The ¹H NMR spectrum also contained signals for five tertiary methyl groups (δ_H 0.46, 0.73, 1.30, 1.58, 1.65). In addition, a signal at δ_H 5.06 was attributed to an olefinic proton and was confirmed by ¹³C NMR spectroscopy (δ_C 125.0 CH). The presence of an ambiguous carbon bearing an oxygen (δ_C 72.9 qC) was shown in the ¹³C NMR spectrum. The spectral data of **1** exhibited some similarity to those of a prenylbicyclogermacrene diterpenoid, palmatol, isolated from *Alcyonium palmatum*,¹⁵ except for the differences of chemical shifts in the vicinity of C-9/C-10. Measurement of the ¹³C–¹³C homonuclear shift correlation 2D spectrum (INADEQUATE) (Supporting Information) of **1** together with COSY, HMQC, and HMBC (Figure 1) experiments established its chemical structure and enabled also the assignment of all resonances in the NMR spectra. The relative stereochemistry of **1** was deduced from a 2D NOESY experiment (Figure 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. From these data, pacificin A can be formulated as **1**.

The molecular formula of pacificin B (**2**) proved to be C₂₀H₃₄O₃ by HREIMS and ¹³C NMR data. Detailed comparison of ¹H and ¹³C NMR spectral data (Tables 1 and 2) of **2** and **1** revealed that **2** differed from **1** in the side chain. COSY correlation between H-3/H-4 and H-4/H-5, HMBC correlations from H-1/H-17 to C-2/C-3 and H-5 to C-3/C-6/C-7, and a J_{3,4} of 15.6 Hz placed an *E* double bond between C-3 and C-4. The relative stereochemistry of **1** was determined by a 2D NOESY experiment, 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. From the aforementioned data, pacificin B can be formulated as **2**.

Pacificin C (**3**) had the molecular formula C₂₀H₃₄O₄, 16 mass units higher than that of **2**. The ¹H and ¹³C NMR

* To whom correspondence should be addressed. Tel: 886-7-525-2000, ext. 5036. Fax: 886-7-525-5020. E-mail: yihduh@mail.nsysu.edu.tw.

[†] National Sun Yat-sen University.

[‡] On leave from Faculty of Pharmacy, Mansoura University, Egypt.

[§] Kaohsiung Medical University.

[⊥] National Taiwan University.

Table 1. ¹H NMR Data of 1–10

H	1 ^a	2 ^a	3 ^a	4 ^a	5 ^a	6 ^b	7 ^a	8 ^a	9 ^a	10 ^a
1	1.65 s	1.33 s	1.34 s	4.86 s, 4.93 s	5.02 s, 5.04 s	1.23 s	1.35 s	5.01 s, 5.04 s	1.69 s	1.33 s
3	5.06 t (6.9)	5.63 d (15.6)	5.58 d (15.6)	4.00 t (6.9)	4.25 t (6.9)	5.59 d (15.9)	5.55 d (15.6)	4.23 t (6.9)	5.11 t (6.9)	5.61 d (15.6)
4	2.04 m	5.69 m	5.76 m	1.22 m	1.29 m	5.72 m	5.74 m	1.60 m	2.10 m	5.69 m
5	1.15 m, 1.28 m	1.96 m, 2.04 m	1.97 m, 2.07 m	1.21 m	1.11 m	1.86 m, 2.01 m	2.02 m	1.22 m	1.31 m	1.99 m
7	1.50 m, 1.74 m	1.56 m, 1.69 m	1.53 m, 1.68 m	1.49 m, 1.75 m	1.48 m, 1.74 m	1.40 m, 1.76 m	1.51 m, 1.74 m	1.44 m, 1.78 m	1.40 m, 1.91 m	1.42 m, 1.79 m
8	1.26 m, 2.01 m	1.23 m, 2.02 m	1.24 m, 2.05 m	1.27 m, 2.06 m	1.28 m, 2.06 m	1.96 m, 2.29 m	2.33 dt (2.4, 12.6)	2.36 dt (2.4, 12.6), 2.18 m	2.08 m, 2.31 m	2.08 m, 2.32 m
10	2.81 br d (10.2)	2.86 br d (10.5)	2.83 br d (9.9)	2.83 br d (9.9)	2.84 br d (8.4)	5.23 br d (11.1)	5.23 br d (10.8)	5.23 br d (10.5)	5.36 br d (10.5)	5.35 br d (11.1)
11	1.36 m, 1.96 m	1.40 m, 2.06 m	1.42 m, 2.05 m	1.36 m, 2.04 m	1.37 m, 2.05 m	1.99 m, 2.25 m	5.44 dt (4.5, 10.8)	5.44 dt (3.9, 10.5)	2.15 m, 2.34 m	2.14 m, 2.35 m
12	1.94 m	1.96 m	1.97 m	1.97 m	1.96 m	1.80 m	2.17 dd (11.4, 4.2), 2.04 m	2.13 dd (12.3, 3.9), 1.97 m	2.16 m, 2.48 m	2.17 m, 2.46 m
14	1.10 m	1.15 m	1.14 m	1.15 m	1.14 m	0.91 m	1.03 m	1.00 m	0.98 m	0.97 m
15	0.20 m, 0.38 m	0.30 m, 0.44 m	0.28 m, 0.43 m	0.23 m, 0.39 m	0.25 m, 0.43 m	0.23 m, 0.49 m	0.27 m, 0.41 m	0.23 m, 0.37 m	0.60 m, 0.68 m	0.64 m, 0.69 m
16	0.73 m	0.74 m	0.73 m	0.73 m	0.74 m	0.56 m	0.55 m	0.52 m	0.55 m	0.53 m
17	1.58 s	1.33 s	1.34 s	1.74 s	1.74 s	1.23 s	1.35 s	1.74 s	1.62 s	1.33 s
18	0.46 s	0.51 s	0.51 s	0.50 s	0.50 s	0.58 s	0.61 s	0.60 s	0.59 s	0.59 s
19	1.30 s	1.31 s	1.30 s	1.32 s	1.32 s	1.64 s	1.83 s	1.84 s	1.60 s	1.58 s
20	0.73 s	0.77 s	0.76 s	0.76 s	0.77 s	0.68 s	0.79 s	0.79 s	4.19 s, 4.58 s	4.17 s, 4.58 s
OAc							2.02 s	2.02 s		

^a Recorded in CDCl₃ at 300 MHz. ^b Recorded in acetone-*d*₆ at 300 MHz.

spectral data (Tables 1 and 2) closely resembled those of **2** except that the tertiary hydroxyl attached to C-2 was replaced by a hydroperoxide. HMBC correlations from H-1/H-17 to C-2/C-3 and H-5 to C-3/C-6/C-7 confirmed the position of the hydroperoxide. The relative stereochemistry of **3** was deduced from a 2D NOESY experiment, 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. From these data, pacificin C was formulated as **3**.

HREIMS and NMR data revealed pacificin D (**4**) to have a molecular formula of C₂₀H₃₄O₃. The ¹H and ¹³C NMR spectral data exhibited the presence of a terminal methylene (δ_{H} 4.86, 4.93; δ_{C} 111.8, 147.3) and a secondary hydroxyl (δ_{H} 4.00; δ_{C} 76.9). The ¹H and ¹³C NMR spectral data of **4** (Tables 1 and 2) closely resembled those of **1** except for NMR signals due to the side chain. HMBC correlations from H-17 to C-1/C-2/C-3 confirmed the 3-hydroxyisopentenyl side chain. The relative stereochemistry of **4** was established by a 2D NOESY experiment, 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, the structure of pacificin D was established as **4**.

Pacificin E (**5**) was isolated as a colorless resin of molecular formula C₂₀H₃₄O₄, as indicated by HRFABMS and NMR spectra. The ¹H and ¹³C NMR spectral data (Tables 1 and 2) were very close to those of **4** except that the tertiary hydroxyl attached to C-3 was replaced by a hydroperoxyl.¹⁶ HMBC correlations from H-17 to C-1/C-2/C-3 confirmed the position of the hydroperoxyl. The relative stereochemistry of **5** was deduced from a 2D NOESY experiment, 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. From the above data, pacificin E was thus formulated as **5**.

Pacificin F (**6**) was shown to have the molecular formula of C₂₀H₃₄O₂ by HREIMS and NMR spectra. The ¹H and ¹³C NMR spectral data (Tables 1 and 2) exhibited some similarity to those of **2** except that the trisubstituted epoxide was replaced by a *E*-trisubstituted double bond. HMBC correlations (Supporting Information) from H-11 to C-9/C-10/C-12/C-13 confirmed the position of the *E*-trisubstituted double bond. The relative stereochemistry of **6** was determined on the basis of a 2D NOESY experiment (Supporting Information), 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. From these data, pacificin F can be formulated as **6**.

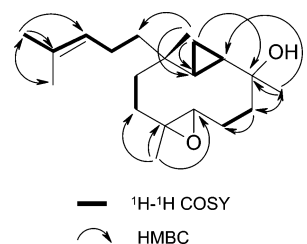
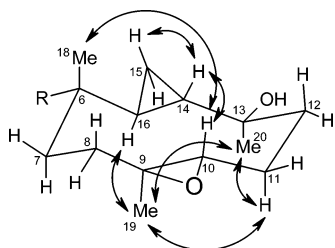
The molecular formula of pacificin G (**7**) was obtained from HRFABMS and NMR spectra. The ¹H and ¹³C NMR spectral data (Tables 1 and 2) resembled those of **6** except for NMR signals due to the side chain terminus and an additional acetoxy group on the 10-membered ring. 2D COSY correlation (H-10/H-11) and HMBC correlations from H-11 to C-9/C-10 confirmed the position of the acetoxy group. The side chain was identical to that of **3**. The relative stereochemistry of **7** was deduced from a 2D NOESY experiment (Supporting Information), 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 the aforementioned data, pacificin G can be formulated as **7**.

Pacificin H (**8**) was isolated as a colorless amorphous solid of molecular formula C₂₀H₃₆O₅, as established by HRFABMS and NMR spectra. The ¹H and ¹³C NMR

Table 2. ^{13}C NMR Spectral Data of **1**–**10**

	1 ^a	2 ^a	3 ^a	4 ^a	5 ^a	6 ^b	7 ^a	8 ^a	9 ^a	10 ^a
1	25.7	30.0	24.5	111.8	114.7	29.8	24.5	114.9	25.8	30.0
2	131.2	70.9	82.2	147.3	143.0	69.4	82.3	143.5	130.9	70.9
3	125.0	141.3	136.7	76.9	90.4	141.7	136.3	90.6	125.5	140.6
4	22.2	122.3	127.4	29.6	24.7	122.3	127.9	24.7	22.4	123.6
5	46.6	48.1	49.1	41.7	41.7	48.6	49.0	40.5	45.9	48.2
6	34.7	35.3	35.7	34.3	34.3	36.0	36.4	35.1	35.9	36.8
7	36.6	36.5	37.1	36.8	36.7	37.2	37.4	36.9	37.7	38.1
8	35.4	35.5	35.4	35.4	35.4	36.0	36.4	35.1	36.4	36.4
9	61.9	62.0	61.9	61.9	62.8	132.2	139.9	140.4	136.1	136.0
10	66.2	66.4	66.3	66.3	66.3	127.2	125.8	125.7	126.1	126.1
11	24.4	24.5	24.3	24.4	24.4	24.7	69.0	69.0	30.4	30.3
12	41.7	41.8	41.7	42.1	42.2	44.4	49.0	49.0	40.4	40.5
13	72.9	73.0	73.0	72.9	72.9	72.0	71.5	71.6	154.0	153.8
14	28.8	28.9	28.9	28.6	28.9	30.5	30.3	30.3	24.9	25.0
15	3.9	3.9	4.3	3.9	3.9	5.8	4.9	4.5	12.9	13.1
16	27.1	27.0	27.8	27.0	26.9	27.5	28.0	27.1	35.5	35.9
17	17.6	30.0	24.5	17.2	17.1	29.8	24.5	17.0	17.6	30.0
18	19.4	19.3	19.5	19.6	19.5	15.5	20.3	19.3	18.4	17.6
19	17.1	17.1	17.0	17.0	17.1	17.9	18.2	17.0	15.8	15.8
20	20.6	20.5	20.6	20.7	20.7	19.9	21.3	21.3	103.2	103.3
OAc							21.4	21.4		
							170.5	170.4		

^a Recorded in CDCl_3 at 75 MHz (assigned by DEPT, COSY, HSQC, and HMBC experiments). ^b Recorded in acetone- d_6 at 75 MHz (assigned by DEPT, COSY, HSQC, and HMBC experiments).

**Figure 1.** Key COSY and HMBC correlations of **1**.**Figure 2.** Selected NOESY correlations of **1**.

spectral data (Tables 1 and 2) were quite similar to those of **7** except for NMR signals due to the side chain. HMBC correlations from H-17 to C-1/C-2/C-3 confirmed the 3-hydroperoxyisopentenyl side chain. The relative stereochemistry of **8** was established by a 2D NOESY experiment, 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 H was formulated as **8**.

The ^1H and ^{13}C NMR spectral data (Tables 1 and 2) of pacificin H (**9**) were identical with those of an acetylation byproduct of an isolate from an octocoral *Alcyonium palmatum*.¹⁵ However, after our detailed analysis of the 2D NMR spectra of **9**, the ^1H and ^{13}C NMR chemical shifts at C-11, C-14, and C-16 should be revised as in Table 2. Compound **9** is a new natural product.

Pacificin I (**10**) analyzed for $\text{C}_{20}\text{H}_{32}\text{O}$ by mass spectrometry in combination with interpretation of ^{13}C NMR data. The ^1H and ^{13}C NMR spectral data (Tables 1 and 2) were analogous to those of **9** except for NMR signals due to the side chain. COSY correlation between H-3/H-4 and H-4/H-5, HMBC correlations from H-1/H-17 to C-2/C-3 and H-5

to C-3/C-6/C-7, and a $J_{3,4}$ of 15.6 Hz placed an *E* double bond between C-3 and C-4. The relative stereochemistry of **9** was determined by a 2D NOESY experiment, 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, the structure of pacificin I was established as **10**.

Pacificins C and H exhibited cytotoxicity against P-388 cells with ED_{50} 's of 1.44 and 2.01 $\mu\text{g}/\text{mL}$, respectively. The other isolates were inactive against P-388 and HT-29 cell lines.

Experimental Section

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 a Bruker Avance 300 NMR spectrometer at 300 MHz for ^1H and 75 MHz for ^{13}C , respectively, using TMS as internal standard. EIMS spectra were obtained with a JEOL JMS-SX/SX 102A mass spectrometer at 70 eV. Si gel 60 (Merck, 230–400 mesh) was used for column chromatography; precoated Si gel plates (Merck, Kieselgel 60 F₂₅₄, 0.25 mm) were used for TLC analysis.

Animal Material. The soft coral *N. pacifica* was collected at Green Island, off Taiwan, in March 2002, at a depth of 5 m and was stored for 1 week in a freezer until extraction. A voucher specimen, NSUGN-058, was deposited in the Department of Marine Resources, National Sun Yat-sen University, Taiwan.

Extraction and Isolation. The bodies of the soft coral *N. pacifica* were freeze-dried to give 1.10 kg of a solid, which was extracted with CH_2Cl_2 (3.0 L \times 3, overnight for each cycle) at room temperature. After removal of solvent in vacuo, the residue (47 g) was chromatographed over Si gel 60 using *n*-hexane–EtOAc and MeOH–EtOAc mixtures as eluting solvents. Elution by *n*-hexane–EtOAc (85:15) afforded fractions containing **9**. Elution by *n*-hexane–EtOAc (65:35) afforded fractions containing **1**, **7**, and **8**. Elution by *n*-hexane–EtOAc (55:45) afforded fractions containing **6** and **10**. Elution by *n*-hexane–EtOAc (35:65) afforded fractions containing **2**–**5**. Compound **1** (360 mg, 7.6%) was further purified by Si gel column chromatography, eluting with *n*-hexane–acetone (4:1). Compounds **2** (3 mg, 0.006%), **3** (3 mg, 0.006%), **4** (5 mg, 0.011%), and **5** (6 mg, 0.013%) were further purified by HPLC (LiChrosorb Si 60, 7 μm , 25 \times 250 mm), eluting with *n*-hexane–acetone (3:1). Compound **6** (3 mg, 0.006%) was further

purified by HPLC (LiChrosorb RP-18, 7 μm , 25 \times 250 mm), eluting with MeOH–H₂O (90:10). Compounds **7** (4 mg, 0.009%) and **8** (3 mg, 0.006%) were further purified by HPLC (LiChrosorb RP-18, 7 μm , 25 \times 250 mm), by eluting with MeOH–H₂O (73:27). Compound **9** (20 mg, 0.042%) was further purified by Si gel column chromatography, eluting with *n*-hexane–EtOAc (9:1). Compound **10** (4 mg, 0.009%) was further purified by HPLC (LiChrosorb RP-18, 7 μm , 25 \times 250 mm), eluting with MeOH–H₂O (90:10).

Pacificin A (1): $[\alpha]_{\text{D}}^{25} -62^\circ$ (*c* 0.2, CHCl₃); IR (neat) ν_{max} 3450 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; EIMS *m/z* 306 [M]⁺ (9), 288 (12), 270 (32), 81 (100); HREIMS *m/z* 306.2558 (calcd for C₂₀H₃₄O₂, 306.2550).

Pacificin B (2): $[\alpha]_{\text{D}}^{25} -53^\circ$ (*c* 0.2, CHCl₃); IR (neat) ν_{max} 3520 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; EIMS *m/z* 322 [M]⁺ (3), 304 (8), 81 (100); HREIMS *m/z* 322.2492 (calcd for C₂₀H₃₄O₃, 322.2499).

Pacificin C (3): $[\alpha]_{\text{D}}^{25} -46^\circ$ (*c* 0.1, CHCl₃); IR (neat) ν_{max} 3480 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; HRFABMS *m/z* 339.2529 (calcd for C₂₀H₃₅O₄, 339.2526).

Pacificin D (4): $[\alpha]_{\text{D}}^{25} -43^\circ$ (*c* 0.2, CHCl₃); IR (neat) ν_{max} 3490 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; EIMS *m/z* 322 [M]⁺ (3), 304 (6), 216 (12), 81 (100); HREIMS *m/z* 322.2495 (calcd for C₂₀H₃₄O₃, 322.2499).

Pacificin E (5): $[\alpha]_{\text{D}}^{25} -38^\circ$ (*c* 0.1, CHCl₃); IR (neat) ν_{max} 3510 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; HRFABMS *m/z* 339.2532 (calcd for C₂₀H₃₅O₄, 339.2526).

Pacificin F (6): $[\alpha]_{\text{D}}^{25} -51^\circ$ (*c* 0.2, CHCl₃); IR (neat) ν_{max} 3460 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; EIMS *m/z* 306 [M]⁺ (4), 288 (6), 271 (12), 215 (8), 189 (70), 95 (100); HREIMS *m/z* 306.2543 (calcd for C₂₀H₃₄O₂, 306.2550).

Pacificin G (7): $[\alpha]_{\text{D}}^{25} -26^\circ$ (*c* 0.2, CHCl₃); IR (neat) ν_{max} 3480, 1730 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; HRFABMS *m/z* 381.2638 (calcd for C₂₂H₃₇O₅, 381.2631).

Pacificin H (8): $[\alpha]_{\text{D}}^{25} -18^\circ$ (*c* 0.1, CHCl₃); IR (neat) ν_{max} 3550, 1732 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; HRFABMS *m/z* 381.2636 (calcd for C₂₂H₃₇O₅, 381.2631).

Pacificin I (9): $[\alpha]_{\text{D}}^{25} -28^\circ$ (*c* 0.1, CHCl₃); ¹H NMR, see Table 1; ¹³C NMR, see Table 2; EIMS *m/z* 272 [M]⁺ (9), 257 (12), 95 (100).

Pacificin J (10): $[\alpha]_{\text{D}}^{25} -22^\circ$ (*c* 0.2, CHCl₃); IR (neat) ν_{max} 3450 cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; EIMS *m/z* 288 [M]⁺ (3), 270 (6), 220 (12), 95 (100); HREIMS *m/z* 288.2440 (calcd for C₂₀H₃₂O, 288.2445).

Cytotoxicity Testing. P-388 cells were kindly supplied by J. M. Pezzuto, Department of Medicinal Chemistry and

Pharmacognosy, University of Illinois at Chicago; A549 and HT-29 were purchased from the American Type Culture Collection. Cytotoxic assays were carried out according to the procedure described previously.¹⁴ Three concentrations (50, 5, and 0.5 $\mu\text{g/mL}$) of the tested compounds were used in the cytotoxicity assays.

Acknowledgment. We thank J. M. Pezzuto, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, for the provision of P-388 cell lines. This work was supported by grants from the National Science Council of Taiwan awarded to C.-Y.D.

Supporting Information Available: ¹H NMR, ¹³C NMR, COSY, HSQC, HMBC, and ¹³C–¹³C homonuclear shift correlation 2D spectrum (INADEQUATE) of **1**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References and Notes

- Coll, J. C.; Bowden, B. F.; Tapiolas, D. M.; Willis, R. H. *Tetrahedron* **1985**, *41*, 1085–1092.
- Poet, S. E.; Ravi, B. N. *Aust. J. Chem.* **1982**, *35*, 77–83.
- Ahond, A.; Bowden, B. F.; Coll, J. C.; Fourneron, J.; Mitchell, S. J. *Aust. J. Chem.* **1981**, *34*, 2657–2664.
- Blackman, A. J.; Bowden, B. F.; Coll, J. C.; Frick, B.; Mahendran, M.; Mitchell, S. J. *Aust. J. Chem.* **1982**, *35*, 1873–1880.
- Kitagawa, I.; Cui, Z.; Son, B. W.; Kobayashi, M.; Kyogoku, Y. *Chem. Pharm. Bull.* **1987**, *35*, 124–135.
- Bowden, B. F.; Coll, J. C.; Mitchell, S. J. *Aust. J. Chem.* **1980**, *33*, 1833–1839.
- Handayani, D.; Edrada, R. A.; Proksch, P.; Wray, V.; Witte, L. J. *Nat. Prod.* **1997**, *60*, 716–718.
- Duh, C.-Y.; Wang, S.-K.; Weng, Y.-L. *Tetrahedron Lett.* **2000**, *41*, 1401–1404.
- Duh, C.-Y.; Wang, S.-K.; Weng, Y.-L.; Chiang, M. Y.; Dai, C.-F. *J. Nat. Prod.* **1999**, *62*, 1518–1521.
- Rao, M. R.; Venkatesham, U.; Venkateswarlu, Y. *J. Nat. Prod.* **1999**, *62*, 1584–1585.
- Zhang, W.-H.; Williams, I. D.; Che, C.-T. *Tetrahedron Lett.* **2001**, *42*, 4681–4686.
- Duh, C.-Y.; Wang, S.-K.; Chu, M.-J.; Sheu, J.-H. *J. Nat. Prod.* **1998**, *61*, 1022–1024.
- Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep.* **1972**, *3*, 1–91.
- Hou, R.-S.; Duh, C.-Y.; Chiang, M. Y.; Lin, C.-N. *J. Nat. Prod.* **1995**, *58*, 1126–1130.
- Zubia, E.; Spinella, A.; Guisto, G. B.; Crispino, A.; Cimino, G. *Tetrahedron Lett.* **1994**, *35*, 7069–7072.
- Sheu, J.-H.; Huang, S.-Y.; Duh, C.-Y. *J. Nat. Prod.* **1996**, *59*, 23–26.

NP040160E