JOURNAL OF NATURAL PRODUCTS

ARTICLE

pubs.acs.org/jnp

Paraminabeolides A−F, Cytotoxic and Anti-inflammatory Marine Withanolides from the Soft Coral *Paraminabea acronocephala*

Chih-Hua Chao,[†] Kuei-Ju Chou,[†] Zhi-Hong Wen,[†,‡] Guey-Horng Wang,[§] Yang-Chang Wu,[⊥] Chang-Feng Dai,[∥] and Jyh-Horng Sheu*[,†,‡]

[†]Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung 804, Taiwan, Republic of China

[‡]Asia-Pacific Ocean Research Center, National Sun Yat-sen University, Kaohsiung 804, Taiwan, Republic of China

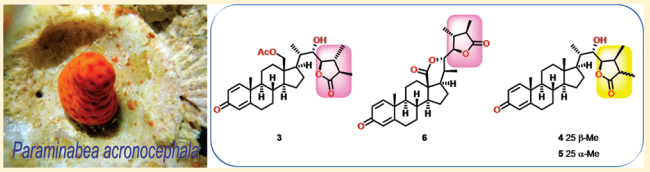
[§]Department of Cosmetic Science, Chia-Nan University of Pharmacy and Science, Tainan 717, Taiwan, Republic of China

[⊥]College of Chinese Medicine, China Medical University, Taichung 404, Taiwan, Republic of China

[∥]Institute of Oceanography, National Taiwan University, Taipei, 106, Taiwan, Republic of China

Supporting Information

ABSTRACT:



Six new withanolides, paraminabeolides A−F (**1**−**6**), along with five known compounds, minabeolides-1, -2, -4, -5, and -8 (**7**−**11**), were isolated from a Formosan soft coral, *Paraminabea acronocephala*. The structures of these compounds were elucidated by extensive spectroscopic analysis and chemical transformation. The absolute configuration of **4** was determined by the application of Mosher's method. Compounds **1** and **7** were cytotoxic toward Hep G2 cancer cells. Compounds **1**−**4** and **7**−**10** were found to significantly inhibit the accumulation of the pro-inflammatory iNOS protein. Compounds **7**−**10** also could effectively reduce the expression of COX-2 protein.

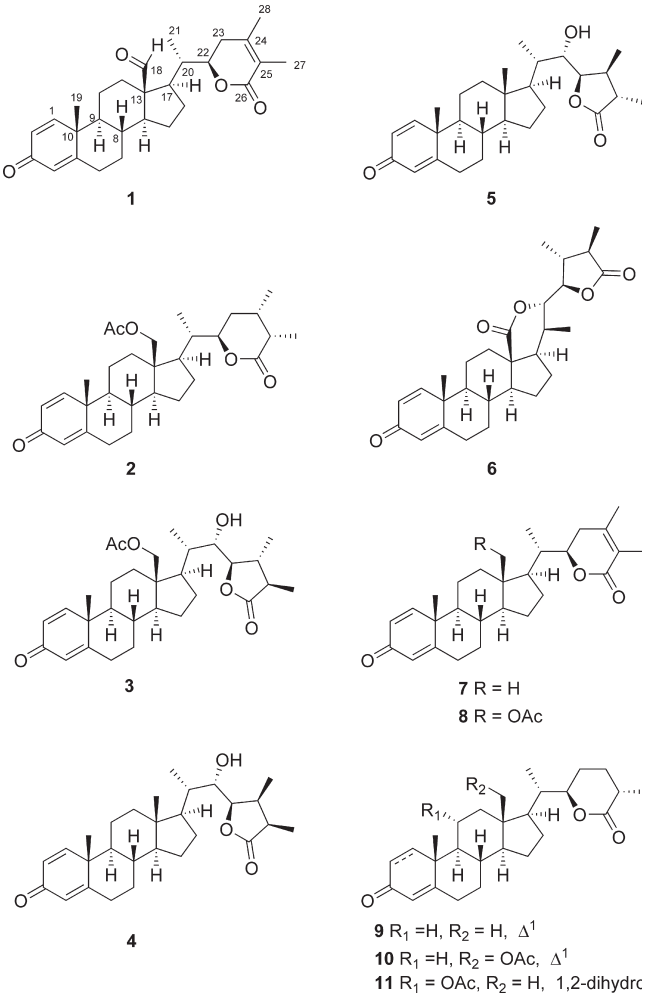
Withanolides are a group of naturally occurring steroidal lactones, in which a C-22/C-26 δ-lactone is generally present in the side chain, although in some cases a C-23/C26 γ-lactone might occur. Withaferin A,[1] isolated from the leaves of *Withania somnifera*, was discovered as the first member of this group. Withanolides have received considerable attention due to their versatile biological activities, such as antitumor,[2] cytotoxic,[3] immunosuppresive,[3,4] antimicrobial,[5] anti-inflammatory,[6] and chemopreventive[7] activities. The minabeolides, isolated from the soft coral *Minabea* sp.,[8] represent the first class of marine withanolides. Although they were discovered more than two decades ago, no biological activities have been reported for the minabeolides. This prompted us to investigate new structures and the biological activities for compounds of this class from marine organisms. In the course of this study, six new withanolides (**1**−**6**) and five known compounds, minabeolides-1, -2, -4, -5, and -8 (**7**−**11**), were isolated from the Formosan soft coral *Paraminabea acronocephala*. The new structures were established by extensive spectroscopic analysis. The absolute configuration at C-22 of **4** was determined by the application of Mosher's method. The cytotoxicity of compounds **1**−**11** against human liver carcinoma (HepG2 and HepG3), human breast carcinoma (MCF-7 and MDA-MB-231), and human lung carcinoma (A-549) cell lines and the ability of **1**−**5** and **7**−**11** to inhibit up-regulation of the pro-inflammatory iNOS (inducible nitric oxide synthase) and COX-2 (cyclooxygenase-2) proteins in LPS (lipopolysaccharide)-stimulated RAW264.7 macrophage cells were evaluated.

RESULTS AND DISCUSSION

The ethanolic extract of the soft coral *P. acronocephala* was partitioned between EtOAc and H_2O to afford the EtOAc-soluble fraction, which was subjected to silica gel column chromatography. The fractions containing steroids were selected on the basis of characteristic peaks of methyl groups in the ^{1}H NMR spectra. These fractions were subsequently subjected to a series of chromatographic separations to afford six new withanolides, paraminabeolides A−F (**1**−**6**), and five known compounds (**7**−**11**).[8] The single-crystal X-ray diffractions analysis (Figure 1)[9] for both known compounds **9** and **11** further confirmed the structures of these two metabolites.

The HRESIMS spectrum of paraminabeolide A (**1**) exhibited a pseudomolecular ion peak at m/z 459.2509 $[M + Na]^+$, consistent with a molecular formula of $C_{28}H_{36}O_4$, appropriate for 11 degrees of unsaturation. The ^{13}C NMR and DEPT

Received: January 25, 2011
Published: March 22, 2011



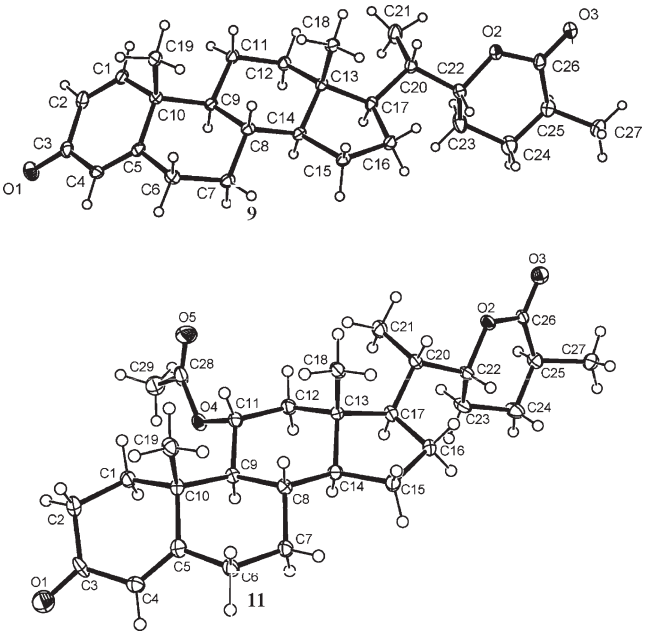


Figure 1. X-ray ORTEP drawings of compounds **9** and **11**.

spectroscopic data (Table 1) displayed 28 carbon signals, including four methyls, seven methylenes, 10 methines, and seven quaternary carbons. The IR spectrum revealed the presence of carbonyl (1705 and 1659 cm^{-1}) groups. The presence of an aldehyde was evidenced by the downfield singlet of a proton at δ 9.86 (Table 2), which correlates to a carbon signal at δ 205.9 in the HSQC spectrum. The carbon resonances at δ 186.3 (C), 155.3 (CH), 127.7 (CH), 124.1 (CH), and 168.4 (C) as well as the proton resonances at δ 7.01 (1H, d, J = 10.4 Hz), 6.23 (1H, d, J = 10.4 Hz), and 6.08 (1H, s) were characteristic signals of steroids with a 1,4-dien-3-one moiety in ring A.[10] In addition, proton resonances at δ 4.35 (1H, dt, J = 13.6, 4.0 Hz), 1.94 (3H, s), and 1.88 (3H, s) and carbon resonances at δ 166.5 (C), 148.5 (C), 122.2 (C), 77.7 (CH), 20.5 (CH_3), and 12.3 (CH_3) were characteristic of an α,β-unsaturated δ-lactone, as compared to minabeolide-1 (**7**).[8] The $^1H-^1H$ COSY correlation between H-1/H-2 and HMBC correlations from H_3-19 to C-1, C-5, C-9, and C-10 further confirmed the 1,4-dien-3-one moiety in ring A. The HMBC correlations from H_3-27 to C-24, C-25, and C-26 as well as from H_3-28 to C-23, C-24, and C-25 corroborated the presence of an unsaturated δ-lactone moiety. A comparison of the NMR spectroscopic data of **1** with those of **7**[8] disclosed that the methyl group attached at C-13 in **7** was replaced by an aldehyde in **1**. This was further supported by the more downfield shift of C-13 (δ 59.5, C) and the HMBC correlation from the aldehyde proton to this carbon. The coupling constants and splitting patterns of the side chain moiety of **1** are close to those of **7**, suggesting that both compounds have the same configuration at C-22. This was further evidenced by a comparison of the CD data of **1** with those of parasorbic acid and related analogues.[11] The CD spectrum of **1** exhibited a positive Cotton effect at 248 nm, indicating that **1** should have a 22*R* configuration.[11]

HRESIMS analysis of paraminabeolide B (**2**) provided a molecular formula of $C_{30}H_{42}O_5$. The NMR spectroscopic data of **2** resembled those of minabeolide-2 (**8**), with differences in the side chain moiety. The appearance of an IR absorption band at 1736 cm^{-1}, two doublets of secondary methyls (δ 1.02 and 1.22) in the ^1H NMR spectrum (Table 2), and the carbon signals from C-22 to C-28 (Table 1) suggested the presence of a saturated δ-lactone in **2**. The relative configuration of C-20/C-22 was elucidated on the basis of analysis of the $^3J_{H,H}$ coupling constants and NOE correlations. As illustrated in Figure 2, a small coupling constant (3.2 Hz) between H-20 and H-22 suggested a *gauche* conformation for both protons. Furthermore, NOE correlations between H_3-21/H-23$_{ax}$, H-17/H-23$_{eq}$, H_2-16/H-22, and H-22/H-20 suggested a 20*S*,22*R* configuration, as revealed in Figure 2. In the NOESY spectrum of **2**, correlations between H-22/H_3-28, H-23$_{ax}$/H-25, and H-24/H_2-23 suggested that the two methyl groups (H_3-27 and H_3-28) are *cis* to H-22, an orientation discovered for the first time in natural withanolides.[12] In order to further confirm the above elucidation, unsaturated δ-lactone **8** was hydrogenated to yield the 1,2,4,5β,24,25-hexahydro derivative **8a** (Scheme 1),[13] of which the configurations at C-5 and the lactone moiety were deduced by the analysis of NOE correlations and comparison of CD data with known analogues. The two lactonic methyls H_3-27 and H_3-28 in **8a** were deduced as *trans* to H-22 by the analysis of NOE correlations as shown in Figure 2. The CD data of **8a** showed negative Cotton effects at 290 and 215 nm,[14] confirming the 5*R* and the 22*R*,24*R*,25*R* configurations, respectively. Consequently, the side chain moiety in **2** was determined as depicted.

Analysis of the HREIMS and ^{13}C NMR spectroscopic data of paraminabeolide C (**3**) suggested a molecular formula of

dx.doi.org/10.1021/np2000705 |J. Nat. Prod. 2011, 74, 1132–1141

Table 1. ^{13}C NMR Spectroscopic Data of Compounds 1−6

position	1,[a] δ_C, mult.	2,[a] δ_C, mult.	3,[b] δ_C, mult.	3,[c] δ_C, mult.	4,[a] δ_C, mult.	4,[d] δ_C, mult.	5,[a] δ_C, mult.	6,[b] δ_C, mult.
1	155.3, CH	155.5, CH	155.5, CH	155.7, CH	155.9, CH	156.1, CH	155.9, CH	155.9, CH
2	127.7, CH	127.6, CH	127.6, CH	127.8, CH	127.5, CH	127.7, CH	127.5, CH	127.5, CH
3	186.3, C	186.3, C	186.3, C	185.7, C	186.4, C	185.9, C	186.5, C	186.4, C
4	124.1, CH	124.0, CH	124.0, CH	124.2, CH	123.8, CH	124.0, CH	123.8, CH	123.9, CH
5	168.4, C	168.6, C	168.7, C	168.8, C	169.3, C	169.3, C	169.3, C	169.3, C
6	32.4, CH_2	32.6, CH_2	32.7, CH_2	32.7, CH_2	32.9, CH_2	32.8, CH_2	32.9, CH_2	32.7, CH_2
7	33.5, CH_2	33.6, CH_2	33.5, CH_2	33.8, CH_2	33.5, CH_2	33.8, CH_2	33.5, CH_2	33.7, CH_2
8	37.5, CH	35.6, CH	35.7, CH	35.7, CH	35.6, CH	35.5, CH	35.6, CH	34.6, CH
9	52.0, CH	52.3, CH	52.0, CH	52.5, CH	52.1, CH	52.5, CH	52.0, CH	52.2, CH
10	43.3, C	43.4, C	43.4, C	43.6, C	43.6, C	43.7, C	43.6, C	43.7, C
11	23.8, CH_2	22.6, CH_2	22.7, CH_2	23.0, CH_2	22.8, CH_2	23.0, CH_2	22.8, CH_2	21.8, CH_2
12	33.4, CH_2	34.5, CH_2	34.5, CH_2	35.0, CH_2	39.2, CH_2	39.5, CH_2	39.2, CH_2	33.9, CH_2
13	59.5, C	45.9, C	45.9, C	46.3, C	43.0, C	43.1, C	43.0, C	51.3, C
14	54.9, CH_2	54.7, CH_2	54.3, CH_2	54.6, CH_2	54.6, CH_2	54.9, CH_2	54.6, CH_2	55.2, CH_2
15	24.8, CH_2	24.2, CH_2	24.3, CH_2	24.5, CH_2	24.5, CH_2	24.7, CH_2	24.5, CH_2	24.8, CH_2
16	28.2, CH_2	26.8, CH_2	27.5, CH_2	28.1, CH_2	27.8, CH_2	28.3, CH_2	27.8, CH_2	22.6, CH_2
17	51.5, CH	52.3, CH	52.5, CH	53.3, CH	51.6, CH	52.2, CH	51.7, CH	50.3, CH
18	205.9, CH	62.0, CH_2	62.4, CH_2	62.7, CH_2	11.8, CH_3	11.9, CH_3	11.9, CH_3	172.5, C
19	18.6, CH_3	18.7, CH_3	18.8, CH_3	18.7, CH_3	18.7, CH_3	18.6, CH_3	18.7, CH_3	18.8, CH_3
20	39.5, CH	39.5, CH	42.6, CH	43.5, CH	42.6, CH	43.3, CH	42.8, CH	28.8, CH
21	13.3, CH_3	12.7, CH_3	13.6, CH_3	14.4, CH_3	13.4, CH_3	13.9, CH_3	11.9, CH_3	16.2, CH_3
22	77.7, CH	78.7, CH	76.1, CH	75.9, CH	70.4, CH	69.6, CH	70.4, CH	81.3, CH
23	29.9, CH_2	28.8, CH_2	82.8, CH	83.9, CH	79.0, CH	80.1, CH_2	79.3, CH	84.8, CH
24	148.5, C	30.2, CH	42.6, CH	43.4, CH	37.0, CH	37.6, CH	40.1, CH	40.0, CH
25	122.2, C	40.8, CH	42.6, CH	42.9, CH	40.0, CH	40.2, CH	42.2, CH	42.9, CH
26	166.5, C	175.1, C	178.9, C	179.1, C	179.2, C	179.5, C	179.6, C	177.7, C
27	12.3, CH_3	13.7, CH_3	13.6, CH_3	13.9, CH_3	9.7, CH_3	10.1, CH_3	14.0, CH_3	13.4, CH_3
28	20.5, CH_3	14.1, CH_3	17.6, CH_3	17.9, CH_3	8.3, CH_3	8.4, CH_3	13.6, CH_3	17.5, CH_3
OAc		171.2, C	171.2, C	170.9, C				
		21.1, CH_3	21.2, CH_3	20.9, CH_3				

[a] Spectra were measured in $CDCl_3$ (100 MHz). [b] Spectra were measured in $CDCl_3$ (125 MHz). [c] Spectra were measured in pyridine-d_5 (125 MHz). [d] Spectra were measured in pyridine-d_5 (100 MHz).

$C_{30}H_{42}O_6$, representing one more oxygen atom than the molecular formula of **2**. Compounds **2** and **3** were found to have the same substituent patterns in rings A−D, as concluded by comparison of their 1D and 2D NMR spectroscopic data. Furthermore, it was found that the chemical shift of the lactone carbonyl carbon of **3** was shifted downfield (Table 1) as compared to that of **2**, suggesting the presence of a five-membered lactone ring in **3**[7] rather than a six-membered lactone ring. This was further confirmed by the IR absorption band at 1772 cm^{-1}. All of the evidence indicated that compound **3** varied from **2** only in their respective side chains. The C-22 to C-28 moiety of **3** was found to be quite similar to that of subtrifloralactones F and G, isolated previously from the plant *Deprea subtriflora*.[7] Interpretation of the 2D NMR spectroscopic data of **3** confirmed the above elucidation and thus established its planar structure.

The relative configuration of **3** was elucidated on the basis of analysis of $^3J_{H,H}$ coupling constants and NOE correlations. In the NOESY spectrum of **3**, both H_3-19 and H-18a show NOE correlations with H-8, suggesting they are all β-oriented. In addition, NOE correlations between H-12β/H-17, H-12α/H-17, and H-14/H-17 revealed that the relative configuration for the steroidal nucleus of **3** is the same as the known minabeolides (**7**−**11**).[8] A small coupling constant between H-20 and H-22 (2.0 Hz, $CDCl_3$) suggested a *gauche* conformation of these two protons (rotamer a, Figure 3). The 20*S*,22*S* configuration was suggested according to the diagnostic NOE correlations between H-20/22-OH, H_3-21/22-OH, H-23/H_3-21, H-17/H-23, and H_2-16/H-22 (Figure 3). In the same manner, a large $^3J_{H,H}$ value (8.0 Hz) between H-22 and H-23 led to the establishment of two possible rotamers, b (22*S*,23*R*) and c (22*S*,23*S*), as illustrated in Figure 3. Among these two rotamers, only rotamer b coincidentally satisfies the NOE correlations between H-22/H-24, H_3-28/22-OH, and H-23/H-17. In addition, NOE correlations between H-23/H-25 and H-23/H_3-28 suggested the α-orientation of H_3-28 and the β-orientation of H_3-27 (Figure 4). Consequently, the 20*S*,22*S*,23*R*,24*R*,25*R* configuration was determined for **3**.

Paraminabeolide D (**4**) was assigned a molecular formula of $C_{28}H_{40}O_4$, according to the HRESIMS and NMR spectroscopic data (Tables 1 and 3). A comparison of spectroscopic data of **4** with those of **7** indicated that they differ only in the nature of the side chains. In addition, the substituent pattern in the side chain moiety of **4** was similar to that of **3**, but with the differences in the ^{13}C shifts (C_5D_5N, Table 1) of the C22−C28 moiety, such as C-22 (69.6 for **4**; 75.9 for **3**), C-23 (80.1 for **4**; 83.9 for **3**), C-27 (10.1 for **4**; 13.9 for **3**), and C-28 (8.4 for **4**; 17.9 for **3**). The two

dx.doi.org/10.1021/np2000705 |*J. Nat. Prod.* 2011, 74, 1132–1141

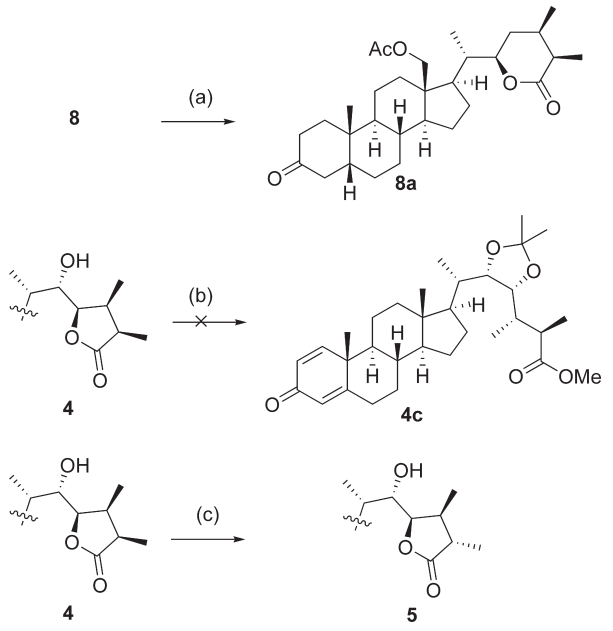
Table 2. ^{1}H NMR Spectroscopic Data of Compounds 1 and 2

position	**1**, δ_H (J in Hz)[a]	**2**, δ_H (J in Hz)[a]
1	7.01, d (10.4)	7.03, d (10.4)
2	6.23, d (10.4)	6.24, d (10.4)
4	6.08, s	6.08, s
6	a: 2.45, m	a: 2.47, m
	b: 2.36, m	b: 2.37, m
7	a: 2.03, m	a: 1.97, m
	b: 1.04, m	b: 1.02, m
8	1.58, m	1.72, m
9	1.07, m	1.08, m
11	a: 1.80, m	a: 1.74, m
	b: 1.60, m	b: 1.60, m
12	a: 2.67, dt (13.2, 3.2)	a: 2.41, m
	b: 1.12, m	b: 1.10, m
14	1.44, m	1.20, m
15	a: 1.90, m	a: 1.70, m
	b: 1.82, m	b: 1.19, m
16	a: 2.02, m	a: 1.77, m
	b: 1.70, m	b: 1.55, m
17	1.61, m	1.21, m
18	9.86, s	a: 4.33, d (12.0)
		b: 3.89, d (12.0)
19	1.14, s	1.24, s
20	1.94, m	2.09, m
21	1.02, d (7.2)	1.05, d (6.8)
22	4.35, dt (13.6, 4.0)	4.60, dt (12.4, 3.2)
23	a: 2.40, m	a: 1.86, ddd (13.2, 13.2, 3.2)
	b: 1.93, m	b: 1.58, m
24		2.18, m
25		2.54, m
27	1.88, s	1.22, d (7.2)
28	1.94,z s	1.02, d (7.2)
OAc		2.10, s

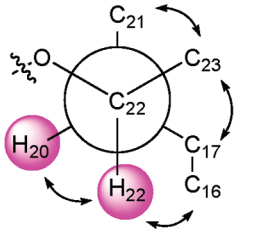
[a] Spectra were measured in $CDCl_3$ (400 MHz).

methyl groups (C-27 and C-28) of the lactone ring in **4** displayed carbon chemical shifts smaller than 10.1 ppm, revealing a *cis* geometry of these two protons.[15] Also, the large upfield shift (9.5 ppm, C_5D_5N, Table 1) of the C-28 signal in **4**, relative to that in **3**, might arise from the steric proximity of C-28 to both the C-27 and C-22 groups, as the C-27 signal in **4** was upfield-shifted by only 3.8 ppm. Thus, both H_3-27 and H_3-28 were suggested to be

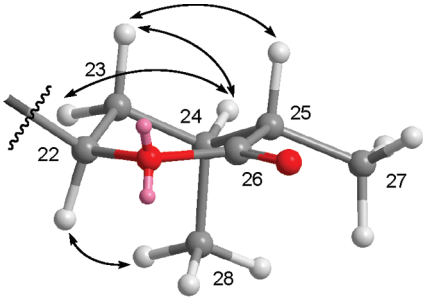
Scheme 1. Chemical Conversions of 8 and 4[a]



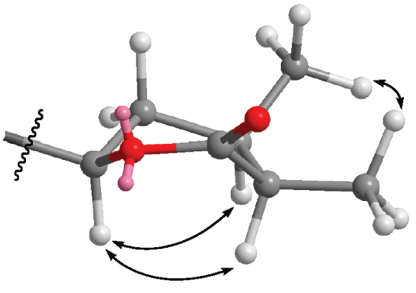
[a] Conditions: (a) Pd/C, H_2, 1 atm, pyridine; (b) $Me_2C(OMe)_2$, catalytic *p*-TsOH, MeOH, rt; (c) $Me_2C(OMe)_2$, catalytic *p*-TsOH, MeOH, 60 °C.



C-20/C-22 rotamer of **2**



δ-lactone moiety of **2**



δ-lactone moiety of **8a**

Figure 2. Selected NOE correlations of C-20/C-22 rotamer of **2** (left) and the δ-lactone moieties of **2** and **8a** (right).

dx.doi.org/10.1021/np2000705 |*J. Nat. Prod.* 2011, 74, 1132–1141

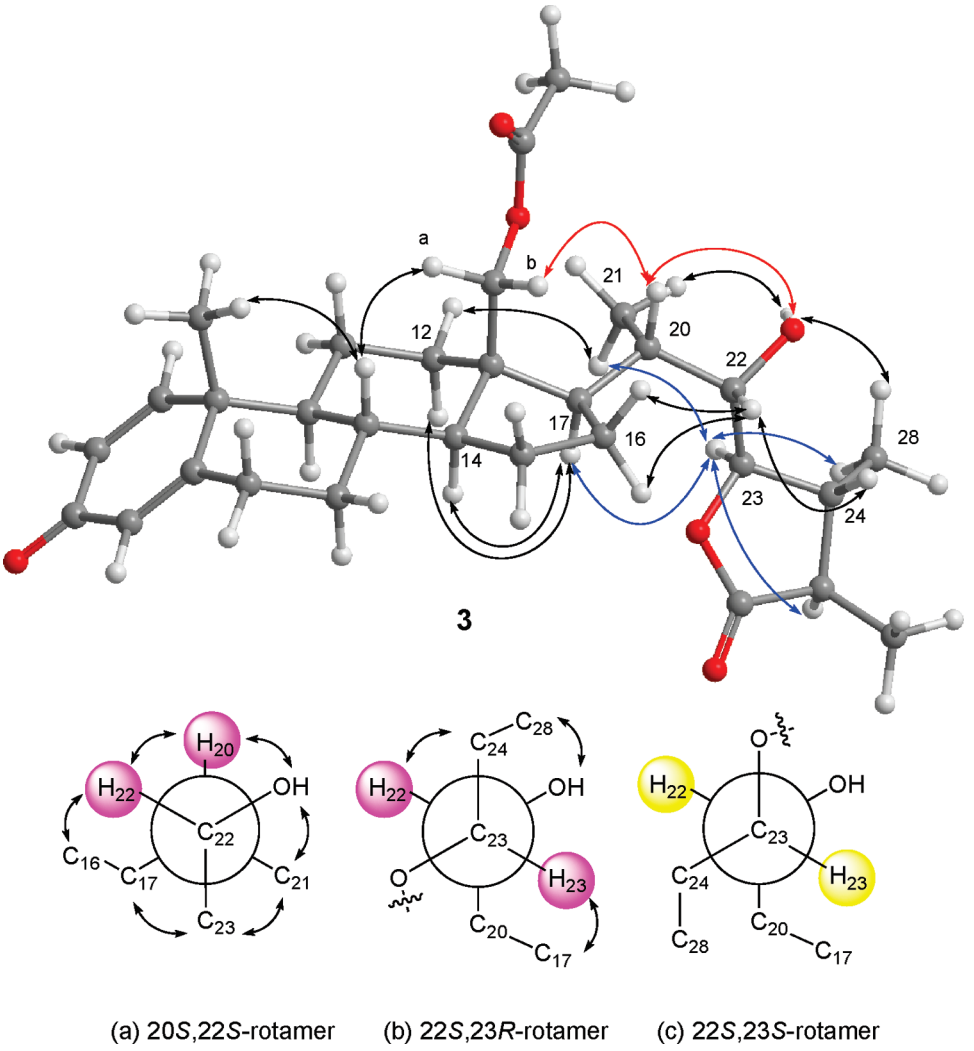


Figure 3. Selected NOE correlations (top) and Newman projections (bottom) for (a) C-20/C-22 and (b and c) C-22/C-23 rotamers of compound **3**.

trans oriented to H-23 (Figure 4). This was further confirmed by the comparison of NOE correlations for the side chain moieties in both **3** and **4**, as shown in Figure 4. The absolute configuration at C-22 was determined by the application of Mosher's method.[16] The (*S*)- and (*R*)-MTPA esters of **4** (**4a** and **4b**, respectively) were prepared using the corresponding (*R*)- and (*S*)-MTPA chlorides, respectively. The determination of chemical shift differences for the protons neighboring C-22 led to the assignment of the 22*S* configuration in **4** (Figure 4). Furthermore, the significant differences between the NOE correlations of **3** and **4** could be observed for those between H-23/H-24 and H-22/H_3-28 in **4**, in contrast to those between H-22/H-24 and H-23/H_3-28 in **3** (Figure 4). Accordingly, the 22*S*,23*R*,24*S*,25*R* configuration was suggested for **4**.

Paraminabeolide E (**5**) gave the same molecular formula as that of **4**, based on the interpretation of the HRESIMS and ^{13}C NMR spectroscopic data (Table 1). A comparison of NMR spectroscopic data of **5** with those of **4** revealed that they possess an epimeric lactone ring. Likewise, the *trans* location for the two methyls, C-27 (δ 14.0) and C-28 (δ 13.6), in **5** was readily deduced according to their more downfield chemical shifts, as compared to the relevant data in **4** and in the literature.[15] The ^{13}C NMR spectroscopic data for the lactone moiety in **5** were different from those in **3**, although the H_3-27 and H_3-28 are in *trans* location in both compounds. The differences in relative configurations for the lactone moieties between compounds **3** and **5** could be characterized by the analysis of their NOE correlations. In the NOESY spectrum of **5**, NOE correlations between H-23/H_3-27, H-23/H_3-21, H-23/H-24, and H-22/H_3-28 suggested the β-orientations of H-22/H_3-28 and the α-orientations of H_3-21, H-23, and H_3-27 (Figure 4). Furthermore, the absolute configuration for compound **5** was deduced by an unexpected but reasonable chemical transformation from **4** to **5** in an attempt to prepare the acetonide derivative of **4** by a known reaction under acidic reaction conditions.[17] The reaction to obtain the desired compound **4c** was unsuccessful at room temperature (Scheme 1). By raising the reaction temperature to 60 °C, we obtained an unexpected product, which is identical to **5**. Accordingly, compound **5** was determined as the C-25 epimer of **4**.

The molecular formula of paraminabeolide F (**6**) was found to be $C_{28}H_{36}O_5$, as deduced from HRESIMS and ^{13}C NMR data, appropriate for 11 degrees of unsaturation. The IR absorption bands showed the absence of hydroxy groups and the presence of an ester carbonyl (1722 cm^{-1}) and a γ-lactone (1779 cm^{-1}). The γ-lactone unit was confirmed by the carbon resonances at δ

dx.doi.org/10.1021/np2000705 |*J. Nat. Prod.* 2011, 74, 1132–1141

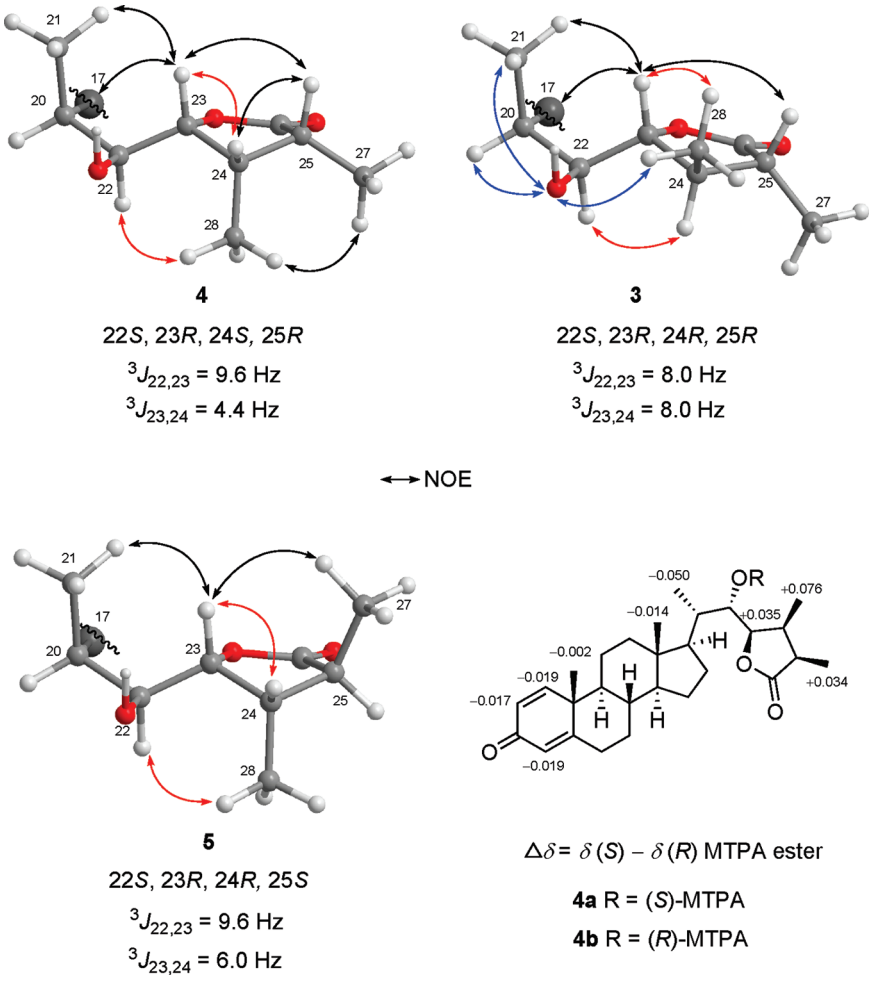


Figure 4. Comparison of NOE correlations for the side chain moieties of **3**−**5** and ^{1}H NMR chemical shift differences of MTPA esters of **4**.

84.8 (C-23, CH) and 177.7 (C-26, C) (Table 2). A comparison of NMR spectroscopic data of **6** with those of **3**−**5** revealed that **6** has a similar steroidal nucleus to those in **3**−**5**; however, differences in the carbon resonances of C-18 (δ 172.5, C) and C-22 (δ 81.3, C) in **6** disclosed the presence of a δ-lactone linkage between these two carbons. This was further confirmed by an IR absorption band at 1722 cm^{-1}. The presence of NOE correlations between H_3-19/H-8 and H-14/H-17 and the absence between H_3-19/H-9 suggested a common configuration for the steroidal nucleus (Figure 5). The ^{13}C NMR shifts for the methyl groups (H_3-27 and H_3-28) of the γ-lactone ring in **6** were found to be consistent with those in **3**, which was further confirmed by an NOE correlation between H-23/H-25 (Figure 5). This suggested that the relative configuration of the γ-lactone ring in **6** is the same as that in **3**. A large coupling constant (9.0 Hz) between H-22 and H-20 suggested the pseudoaxial orientation for these two protons. In addition, the presence of NOE correlations between H-17/H-20 and H_3-21/H-22 and the absence of correlations between H-22/H-20 suggested the 20*S*,22*S* configuration. The absolute configuration at C-23 of **6** could be suggested to be the same as that of **3** by biogenetic considerations.

The cytotoxicities of compounds **1**−**11** against HepG2, Hep3B, MDA-MB-231, MCF-7, and A-549 cancer cells were evaluated. The data revealed that compounds **1** and **7** showed selective cytotoxicity toward HepG2 cancer cells with IC_{50} values of 8.0 and 5.2 μM, respectively, while **7** showed weak cytotoxicity toward MCF-7 cancer cells with an IC_{50} value of 18.7 μM. Also, **2** showed weak cytotoxicity toward MDA-MB-231 and MCF-7 cancer cells with IC_{50} values of 19.3 and 14.9 μM, respectively. We also investigated the inhibition of compounds **1**−**5** and **7**−**11** toward LPS-induced pro-inflammatory protein (iNOS and COX-2) expression in RAW264.7 macrophage cells by Western blot analysis (Figure 6). At a concentration of 10 μM, compounds **1**−**4** and **7**−**10** significantly reduced the levels of iNOS to 11.0 ± 7.7%, 7.3 ± 1.0%, 37.9 ± 9.9%, 43.4 ± 9.5%, 9.6 ± 1.9%, 45.7 ± 7.7%, 23.2 ± 4.6%, and 6.3 ± 1.5%, respectively, while compounds **7**−**10** significantly reduced the levels of COX-2 to 18.3 ± 7.2%, 51.2 ± 11.5%, 22.4 ± 9.9%, and 31.3 ± 10.7%, respectively, in comparison with those of control cells stimulated with LPS only (100% for both iNOS and COX-2). However, a decrease of β-actin (66.5 ± 11.4% relative to the control group) occurred at 10 μM of compound **1**, revealing that it may exhibit cytotoxicity against the tested macrophage cells.

Withanolides with a saturated lactone ring in the side chain are common in plants.[7,12,13] The withanolides with a saturated δ-lactone ring from plants have all been found to possess 24α,25β-dimethyl substituents. Some semisynthetic withanolides with a saturated δ-lactone, prepared by hydrogenation of the

dx.doi.org/10.1021/np2000705 |*J. Nat. Prod.* 2011, 74, 1132–1141

Table 3. ^{1}H NMR Spectroscopic Data of Compounds 3−6

position	3, δ_H (J in Hz)[a]	3, δ_H (J in Hz)[b]	4, δ_H (J in Hz)[c]	4, δ_H (J in Hz)[d]	5, δ_H (J in Hz)[c]	6, δ_H (J in Hz)[a]
1	7.02, d (10.5)	7.01, d (10.0)	7.05, d (10.0)	7.01, d (10.4)	7.05, d (10.4)	7.05, d (10.0)
2	6.23, dd (10.5, 2.0)	6.42, dd (10.0, 2.0)	6.23, dd (10.0, 1.6)	6.43, dd (10.4, 1.6)	6.23, dd (10.4, 2.0)	6.24, dd (10.0, 2.0)
4	6.08, s	6.30, s	6.07, s	6.28, s	6.07, s	6.08, s
6	a: 2.46, td (13.0, 4.5)	a: 2.28, m	a: 2.46, td (13.2, 4.8)	a: 2.29 td (13.6, 4.4)	a: 2.45, td (13.2, 4.4)	a: 2.55, td (13.5, 4.5)
	b: 2.37, m	b: 2.19, m	b: 2.36, dt (13.2, 1.8)	b: 2.19, dt (13.6, 2.4)	b: 2.35, dt (13.2, 2.0)	b: 2.37, dt (13.5, 3.5)
7	a: 1.94, m	a: 1.68, m	a: 1.93, m	a: 1.70, m	a: 1.93, m	a: 2.02, m
	b: 1.03, m	b: 0.84, m	b: 1.03, m	b: 0.84, m	b: 1.03, m	b: 0.97, m
8	1.71, m	1.54, m	1.60, m	1.40, m	1.61, m	2.70, qd (11.0, 4.0)
9	1.10, m	0.93, td (11.0, 3.5)	1.05, m	0.86, m	1.04, m	1.07, m
11	a: 1.73, m	1.62, m	1.69, m	a: 1.52, m	1.69, m	2.03, m
	b: 1.58, m			b: 1.49, m		1.80, m
12	a: 2.40, m	a: 2.47, dt (13.0, 3.0)	a: 2.00, m	a: 1.97, m	a: 2.01, m	a: 2.14, dt (13.5, 3.5)
	b: 1.18, m	b: 1.09, m	b: 1.25, m	b: 1.10, m	b: 1.26, m	b: 1.25 m
14	1.25, m	1.02, m	1.10, m	0.80, m	1.08, m	1.08, m
15	a: 1.70, m	a: 1.50, m	a: 1.62, m	a: 1.44, m	a: 1.61, m	a: 1.83 m
	b: 1.16, m	b: 1.01, m	b: 1.12, m	b: 1.02, m	b: 1.07, m	b: 1.26 m
16	a: 2.02, m	a: 2.17, m	a: 1.90, m	a: 2.05, m	a: 1.79, m	a: 1.80 m
	b: 1.50, m	b: 1.56, m	b: 1.36, m	b: 1.36, m	b: 1.39, m	b: 1.61 m
17	1.67, m	1.88, m	1.63, m	1.85, m	1.62, m	1.79 m
18	a: 4.31, d (12.0)	a: 4.44, d (12.0)	0.75, s	0.67, s	0.75, s	
	b: 3.95, d (12.0)	b: 4.06, d (12.0)				
19	1.23, s	1.06, s	1.23, s	1.08, s	1.22, s	1.30, s
20	1.79, m	2.19, m	1.77, m	2.10, m	1.76, m	2.23, m
21	1.15, d (6.5)	1.46, d (6.5)	1.08, d (6.8)	1.31, d (6.4)	1.10, d (6.8)	1.11, d (6.5)
22	3.79, dd (8.0, 2.0)	4.12, m	3.85, br d (9.6)	4.14, br d (9.6)	3.86, br d (9.6)	4.36, dd (9.0, 4.0)
23	3.97, t (8.0)	4.27, t (8.0)	4.22, dd (9.6, 4.4)	4.60, dd (9.6, 4.4)	4.39, dd (9.6, 6.0)	4.10, dd (11.0, 4.0)
24	2.06, m	2.32, m	2.61, m	2.83, m	2.29, m	2.16, m
25	2.16, m	2.32, m	2.72, m	2.91, m	2.26, m	2.24, m
27	1.25, d (7.0)	1.27, d (6.5)	1.16, d (7.2)	1.17, d (6.8)	1.27, d (7.2)	1.28, d (6.5)
28	1.25 d (7.0)	1.39, d (6.5)	0.94, d (6.8)	1.09, d (6.8)	1.14, d (7.2)	1.28, d (6.5)
OAc	2.11, s	2.02, s				
OH		6.70, d (5.0)				

[a] Spectra were measured in $CDCl_3$ (500 MHz). [b] Spectra were measured in pyridine-d_5 (500 MHz). [c] Spectra were measured in $CDCl_3$ (400 MHz). [d] Spectra were measured in pyridine-d_5 (400 MHz).

corresponding compounds with an unsaturated δ-lactone using a palladium catalyst, have 24β,25β-dimethyl substituents.[13] Our present study reports a novel saturated withanolide (**2**) where the two methyl groups at C-24 and C-25 are both α-oriented. Likewise, withanolides possessing a 24α,25β-dimethyl-γ-lactone ring in the side chain, such as compounds **3** and **6**, have been discovered previously only from the plants *Physalis philadelphic*[18] and *Deprea subtriflora*.[7] Both compounds **4** (24β,25β-dimethyl-γ-lactone) and **5** (24β,25α-dimethyl-γ-lactone) represent the first withanolides that have a different configuration in the γ-lactone ring compared to the common analogues (24α,25β-dimethyl-γ-lactone).

EXPERIMENTAL SECTION

General Experimental Procedures. The melting points were determined using a Fisher-Johns melting point apparatus. Optical rotations were determined with a JASCO P1020 digital polarimeter. The CD and IR spectra were obtained on a JASCO V-650, a JASCO J-815, and a JASCO FT/IR-4100 spectrophotometer, respectively. The NMR spectra were recorded on a Bruker AVANCE 300 FT-NMR (or Varian 400 MR NMR/Varian Unity INOVA 500 FT-NMR) instrument at 300 MHz (or 400/500 MHz) for ^{1}H (referenced to TMS, δ_H 0.00 ppm, for both $CDCl_3$ and C_5D_5N) and 75 MHz (or 100/125 MHz) for ^{13}C (referenced to δ_C 77.0 for $CDCl_3$ and to internal TMS at δ_C 0.0 ppm for C_5D_5N). ESIMS were recorded on a Bruker APEX II mass spectrometer. Silica gel 60 (Merck, 230−400 mesh) and LiChroprep RP-18 (Merck, 40−63 μm) were used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F254, 0.25 mm) and precoated RP-18 F254S plates (Merck, 1.05560) were used for TLC analysis. High-performance liquid chromatography (HPLC) was performed on a Hitachi L-7100 pump equipped with a Hitachi L-7400 UV detector at 210 nm and a semipreparative reversed-phase column (Merck, Hibar Purospher RP-18e, 5 μm, 250 × 10 mm).

Animal Material. The soft coral *Paraminabea acronocephala* was collected by hand using scuba off the western coast of Pingtung county, in May 2009, at a depth of 10 m, and was stored in a freezer until being extracted. This soft coral was identified by one of the authors (C.-F.D.). A voucher specimen (specimen no. 200905PA) was deposited in the Department of Marine Biotechnology and Resources, National Sun Yat-sen University.

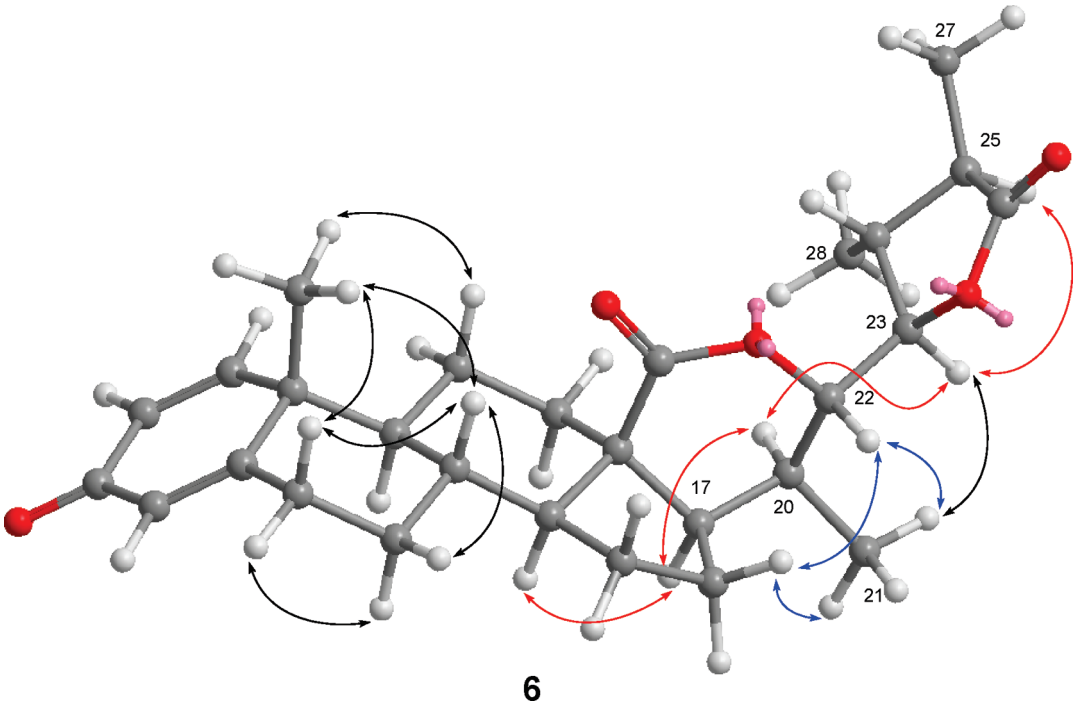


Figure 5. Selected NOE correlations for compound **6**.

Extraction and Isolation. The frozen bodies of the soft coral *P. acronocephala* (3.8 kg fresh wt) were minced and extracted exhaustively with EtOH (6 × 2 L). The organic extract was concentrated to an aqueous suspension and was further partitioned between EtOAc and H_2O. The EtOAc extract (30 g) was fractionated by open column chromatography on silica gel using *n*-hexane−EtOAc and EtOAc−MeOH mixtures of increasing polarity to yield 28 fractions. Fraction 21 (3.6 g), eluted with *n*-hexane−EtOAc (1:6), was further separated by silica gel column chromatography with gradient elution (*n*-hexane−acetone, 5:1 to 2:1) to yield eight subfractions (21A to 21H). Subfraction 21E was separated by RP-18 open column chromatography (MeOH−H_2O, 50% to 100%) to yield 11 subfractions (21E1 to 21E11). Subfraction 21E6 was subjected to RP-18 HPLC (CH_3CN−MeOH−H_2O, 5:64:31) to obtain compounds **3** (0.9 mg), **1** (1.8 mg), and **6** (0.6 mg). Subfraction 21E7 was further fractionated by silica gel column chromatography using gradient elution (*n*-hexane−acetone, 6:1 to 3:1) to afford 10 subfractions (21E7A to E21E7J). Subfraction 21E7G, which was identified to contain steroids by the analysis of the ^{1}H NMR spectrum, was subjected to RP-18 HPLC (CH_3CN−MeOH−H_2O, 5:63:32) to afford compounds **5** (0.9 mg), **4** (1.9 mg), **2** (1.5 mg), **10** (15.6 mg), **8** (3.2 mg), and **11** (2.8 mg). Compounds **9** (200 mg) and **7** (2.0 mg) were obtained from fraction 19 by repeated column chromatography over silica gel (*n*-hexane−acetone, 8:1 to 5:1) and RP-18 HPLC (MeOH−H_2O, 85:15).

Paraminabeolide A (***1***): amorphous solid; $[\alpha]^{24}_D$ +83 (*c* 0.18, $CHCl_3$); CD (1.1 × 10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$) 270 (−1.74), 248 (+6.96), and 232 (+11.1); IR (KBr) ν_{max} 2933, 2863, 1705, 1659, 1621, 1440, 1388, 1291, 1147, 1120, 1027 cm^{-1}; ^{13}C NMR and ^{1}H NMR data, see Tables 1 and 2; ESIMS *m/z* 459 $[M + Na]^+$; HRESIMS *m/z* 459.2509 $[M + Na]^+$ (calcd for $C_{28}H_{36}O_4Na$, 459.2511).

Paraminabeolide B (***2***): amorphous solid; $[\alpha]^{24}_D$ +8 (*c* 0.09, $CHCl_3$); IR (KBr) ν_{max} 2936, 2873, 1736, 1662, 1609, 1454, 1370, 1240, 1183, 1084, 1039 cm^{-1}; ^{13}C NMR and ^{1}H NMR data, see Tables 1 and 2; ESIMS *m/z* 505 $[M + Na]^+$; HRESIMS *m/z* 505.2927 $[M + Na]^+$ (calcd for $C_{30}H_{42}O_5Na$, 505.2930).

Paraminabeolide C (***3***): amorphous solid; $[\alpha]^{24}_D$ +13 (*c* 0.09, $CHCl_3$); IR (KBr) ν_{max} 3456, 2936, 2873, 1772, 1738, 1662, 1454, 1375, 1241, 1183, 1044, 1000 cm^{-1}; ^{13}C NMR and ^{1}H NMR data, see Tables 1 and 3; ESIMS *m/z* 521 $[M + Na]^+$; HRESIMS *m/z* 521.2876 $[M + Na]^+$ (calcd for $C_{30}H_{42}O_6Na$, 521.2879).

Paraminabeolide D (***4***): amorphous solid; $[\alpha]^{24}_D$ +28 (*c* 0.09, $CHCl_3$); IR (KBr) ν_{max} 3464, 2938, 2859, 1773, 1656, 1618, 1451, 1380, 1340, 1241, 1168, 1050 cm^{-1}; ^{13}C NMR and ^{1}H NMR data, see Tables 1 and 3; ESIMS *m/z* 463 $[M + Na]^+$; HRESIMS *m/z* 463.2825 $[M + Na]^+$ (calcd for $C_{28}H_{40}O_4Na$, 463.2824).

Paraminabeolide E (***5***): amorphous solid; $[\alpha]^{24}_D$ −33 (*c* 0.12, $CHCl_3$); IR (KBr) ν_{max} 3455, 2938, 2859, 1776, 1658, 1613, 1454, 1380, 1294, 1241, 1201, 1171, 1041 cm^{-1}; ^{13}C NMR and ^{1}H NMR data, see Tables 1 and 3; ESIMS *m/z* 463 $[M + Na]^+$; HRESIMS *m/z* 463.2822 $[M + Na]^+$ (calcd for $C_{28}H_{40}O_4Na$, 463.2824).

Paraminabeolide F (***6***): amorphous solid; $[\alpha]^{24}_D$ +70 (*c* 0.06, $CHCl_3$); IR (KBr) ν_{max} 2957, 2932, 2876, 2855, 1779, 1722, 1662, 1621, 1455, 1369, 1294, 1240, 1131, 1011 cm^{-1}; ^{13}C NMR and ^{1}H NMR data, see Tables 1 and 3; ESIMS *m/z* 475 $[M + Na]^+$; HRESIMS *m/z* 475.2459 $[M + Na]^+$ (calcd for $C_{28}H_{36}O_5Na$, 475.2460).

Minabeolide-1 (***7***): colorless oil; $[\alpha]^{24}_D$ +35 (*c* 0.20, $CHCl_3$).

Minabeolide-2 (***8***): colorless oil; $[\alpha]^{24}_D$ +45 (*c* 0.32, $CHCl_3$).

Minabeolide-4 (***9***): colorless prism; mp 261−262 °C; $[\alpha]^{24}_D$ +18 (*c* 4.81, $CHCl_3$).

Minabeolide-5 (***10***): colorless oil; $[\alpha]^{24}_D$ +13 (*c* 1.56, $CHCl_3$).

Minabeolide-8 (***11***): colorless prism; mp 260−261 °C; $[\alpha]^{24}_D$ +37 (*c* 0.28, $CHCl_3$).

Crystallographic Data and X-ray Structure Analysis of 9. A suitable colorless crystal (0.5 × 0.3 × 0.3 mm^3) of **9** was grown by slow evaporation from a MeOH−acetone−*n*-hexane (1:3:10) solution. Diffraction intensity data were acquired with a CCD area detector with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). Crystal data for **9**: $C_{27}H_{38}O_3$ (formula weight 410.57), approximate crystal size, 0.5 × 0.3 × 0.3 mm^3, orthorhombic, space group, $P2_12_12_1$ (#19), *T* = 298(2) K, *a* = 6.5336(5) Å, *b* = 14.5778(10) Å, *c* = 23.6095(16) Å, β = 90.00(3)°, *V* = 2248.7(3) $Å^3$, D_c = 1.213 Mg/m^3, *Z* = 4, *F*(000) = 896, μ(Mo Kα) = 0.077 mm^{-1}. A total of 8990 reflections were collected in the range 2.22° < θ < 25.00°, with 3586 independent reflections

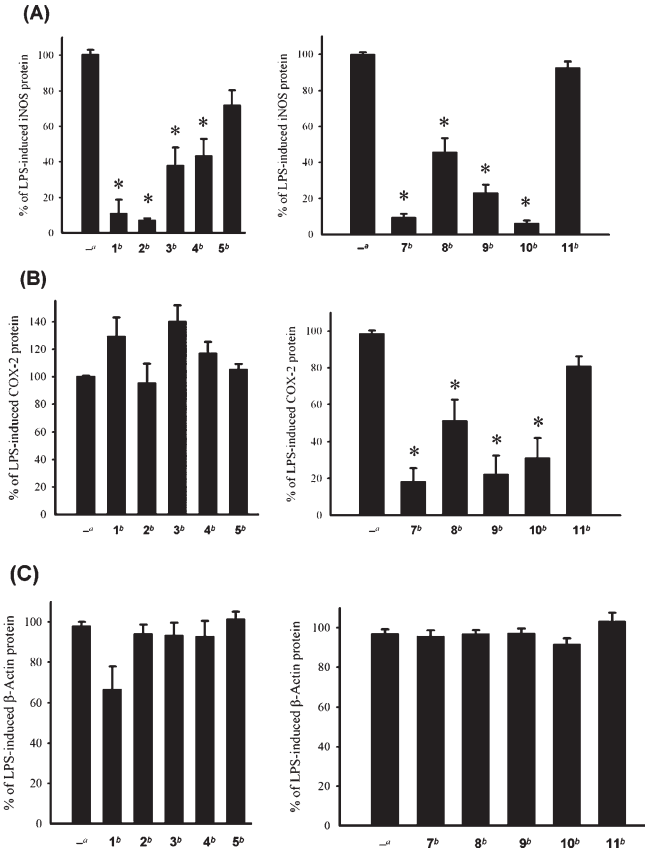


Figure 6. Effect of compounds **1**−**5** and **7**−**11** at 10 μM on the LPS-induced pro-inflammatory iNOS and on COX-2 protein expression of RAW264.7 macrophage cells by immunoblot analysis. (A) Quantification of immunoblots of iNOS. (B) Quantification of immunoblots of COX-2. The values are means ± SEM (n = 6). The relative intensity of the LPS alone stimulated group was taken as 100%. *Significantly different from LPS alone stimulated group (*p < 0.05). [a]Stimulated with LPS. [b]Stimulated with LPS in the presence of **1**−**5** and **7**−**11** (10 μM). (C) Quantification of immunoblots of β-actin.

[R(int) = 0.0277]; completeness to θ_{max} was 97.4%; psi-scan absorption correction applied; full-matrix least-squares refinement on F^2, the number of data/restraints/parameters were 3586/0/275; goodness-of-fit on F^2 = 1.046; final R indices [$I > 2\sigma(I)$], R_1 = 0.0370 wR_2 = 0.0886; R indices (all data), R_1 = 0.0385, wR_2 = 0.0894, largest difference peak and hole, 0.198 and −0.175 e/Å^3.

Crystallographic Data and X-ray Structure Analysis of 11. A suitable colorless crystal (0.6 × 0.5 × 0.4 mm^3) of **11** was grown by slow evaporation from a MeOH−acetone−n-hexane (1:3:10) solution. Diffraction intensity data were acquired with a CCD area detector with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). Crystal data for **11**: $C_{29}H_{42}O_5$ (formula weight 470.65), approximate crystal size, 0.6 × 0.5 × 0.4 mm^3, monoclinic, space group, $P2_1$ (#4), T = 150(2) K, a = 13.846(4) Å, b = 6.1634(17) Å, c = 15.497(4) Å, β = 105.886(15)°, V = 1272.1(6) Å^3, D_c = 1.229 Mg/m^3, Z = 2, F(000) = 512, μ(Mo Kα) = 0.082 mm^{-1}. A total of 8917 reflections were collected in the range 1.37° < θ < 25.16°, with 3933 independent reflections [R(int) = 0.0170]; completeness to θ_{max} was 98.8%; psi-scan absorption correction applied; full-matrix least-squares refinement on F^2, the number of data/restraints/parameters were 3933/1/312; goodness-of-fit on F^2 = 1.065; final R indices [$I > 2\sigma(I)$], R_1 = 0.0285 wR_2 = 0.0692; R indices (all data), R_1 = 0.0301, wR_2 = 0.0725, largest difference peak and hole, 0.241 and −0.153 e/Å^3.

Hydrogenation of Compound 8. Compound **8** (5 mg) was hydrogenated with 10 mg of 10% Pd/C in pyridine (1 mL) at room temperature and atmospheric pressure for 24 h. After the catalyst was removed by filtration, the filtrate was concentrated to give a residue, which was chromatographed on silica gel using n-hexane−EtOAc (3:1) as eluent to afford **8a** (4 mg). The relative configuration was established by analysis of NOE correlations observed in an NOESY experiment. CD (1.9×10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$) 290 (−0.35) and 215 (−2.04); ^{1}H NMR ($CDCl_3$, 400 MHz) of **8a**, δ 4.48 (1H, br d, J = 12.0 Hz, H-22), 4.25 (1H, d, J = 12.0 Hz, H-18a), 3.87 (1H, d, J = 12.0 Hz, H-18b), 2.71 (1H, m, H-25), 2.65 (1H, m, H-4a), 2.43 (1H, br d, J = 12.8 Hz, H-12a), 2.30 (1H, m, H-2a), 2.22 (1H, m, H-24), 2.20 (1H, m, H-2b), 2.08 (3H, s, OCOCH_3), 2.04 (2H, m, H-4b and H-20), 2.00 (1H, m, H-1a), 1.89 (1H, m, H-6a), 1.85 (1H, m, H-5), 1.79 (2H, m, H-16a and H-23a), 1.70 (1H, H-15a), 1.54 (1H, m, H-8), 1.53 (2H, m, H_2-7), 1.52 (1H, m, H-16b), 1.51 (1H, m, H-9), 1.50 (1H, m, H-11a), 1.40 (1H, m, H-1b), 1.37 (1H, m, H-23b), 1.30 (2H, m, H-11b and H-14), 1.27 (1H, m, H-6b), 1.26 (1H, m, H-17), 1.16 (3H, d, J = 6.8 Hz, H_3-27), 1.15 (2H, m, H-12b, and H-15b), 1.08 (3H, d, J = 6.4 Hz, H_3-21), 1.03 (3H, s, H_3-19), 0.94 (3H, d, J = 6.4 Hz, H_3-28); ^{13}C NMR ($CDCl_3$, 100 MHz) of **8a**, δ 213.0 (C, C-3), 176.2 (C, C-26), 171.2 (C, $OCOCH_3$), 79.8 (CH, C-22), 62.3 (CH_2, C-18), 55.6 (CH, C-14), 52.7 (CH, C-17), 45.9 (C, C-13), 43.9 (CH, C-5), 42.2 (CH_2, C-4), 41.0 (CH, C-9), 39.2 (CH, C-20), 38.2 (CH, C-25), 37.1 (CH_2, C-2), 36.8 (CH_2, C-1), 35.7 (CH, C-8), 35.1 (CH_2, C-12), 34.8 (C, C-10), 29.2 (CH, C-24), 27.2 (CH_2, C-23), 27.1 (CH_2, C-16), 26.4 (CH_2, C-6), 25.9 (CH_2, C-7), 24.0 (CH_2, C-15), 22.6 (CH_3, C-19), 21.1 (CH_3, $OCOCH_3$), 21.0 (CH_2, C-11), 18.2 (CH_3, C-28), 12.9 (CH_3, C-21), 12.1 (CH_3, C-27); ESIMS m/z 509 [M + Na]$^+$.

Preparation of (*S*)- and (*R*)-MTPA Esters of 4. To a solution of **4** (0.5 mg) in pyridine (0.4 mL) was added (*R*)-MTPA chloride (25 μL), and the mixture was allowed to stand for 3 h at room temperature. The reaction was quenched by the addition of H_2O (1.0 mL), and the mixture was subsequently extracted with EtOAc (3 × 1.0 mL). The EtOAc-soluble layers were combined, dried over anhydrous $MgSO_4$, and evaporated. The residue was subjected to short silica gel column chromatography using n-hexane−acetone (5:1) to yield the (*S*)-MTPA ester, **4a** (0.4 mg). The same procedure was used to prepare the (*R*)-MTPA ester, **4b** (0.5 mg from 0.5 mg of **4**), with (*S*)-MTPA chloride. Selected ^{1}H NMR ($CDCl_3$, 300 MHz) of **4a**: δ 7.380−7.600 (5H, m, Ph), 7.031 (1H, d, J = 10.0 Hz, H-1), 6.228 (1H, d, J = 10.0 Hz, H-2), 6.072 (1H, s, H-4), 5.200 (1H, br d, J = 9.8 Hz, H-22), 4.409 (1H, dd, J = 9.8, 4.0 Hz, H-23), 3.526 (3H, s, O*Me*), 1.223 (3H, s, H_3-19), 1.141 (3H, d, J = 7.1 Hz, H_3-27), 0.966 (3H, d, J = 6.8 Hz, H_3-21), 0.772 (3H, d, J = 6.8 Hz, H_3-28), 0.713 (3H, s, H_3-18); ESIMS m/z 679 [M + Na]$^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) of **4b**: δ 7.380−7.600 (5H, m, Ph), 7.050 (1H, d, J = 10.0 Hz, H-1), 6.245 (1H, d, J = 10.0 Hz, H-2), 6.091 (1H, s, H-4), 5.199 (1H, br d, J = 10.2 Hz, H-22), 4.374 (1H, dd, J = 10.2, 3.9 Hz, H-23), 3.546 (3H, s, O*Me*), 1.225 (3H, s, H_3-19), 1.107 (3H, d, J = 7.1 Hz, H_3-27), 1.016 (3H, d, J = 6.8 Hz, H_3-21), 0.727 (3H, s, H_3-18), 0.696 (3H, d, J = 7.1 Hz, H_3-28); ESIMS m/z 679 [M + Na]$^+$.

Cytotoxicity Testing. Cell lines were purchased from the American Type Culture Collection (ATCC). Cytotoxicity assays were performed using the MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.[19,20] Doxorubicin was employed as positive control, which exhibited cytotoxic activity toward HepG2, Hep3B, MDA-MB-231, MCF-7, and A-549 cancer cell lines with IC_{50} values of 0.5, 0.7, 2.2, 1.2, and 2.2 μM, respectively. Compounds were considered to be inactive with IC_{50} values > 20 μM/mL.

In Vitro Anti-inflammatory Assay. Macrophage (RAW264.7) cell line was purchased from ATCC. In vitro anti-inflammatory activity of tested compounds was measured by examining the inhibition of lipopolysaccharide (LPS)-induced upregulation of iNOS (inducible

nitric oxide synthetase) and COX-2 (cyclooxygenase-2) proteins in macrophage cells using Western blotting analysis.[21]

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H and ^{13}C NMR spectra for **1**−**6** and **8a** and ^{13}C NMR chemical shifts for the *cis*- and *trans*-methyls in γ-lactone compounds from the literature. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: 886-7-5252000, ext. 5030. Fax: 886-7-5255020. E-mail: sheu@mail.nsysu.edu.tw.

■ ACKNOWLEDGMENT

This work was supported by grants from the National Science Council of Taiwan (NSC98-2113-M-110-002-MY3) and Ministry of Education (97C031702) awarded to J.-H.S.

■ REFERENCES

(1) Lavie, D.; Glotter, E.; Shove, Y. *J. Org. Chem.* **1965**, *30*, 1774–1776.

(2) (a) Das, H.; Dutta, S. K.; Bhattacharya, B.; Chakraborti, S. K. *Indian J. Cancer Chemother.* **1985**, *7*, 59–65. (b) Gunasekera, S. P.; Cordell, G. A.; Farnsworth, N. R. *Planta Med.* **1981**, *43*, 389–391.

(3) Habtemariam, S. *Planta Med.* **1997**, *63*, 15–17.

(4) (a) Luis, J. G.; Echeverri, F.; Garcia, F.; Rojas, M. *Planta Med.* **1994**, *60*, 348–350. (b) Shohat, B.; Kirson, I.; Lavie, D. *Biomedicine* **1978**, *28*, 18–24.

(5) (a) Jamal, S. A.; Qureshi, S.; Ali, S. N.; Choudhary, M. *Khim. Geterotsikl. Soedin.* **1995**, *9*, 1200–1213. (b) Chatterjee, S.; Chakraborti, S. K. *Antonie van Leeuwenhoek* **1980**, *46*, 59–63.

(6) Budhiraja, R. D.; Sudhir, S.; Garg, K. N. *Planta Med.* **1984**, *50*, 134–136.

(7) Su, B. -N.; Park, E. J.; Nikolic, D.; Santarsiero, B. D.; Mesecar, A. D.; Vigo, J. S.; Graham, J. G.; Cabieses, F.; van Breemen, R. B.; Fong, H. H. S.; Farnsworth, N. R.; Pezzuto, J. M.; Kinghorn, A. D. *J. Org. Chem.* **2003**, *68*, 2350–2361.

(8) Ksebati, M. B.; Schmitz, F. J. *J. Org. Chem.* **1988**, *53*, 3926–3929.

(9) Crystallographic data for **9** and **11** have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 783473 and CCDC 783474, respectively). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: +44-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

(10) Su, J.-H.; Lin, F.-Y.; Huang, H.-C.; Dai, C.-F.; Wu, Y.-C.; Hu, W.-P.; Hsu, C.-H.; Sheu, J.-H. *Tetrahedron* **2007**, *63*, 707–707.

(11) (a) Kirson, D. L. I.; Glotter, E.; Snatzke, G. *Tetrahedron* **1970**, *26*, 2221–2228. (b) Hsieh, P.-W.; Huang, Z.-Y.; Chen, J.-H.; Chang, F.-R.; Wu, C.-C.; Yang, Y.-L.; Chiang, M. Y.; Yen, M.-H.; Chen, S.-L.; Yen, H.-F.; Lübken, T.; Huang, W.-C.; Wu, Y.-C. *J. Nat. Prod.* **2007**, *70*, 747–753.

(12) Kennelly, E. J.; Gerhäuser, C.; Song, L. L.; Graham, J. G.; Beecher, C. W.; Pezzuto, J. M.; Kinghorn, A. D. *J. Agric. Food Chem.* **1997**, *45*, 3771–2777.

(13) (a) Tsuji, N.; Suzuki, J.; Shiota, M. *J. Org. Chem.* **1980**, *45*, 2729–2731. (b) Kirson, I.; Glotter, E.; Abraham, A.; Lavie, D. *Tetrahedron* **1970**, *26*, 2209–2219.

(14) (a) Lavie, D.; Kirson, I.; Glotter, E.; Snatzke, G. *Tetrahedron* **1970**, *26*, 2221–2228. (b) Jacobs, H. J. C.; Havinga, E. *Tetrahedron* **1972**, *28*, 135–153.

(15) The ^{13}C NMR chemical shifts for the *cis*- and *trans*-methyls in γ-lactone compounds are summarized in Table S1, which can be found in the Supporting Information.

(16) (a) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096. (b) Randazzo, A.; Bifulco, G.; Giannini, C.; Bucci, M.; Debitus, C.; Cirino, G.; Gomez-Paloma, L. *J. Am. Chem. Soc.* **2001**, *123*, 10870–10876.

(17) Fürstner, A.; Nagano, T.; Müller, C.; Seidel, G.; Müller, O. *Chem. Eur. J.* **2007**, *13*, 1452–1462.

(18) (a) Gu, J. -Q.; Li, W.; Kang, Y. -H.; Su, B. -N.; Fong, H. H. S.; Van Breemen, R. B.; Pezzuto, J. M.; Kinghorn, A. D. *Chem. Pharm. Bull.* **2003**, *51*, 530–539. (b) Su, B.-N.; Misico, R.; Park, E. J.; Santarsiero, B. D.; Mesecar, A. D.; Fong, H. H. S.; Pezzuto, J. M.; Kinghorn, A. D. *Tetrahedron* **2002**, *58*, 3453–3466.

(19) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589–601.

(20) Scudiero, D. A.; Shoemaker, R. H.; Paull, K. D.; Monks, A.; Tierney, S.; Nofziger, T. H.; Currens, M. J.; Seniff, D.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 4827–4833.

(21) Jean, Y.-H.; Chen, W.-F.; Sung, C.-S.; Duh, C.-Y.; Huang, S.-Y.; Lin, C.-S.; Tai, M.-H.; Tzeng, S.-F.; Wen, Z.-H. *Br. J. Pharmacol.* **2009**, *158*, 713–725.