Full Papers

Asterolaurins A−F, Xenicane Diterpenoids from the Taiwanese Soft Coral *Asterospicularia laurae*

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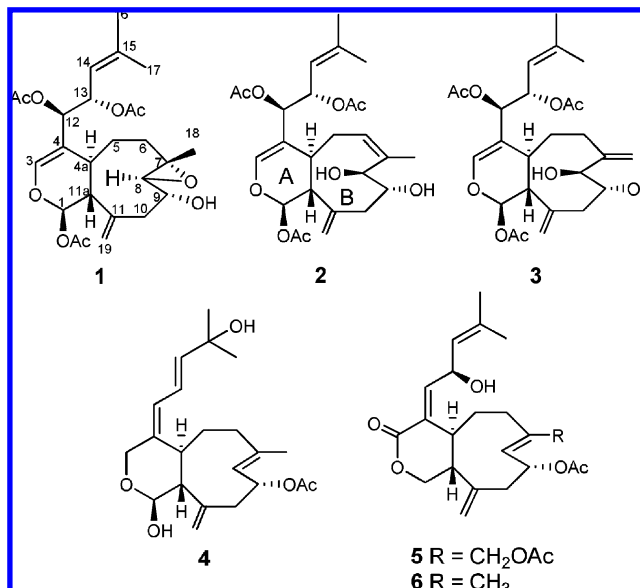
Six new xenicane-type diterpenoids, designated as asterolaurins A−F (**1**−**6**), have been isolated from the organic extract of the soft coral *Asterospicularia laurae*, collected in southern Taiwan. Compounds **1**−**4** possess a xenicin skeleton with a 2-oxabicyclo[7.4.0]tridecane ring system, while **5** and **6** are xeniolide A-type compounds. The structures of the new secondary metabolites, including their configurations, were established on the basis of an extensive spectroscopic analysis, including 1D and 2D NMR (¹H−¹H COSY, HMQC, HMBC, and NOESY), and by comparison of their NMR data with those of the related compounds. The structure of asterolaurin A (**1**) was confirmed by X-ray diffraction analysis, and its absolute configuration was determined using the modified Mosher's method. Asterolaurin A (**1**) exhibited moderate cytotoxicity against HepG2 cells with an IC_{50} of 8.9 μM, while asterolaurin D (**4**) showed potent inhibition of elastase release and superoxide anion generation in vitro.

Octocorals (phylum Cnidaria) have been widely studied and have proven to be a rich source of xenia diterpenoids.[1−5] All of these compounds contain a cyclononane skeleton, and they have been structurally divided into five types: xenicins,[6] xeniolides,[7] xeniaphyllanes (having a bicyclo[7.2.0]undecane ring system),[8] xeniaethers (bearing an oxabicyclo[7.3.0]undecane ring system),[9] and more recently azamilides (with an opened A ring and the nine-membered monocarbocyclic skeleton acylated with a series of C_{16}−C_{20} saturated fatty acids).[10,11] *Asterospicularia* is a genus of soft coral predominant in the Indo-Pacific region. Two papers pertaining to the chemistry of the genus *Asterospicularia* previously reported the isolation of 24-methyl-5α-cholestane-3β,5,6β,22R,24-pentol 6-acetate from *A. randalli*[12] and the isolation of 13-*epi*-9-deacetoxyxenicin, 13-*epi*-9-deacetylxenicin, and gorgosterol from *A. laurae* in 2003.[4] In the search for bioactive substances from the Taiwanese marine soft corals,[13] six new xenicane-type diterpenoids, designated asterolaurins A−F (**1**−**6**), have now been isolated from *Asterospicularia laurae*. In this article we report the isolation and structure determination of these new marine metabolites.

Results and Discussion

The EtOAc-soluble portion of the CH_2Cl_2/MeOH (1:1) extract of the Taiwanese soft coral *A. laurae* was extensively chromatographed over silica gel columns, followed by RP-HPLC, to yield the six new compounds. Asterolaurin A (**1**) analyzed for $C_{26}H_{36}O_9$ from its HRESIMS and NMR spectroscopic data, and this composi-

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tion was later confirmed by X-ray diffraction analysis. The IR spectrum of **1** suggested the presence of a diagnostic hydroxy group (3418 cm⁻¹), ester groups (1747, 1728, 1710 cm⁻¹), double bonds (1600 cm⁻¹), and a terminal methylene (890 cm⁻¹). The ¹H and ¹³C NMR experiments (Tables 1 and 2) revealed the presence of three acetate groups (δ_C 169.5 s, 21.0 q; 169.9 s, 21.1 q; 170.0 s, 21.1 q; δ_H 1.97 s, 1.98 s, 2.03 s, 3H each, respectively), one acetal (δ_C 91.2 d, δ_H 5.88, *J* = 2.1 Hz), one enol ether (δ_C 142.1 d, 113.6 s; δ_H 6.48 s), one exocyclic double bond (δ_C 144.9 s, 116.6 t; δ_H 4.93 s, 5.11 s), and a 1,1-dimethylvinyl [−CH=C(CH₃)₂] group (δ_C 119.2 d, 140.1 s, 25.9 q, 18.6 q; δ_H 5.10 m, 1.70 s, 6H). The above functionalities account for 6 of the 9 degrees of unsaturation of **1**, suggesting a tricyclic structure for asterolaurin A. The COSY

Table 1. ^{1}H NMR of Compounds **1−6** (400 MHz, CDCl$_3$)^{a,b}

| proton	1	2	3	4	5	6
1	5.88, d (2.1)	5.08, d (9.3)	6.10, d (3.6)	4.60, d (8.2)	3.64, t (12.0, Hα) 4.10, dd (12.0, 6.0, Hβ)	3.64, t (12.0, Hα) 4.10, d (12.0, 5.5, Hβ)
3	6.48, s	6.44, d (1.5)	6.44, s	4.31, d (13.7) 4.69, d (13.7)		
4a	2.38, m	2.87, m	2.48, m	2.88, d (14.0)	3.02, d (10.0)	3.0, dd (4.0, 6.5)
5	2.10, m (Hα) 1.55, m (Hβ)	2.73, m 2.51, dd (14.1, 5.1)	2.28, m 1.75, m	2.05, m (Hα) 1.50, m (Hβ)	1.60, m	1.46, m 1.58, m
6	1.25, m (Hα) 2.18, m (Hβ)	5.27, dd (5.1, 12.0)	2.31, m	2.20, m	2.10, m (Hα) 2.40, m (Hβ)	2.24, m
8	2.97, d (8.4)	4.66, d (8.4)	3.95, brs	2.96, d (8.4)	5.46, d (8.1)	5.36, d (7.5)
9	3.74, t (6.6)	4.03, t (7.0)	3.95, m	5.68, t (7.5)	5.77, t (7.2)	5.67, t (7.0)
10	2.40, m (Hα) 2.59, dd (6.9, 8.7, Hβ)	2.68, m 2.10, m	2.35, m 2.58, m	2.35, m (Ha) 2.61, dd (7.0, 8.7, Hβ)	2.52, d (6.9, Hα) 2.40, m, Hβ	2.47, d (5.5) 2.41, m
11a	2.28, (brs)	2.25, d (11.1)	2.55, m	1.71, m	2.10, m	2.03, m
12	5.26, d (6.3)	5.33, d (5.7)	5.27, d (7.2)	5.84, d (14.4)	6.41, d (7.8)	6.39, d (8.0)
13	5.65, dd (6.3, 9.3)	5.79, dd (5.7, 9.3)	5.56, dd (6.3, 9.3)	6.44, dd (10.7, 15.1)	5.22, t (7.8)	5.26, d (8.0)
14	5.10, m	5.15, d (9.3)	5.10, d (9.0)	5.84, d (14.4)	5.31, d (7.8)	5.31, d (8.0)
16	1.70, s	1.76, s	1.72, s	1.36, s	1.79, s	1.76, s
17	1.70, s	1.76, s	1.74, s	1.36, s	1.75, s	1.79, s
18	1.37, s	1.76, s	5.18, 5.38 s	1.80, s	4.64, s	1.76, s
19	4.93, s 5.11, s	4.79, s 5.48, s	4.97, s 5.48, s	4.73, s 4.92, s	5.10, s 5.14, s	5.04, s 5.08, s
OAc	1.97, s 1.98, s 2.03, s	2.04, s 2.05, s 2.06, s	1.93, s 1.99, s 2.03, s	2.10, s	2.08, s 2.09, s	2.10, s

^{a} Chemical shifts are in ppm; J values (Hz) are in parentheses. ^{b} Assignments were made by COSY, HMQC, and HMBC techniques.

Table 2. ^{13}C NMR of Compounds **1−6** (100 MHz, CDCl$_3$)^a

position	1	2	3	4	5	6
1	91.2, CH	95.7, CH	92.3, CH	99.8, CH	70.8, CH$_2$	70.8, CH$_2$
3	142.1, CH	144.2, CH	142.0, CH	69.8, CH	170.7, qC	170.5, qC
4	113.6, qC	111.3, qC	111.7, qC	139.5, qC	133.9, qC	134.2, qC
4a	36.7, CH	38.1, qC	31.8, CH	44.5, CH	43.6, CH	44.2, CH
5	30.0, CH$_2$	25.8, CH$_2$	30.9, CH$_2$	35.4, CH$_2$	37.9, CH$_2$	37.2, CH$_2$
6	39.6, CH$_2$	125.3, CH	26.3, CH$_2$	40.3, CH$_2$	35.6, CH	39.7, CH$_2$
7	59.6, qC	138.1, qC	147.0, qC	134.1, qC	131.4, qC	133.7, qC
8	67.1, CH	72.1, CH	82.4, CH	126.0, CH	131.4, CH	126.3, CH
9	68.8, CH	74.5, CH	72.1, CH	70.4, CH	69.1, CH	70.0, CH
10	43.6, CH$_2$	42.1, CH$_2$	41.0, CH$_2$	43.7, CH$_2$	43.3, CH$_2$	42.3, CH$_2$
11	144.9, qC	142.1, qC	144.1, qC	150.7, qC	146.8, qC	147.6, qC
11a	48.7, CH	49.7, CH	40.9, CH	57.3, CH	49.7, CH	49.0, CH
12	74.6, CH	74.3, CH	74.8, CH	122.2, CH	139.4, CH	138.8, CH
13	70.2, CH	70.5, CH	69.6, CH	120.8, CH	65.0, CH	64.9, CH
14	119.2, qC	119.4, CH	119.6, CH	142.4, CH	124.7, CH	124.6, CH
15	140.1, qC	140.3, qC	140.2, qC	71.0, qC	137.3, qC	137.3, qC
16	18.6, CH$_3$	18.8, CH$_3$	18.7, CH$_3$	30.0, CH$_3$	18.5, CH$_3$	18.4, CH$_3$
17	25.9, CH$_3$	25.9, CH$_3$	25.9, CH$_3$	30.0, CH$_3$	25.9, CH$_3$	25.8, CH$_3$
18	17.6, CH$_3$	18.1, CH$_3$	119.1, CH$_2$	17.6, CH$_3$	61.9, CH$_2$	17.2, CH$_3$
19	116.6, CH$_2$	116.4, CH$_2$	113.4, CH$_2$	113.2, CH$_2$	117.1, CH$_2$	115.9, CH$_2$
OAc	169.5, qC	169.9, qC	169.5, qC	170.8, qC	170.5, qC	170.5, qC
	21.0, CH$_3$	20.8, CH$_3$	21.0, CH$_3$	21.5, CH$_3$	20.9, CH$_3$	20.9, CH$_3$
	169.9, qC	170.1, qC	169.9, qC		170.5, qC	
	21.1, CH$_3$	21.2, CH$_3$	21.0, CH$_3$		21.3, CH$_3$	
	170.0, qC	170.1, qC	170.0, qC			
	21.1, CH$_3$	21.3, CH$_3$	21.2, CH$_3$			

^{a} Multiplicities and assignments made by DEPT, HMQC, and HMBC techniques.
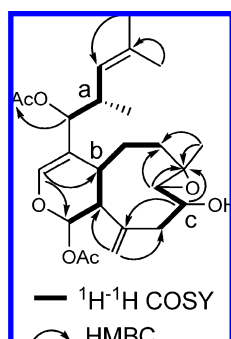
Figure 1. Key COSY and HMBC correlations of **1**.

NMR spectrum revealed the presence of three spin systems (a−c) as shown in Figure 1. HMBC correlations (Figure 1) showed both H-3 and H-11a correlated to C-4a, indicating the connection between C-4 and C-4a. Another HMBC correlation from H-3 to C-1 established the connection between H-1 and C-3 (δ_C 142.1 d) to create the enol ether, forming a 2-acetoxy-3,4-dihydropyran ring. The oxymethine proton at δ_H 2.97 (d, J = 8.4 Hz, H-8), attached to a carbon at δ_C 67.1, revealed HMBC correlations to a quaternary carbon at δ_C 59.6 (C-7) and a methylene at δ_C 39.6 (C-6), whereas H-18 (δ_H 1.37, s) correlated with C-6, C-7, and C-8, indicating the presence of an epoxy ring at C-7 and C-8. HMBC correlations between H-9 and carbons at δ_C 59.6 (s, C-7) and 144.9 (s, C-11) and correlations of the exomethylene protons (δ_H 5.11, 4.93, H-19) with C-11a (CH, δ_C 48.7) and C-10 (CH$_2$, δ_C 43.6), allowed the
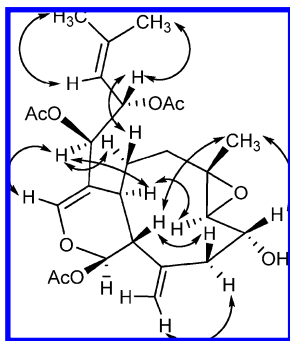
Figure 2. Key NOESY correlations and relative configuration of **1**.
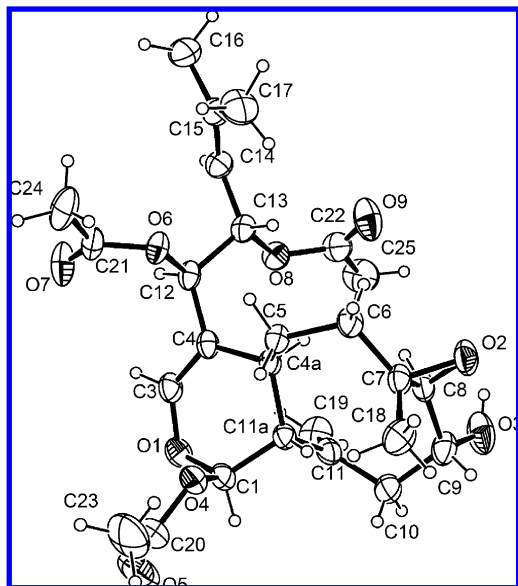

Figure 3. Perspective drawing of the X-ray structure of **1**.

construction of a cyclononane ring with an exomethylene functionality at C-11. The olefinic proton at δ_H 5.28 (m, H-14) was coupled to H-13 and attached to a carbon at δ_C 117.8 (d, C-14). The two methyl signals at δ_H 1.70 (6H, s) were ^2J-correlated to the quaternary olefinic carbon at δ_C 140.1 (C-15) and ^3J-correlated to C-14, as well as to the methyl signals at δ_C 25.9 and 18.6, confirming the attachment of 1,2-diacetoxy-4-methylpent-3-ene group at C-4.[14] On the basis of these COSY and HMBC correlations, asterolaurin A (**1**) was assigned as a nine-membered monocarbocyclic ring belonging to the xenicane-type diterpenoids.

The coupling constants $J_{12,13}$ and $J_{13,14}$ were identical to values reported for related compounds,[14] indicating the 12R*, 13S* configurations at C-12 and C-13. The NOESY correlations between H-11a/H-1; H-18/,Hβ-6, H-11a; H-9/Hβ-10; and H-4a/H-12 suggested the relative configuration of **1** (Figure 2). The NMR spectroscopic data of **1** were also compared to those of 13-*epi*-9-desacetylxenicin,[15] xenione,[16] and 9-desacetyl-7,8-epoxy-13-*epi*-xenicin,[16] which led to the conclusion that the structure of **1** has the same planar structure as 9-desacetyl-7,8-epoxy-13-*epi*-xenicin,[16] with the only difference in the side chain configuration and in the B ring. The quite different specific rotation and NMR data clearly demonstrated that they are diastereomers. The relative configuration of **1** was confirmed by X-ray diffraction analysis (Figure 3).[17] The presence of a secondary hydroxy group in the structure of asterolaurin A (**1**) offered the possibility of upgrading the above determined relative configuration to the absolute one, through application of the modified Mosher's method.[18] For this goal, two aliquots of **1** were treated with (*R*)- and (*S*)-MTPA chloride in dry
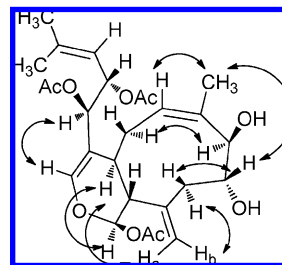
pyridine to give the corresponding (*S*)-**1a** and (*R*)-**1b** MTPA esters. The pattern of $\Delta\delta$ (*S*−*R*) values (−0.03, +0.04, +0.13, +0.08, −0.01, +0.32, and +0.20 for H-8, 9, 10a, 10b, Me-18, H-19a, and H-19b, respectively) established the *R*-configuration at C-9 and, consequently, allowed assignment of the complete stereostructure of asterolaurin A (**1**) as 1R, 4aS, 7S, 8S, 9R, 11aR, 12R, 13S.

Asterolaurins B (**2**) and C (**3**) have the same molecular formula, $C_{26}H_{36}O_9$, as that of **1**, as revealed from the HRESIMS data (*m/z* 515.2259, [M + Na]$^+$), indicating 9 degrees of unsaturation. Compounds **2** and **3** showed similar IR and NMR spectroscopic data to those of **1** (Tables 1 and 2). Preliminary inspection of ^{1}H and ^{13}C NMR spectra of **2** (Tables 1 and 2) revealed the absence of the epoxide doublet (δ_H 2.97) with a carbinolic proton at δ_H 3.74, which is characteristic for ring B of **1**. Instead, a trisubstituted carbon−carbon double bond $\Delta^{6,7}$ (125.3 d, 138.1 s) was observed in ring B of **2**. Comparison of ^{13}C NMR data from **2** with that of **1** indicated the 7,8-epoxide of **1** was replaced by an *E*-trisubstituted double bond bearing a methyl group in **2**. COSY correlations between H-6 and H$_2$-5 and HMBC correlations between H$_3$-18 and C-6/C-8 and between H$_2$-19 and C-11/C-11a/C-10 confirmed the positions of the trisubstituted olefin and the exomethylene functionalities. Placement of the hydroxy group at C-8 was suggested on the basis of NMR analysis. In addition, some known compounds possessing a hydroxy group at C-8 have been isolated from this soft coral in addition to a hydroxy group (δ_H 4.03 t; δ_C 74.5 d) at C-9 in **2**.[19] A COSY correlation between H-9 and H-8/H-10 and HMBC correlations between H-9 and C-8/C-10/C-11/C-7 confirmed the location of the secondary hydroxy at C-9. Thus the skeleton of **2** could be established unambiguously. The relative configuration of **2** was proposed on the basis of key NOESY correlations (Figure 4) and comparison with those of **1**. The α-disposition of the hydroxy group at C-9 and the β-disposition of the hydroxy group at C-8 were inferred on the basis of the proton−proton coupling constants and by NOESY correlations (Figure 4) and comparison with 9-hydroxyxeniolide-F.[19] The signal for H-9 should have a pseudo-axial orientation on the β-face of the molecule. Thus the structure of asterolaurin B was formulated as shown in **2**.

Spectroscopic data from **3** were analogous to those of **2** with the exception that the resonances for the methyl-bearing trisubstituted olefin were replaced by an exocyclic methylene positioned at $\Delta^{7,18}$, adjacent to the carbinolic proton at C-8. HMBC correlations from H$_2$-18 to C-7, C-6, and C-8 clearly positioned the exomethylene group. On the basis of the key NOESY correlations of **3** and comparison with those of **2**, the relative configuration of **3** was proposed and the structure of asterolaurin C was assigned as **3**.

Compound **4** showed a quasi-molecular ion peak at *m/z* 399.2146 [M + Na]$^+$, consistent with the molecular formula $C_{22}H_{32}O_5$, indicating 7 degrees of unsaturation. The IR bands suggested the presence of hydroxy, ester, and double bonds, whereas the UV spectrum suggested a conjugated diene system (λ_{max} 228 nm). The ^{1}H NMR spectrum displayed bands for an *E*-diene olefinic system at δ_H 5.84 (d, *J* = 14.4 Hz, H-12), 6.44 (dd, *J* = 15.1, 10.7 Hz, H-13), and 5.84 (d, *J* = 14.4 Hz, H-14). The NMR features of **4** closely resembled those of xenialactol,[20] with the exception of the

Figure 4. Key NOESY correlations and relative configuration of **2**.
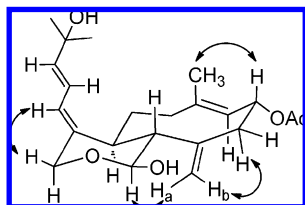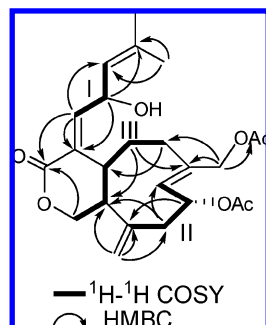
Figure 5. Key NOESY correlations and relative configuration of **4**.

Figure 6. Key COSY and HMBC correlations of **5**.

hydroxy group at C-3 that was replaced by an acetoxy (δ_H 2.10, 3H, s; δ_C 21.5 q, 170.8 s) in **4**. HMBC correlations between H-9 and C-11/C-7/CH$_3$COO permitted the placement of the secondary acetoxy at C-9. The relative configuration of **4** was determined by the aid of a NOESY spectrum (Figure 5) and by comparison with NMR data of xenialactol.[20] The *E*-geometries for the $\Delta^{4,12}$ and Δ^{13} double bonds, respectively, and the *trans*-juncture of two rings in **4** were deduced by direct comparison of the NOESY and spectroscopic data with those of **1**. Asterolaurin D (**4**) was elucidated to be a natural compound acetylated at the C-9 α-position of xenialactol (9α-acetylxenialactol).

Compounds **5** and **6**, possessing the xeniolide A-type ring system, were designated asterolaurins E and F. The molecular formula of asterolaurin E (**5**) was deduced as C$_{24}$H$_{32}$O$_7$ by interpretation of HREIMS and ^{13}C NMR spectroscopic data. The data for **5** were comparable to those of xeniolide A-type compounds with a δ-lactone conjugated to an exocyclic trisubstituted double bond (δ_C 70.5 t, 170.7 s, 133.9 s, 139.4 d) condensed to the nine-membered ring. The ^{13}C NMR data revealed the replacement of the terminal tertiary allylic alcohol group of **4** by those of a dimethyl olefin functionality found in xenicane diterpenoids and an oxymethine group (C-13, δ_C 65.0 d), with two substituted CH$_3$ groups (Me-16, 17, δ_C 18.5 q, 25.9 q). Corresponding changes were also observed in the ^{1}H NMR data for the additional α,β-unsaturation-γ-hydroxy group, and vinyl methyls were found respectively at δ_H 5.22 (1H, t, *J* = 7.8 Hz, H-13), 1.79 (3H, s, H-16), and 1.75 (3H, s, H-17). The COSY spectrum (Figure 6) revealed the presence of three spin systems (I−III) that were joined together by long-range CH correlations (HMBC), establishing its planar structure. COSY correlations of H-13 and both of the olefinic protons H-12/H-14 and the HMBC correlations between H-13 and both C-4 and C-16 established that C-13 should be hydroxylated. The H-13 NMR signal showed a NOESY correlation with H-4a, allowing assignment of the C-13 hydroxy group in the β-orientation (Figure 7). This assignment was supported by molecular modeling studies, which indicated the H-13/H-4a NOE would be possible only with H-13 in the β-configuration. The *E*-configuration of the 4(12) double bond as in xeniolide A was established from the upfield chemical shift of H-12 (δ_H 5.84, against 6.40 in the case of the isomer)[14] and NOE correlations between H-13, H-4a, and H-5.[15] Comparison of the NMR data between **5** and **4** for the B-moiety suggested that the olefinic methyl (Me-18) was oxidized to the primary alcohol, followed by acetylation, which would provide the acetoxy group.
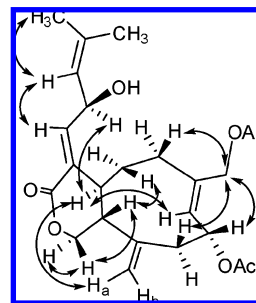

Figure 7. Key NOESY correlations and relative configuration of **5**.

Table 3. Inhibitory Effect of Compounds **1**−**6** on Elastase Release and Superoxide Anion Generation, as Tested on Human Neutrophils in Response to FMLP/CB[a]

compound	elastase	superoxide anion
1	40 ± 3[b]	−12 ± 4[b]
2	30 ± 6	31 ± 7
3	39 ± 5	22 ± 7
4	68 ± 6	56 ± 2
5	36 ± 6	24 ± 5
6	13 ± 6	9 ± 3
genistein[c]	52 ± 6	65 ± 6

[a] Percentage of inhibition at 10 μg/mL. [b] Concentration necessary for 50% inhibition. Results are presented as mean + SEM (*n* = 3), *p* < 0.05. [c] Positive control.

Detailed analyses of 2D NMR spectra of **5**, focusing in particular on the interpretation of ^{1}H−^{1}H COSY, HMQC, and HMBC correlations, illustrated the planar structure of **5** as shown in Figure 6. Further support for the suggested configuration came from NOE cross-peaks depicted in Figure 7. Thus, compound **5** was determined to be consistent with a new structure possessing the xeniolide A-type skeleton.

Asterolaurin F (**6**) was determined to have a molecular formula of C$_{22}$H$_{30}$O$_5$, which agreed with the loss of an acetic acid molecule from **5**, by HRFABMS and NMR spectroscopic data. Comprehensive comparison of the NMR data of **6** with those of **5** revealed a good agreement with **5** in the carbon signals of the terminal dimethyl olefinic group and ring A and with those of **4** in the carbon signals of ring B. The relative configuration of the ring system was established by a NOESY experiment, where similar correlations to those seen for **5** and **4** were observed.

The similarity between the polyp structure of *Asterospicularia* and those of the genera *Xenia* and *Sympodium* has been discussed.[2] In an earlier study on the soft coral *A. laurae* collected from the Great Barrier Reef, Australia, two xenicin-type diterpenes were reported.[4] This is the only prior report describing the secondary metabolites from *A. laurae*. The discovery of four new xenicins (**1**−**4**), together with the two new xeniolide A-type compounds, **5** and **6**, adds to the vast chemical diversity of the marine soft corals. These compounds are similar to those diterpenes isolated from *Xenia* species, but may serve as characteristic constituents of *A. laurae* from a chemotaxonomic point of view. A human cancer cell line was chosen to test the in vitro cytotoxicity of **1**−**6**. Asterolaurin A (**1**) exhibited cytotoxicity against HepG2 cells with an IC$_{50}$ = 8.9 μM, while others were inactive (>40 μM). The anti-inflammatory activities of **1**−**6** were evaluated by assaying for the inhibition of elastase release and for the generation of superoxide anion, as tested on human neutrophils in vitro. Among the isolated xenicanes, **4** showed more potent inhibition of elastase release and superoxide anion generation than genistein (Table 3). The IC$_{50}$ values of **4** for anti-inflammatory effects were 18.7 and 23.6 μM, respectively. It is worthy to note that the occurrence of the

hemiacetal functionality in compound **4** may play a significant role in the anti-inflammatory activity.

Experimental Section

General Experimental Procedures. The melting point was measured on a Büchi melting point B-540 apparatus and is uncorrected. Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR and UV spectra were measured on Hitachi T-2001 and U-3210 spectrophotometers, respectively. The ^{1}H and ^{13}C NMR, COSY, HMQC, HMBC, and NOESY spectra were recorded respectively on a Bruker FT-300 spectrometer and on a Varian Unity INOVA 500 FT-NMR at 500 MHz for ^{1}H and 125 MHz for ^{13}C using TMS as an internal standard. The chemical shifts are given in δ values (ppm) and coupling constants in Hz. Low-resolution EIMS and FABMS were recorded on a VG Quattro 5022 mass spectrometer, and HREIMS were measured on a JEOL JMS-SX 102 spectrometer. Silica gel 60 (Merck) was used for column chromatography (CC), and precoated silica gel plates (Merck, Kieselgel 60 F-254, 1 mm) were used for preparative TLC. Sephadex LH-20 (Amersham Pharmacia Biotech AB, Uppsala, Sweden) was used. (*S*)-(+)-α-Methoxy-α-trifluoromethylphenyl acetyl chloride and (*R*)-(−)-α-methoxy-α-trifluoromethylphenyl acetyl chloride (MTPA-Cl) were obtained from Acros Organics (NJ).

Animal Material. The soft coral *Asterospicularia laurae* was collected from the southern coast of Taiwan, in November 2006, at a depth of 15 m, and immediately stored in a freezer. This species was identified by one of the authors (C.-F.D.). A voucher specimen (NTUO-9) was deposited in the School of Pharmacy, National Taiwan University, Taipei, Taiwan.

Extraction and Isolation. The soft coral (wet, 2 kg) was extracted with CH_2Cl_2/MeOH (1:1) at room temperature using a stirrer, and the extract was concentrated under vacuum. The crude extract was partitioned between EtOAc and H_2O (1:1). The EtOAc-soluble portion (4.5 g) was subjected to a flash Si gel column developed with *n*-hexane/EtOAc (100:0/0:100) to give eight fractions. Fraction F_1 (2.5 g) was chromatographed on a silica gel column using a *n*-hexane/CH_2Cl_2/MeOH gradient followed by separation on RP-HPLC eluting with MeOH/H_2O/CH_3CN (6:3:1) to yield **1** (37 mg) and **2** (5 mg). Fraction F_3 was separated on RP-HPLC eluting with MeOH/H_2O/CH_3CN (6:3:1) to give **5** (4 mg) and **6** (2 mg). Fraction F_4 was subjected to separation on RP-HPLC using MeOH/H_2O/CH_3CN (5:1:4) to furnish **3** (4.5 mg) and **4** (8 mg).

Asterolaurin A (1): colorless prisms; mp 138−140 °C; $[\alpha]^{25}_D$ −3.0 (*c* 5.6, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 228 (3.27) nm; IR (CH_2Cl_2) γ_{max} 3418, 1747, 1728, 1710, 1600, 890 cm^{-1}; ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; HRESIMS *m/z* 515.2259 (calcd for $C_{26}H_{36}O_9Na$, 515.2257).

Single-Crystal X-ray Crystallographic Data for 1.[17] Suitable colorless prisms (MeOH/CH_2Cl_2) of **1** ($C_{26}H_{36}O_9$, 0.8 × 0.6 × 0.5 mm) belong to the orthorhombic system, space group $P2_12_12_1$ (# 19) with *a* = 11.856(2) Å, *b* = 13.164(3) Å, *c* = 17.373(4) Å, *V* = 2711.4(9) Å^3, *Z* = 4, D_{calcd} = 1.207 g/cm^3, (Mo Kα) = 0.71073 Å. Intensity data were measured on a Rigaku AFC7S diffractometer up to $2\sigma_{max}$ of 52. All 5219 unique reflections were collected and corrected for background, Lorentz-polarization and absorption (μ = 0.091 mm^{-1}), while crystal decay was negligible. The structure was solved by direct methods and refined by a full-matrix least-squares procedure. The non-hydrogen atoms were given anisotropic thermal parameters. The refined structural model converged to a final *R* = 0.0448, R_w = 0.1087 for 4103 observed reflections [*I* > 2(*I*)] and 322 variable parameters. The final difference Fourier map was flat, with the highest and lowest residual peaks of 0.176 and −0.240 e/Å^3, respectively.

Preparation of (*S*)- and (*R*)-MTPA Esters (1a and 1b) of 1. To a solution of **1** (2 mg in 0.5 mL of pyridine) was added *S*-(+)- or *R*-(−)-MTPA chloride (one drop), and the solution was allowed to stand at room temperature for 7 h. After purification using preparative TLC, the ester (1.6 mg, 80% yield) was analyzed by ^{1}H NMR spectroscopic measurement, and $\Delta\delta = \delta_S - \delta_R$ was calculated for **1**.

(*S*)-1a: ^{1}H NMR (CDCl$_3$, 300 MHz) δ 3.12 (d, *J* = 8.4 Hz, H-8), 5.14 (t, *J* = 7.8 Hz, H-9), 2.52 (d, *J* = 15 Hz, H-10a), 2.74 (dd, *J* = 15, 6.9 Hz, H-10b), 1.42 (s, Me-18), 5.01 (s, H-19a), 5.06 (s, H-19b).

(*R*)-1b: ^{1}H NMR (CDCl$_3$, 300 MHz) δ 3.15 (d, *J* = 8.4 Hz, H-8), 5.10 (t, *J* = 7.8 Hz, H-9), 2.38 (d, *J* = 15 Hz, H-10a), 2.67 (dd, *J* = 15, 6.8 Hz, H-10b), 1.43 (s, Me-18), 4.69 (s, H-19a), 4.86 (s, H-19b).

Asterolaurin B (2): colorless, amorphous solid; $[\alpha]^{25}_D$ −56 (*c* 0.2, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 227 (3.25) nm; IR (CH_2Cl_2) γ_{max} 3444, 1747, 1731, 1710, 1600, 890 cm^{-1}; ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; HRESIMS *m/z* 515.2259 (calcd for $C_{26}H_{36}O_9Na$, 515.2257).

Asterolaurin C (3): colorless, amorphous solid; $[\alpha]^{25}_D$ −4 (*c* 5.2, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 228 (3.23) nm; IR (CH_2Cl_2) γ_{max} 3444, 1731, 1728, 1710, 1600, 890 cm^{-1}; ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; HRESIMS *m/z* 515.2255 (calcd for $C_{26}H_{36}O_9Na$, 515.2257).

Asterolaurin D/9α-Acetylxenialactol (4): colorless, amorphous solid; $[\alpha]^{25}_D$ −9 (*c* 1.4, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 228 (3.54) nm; IR (CH_2Cl_2) γ_{max} 3418, 1731 cm^{-1}; ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; HRESIMS *m/z* 399.2146 (calcd for $C_{22}H_{32}O_5Na$, 399.2148).

Asterolaurin E (5): colorless, amorphous solid; $[\alpha]^{25}_D$ −20 (*c* 1.0, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 253 (3.65) nm; IR (CH_2Cl_2) γ_{max} 3454, 1738 cm^{-1}; ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; HRESIMS *m/z* 455.2049 (calcd for $C_{24}H_{32}O_7Na$, 455.2049).

Asterolaurin F (6): colorless, amorphous solid; $[\alpha]^{25}_D$ −71 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 252 (3.63) nm; IR (CH_2Cl_2) γ_{max} 3457, 1731 cm^{-1}; ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; HRESIMS *m/z* 397.1989 (calcd for $C_{22}H_{30}O_5Na$, 397.1991).

Cytotoxicity Assay. Cytotoxicity was tested against the human HepG2 (liver carcinoma) cells, using a MTT assay method. The assay procedure was carried out as previously described.[21] Camptothecin was used as positive control, which gave IC_{50} values for HepG2 tumor cells of 0.06 μM.

Anti-inflammatory Assays. Measurement of Elastase Release. Degranulation of azurophilic granules in human neutrophils was determined by elastase release as described previously.[22] Experiments were performed using MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide as the elastase substrate. After supplementation with MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide (100 μM), neutrophils (6 × 10^5/mL) were equilibrated at 37 °C for 2 min and incubated with each test compound for 5 min. Cells were activated by FMLP (100 nM)/CB (0.5 μg/mL), and changes in absorbance at 405 nm were monitored continuously for elastase release. The results were expressed as the percentage of the initial rate of elastase release in the FMLP/CB-activated, test compound-free (DMSO) control system. Genistein was used as a positive control.

Human Neutrophil Superoxide Generation. Human neutrophils were obtained by means of dextran sedimentation and Ficoll centrifugation. Superoxide anion production was assayed by monitoring the superoxide dismutase-inhibitable reduction of ferricytochrome *c*.[23,24] In brief, after supplementation with 0.5 mg/mL ferricytochrome *c* and 1 mM Ca^{2+}, neutrophils were equilibrated at 37 °C for 2 min and incubated with drugs for 5 min. Cells were activated with 100 nM FMLP for 10 min. When FMLP was used as a stimulant, CB (1 μg/mL) was incubated for 3 min before activation by the peptide (FMLP/CB). Changes in absorbance with the reduction of ferricytochrome *c* at 550 nm were continuously monitored in a double-beam, six-cell positioner spectrophotometer with constant stirring (Hitachi U-3010, Tokyo, Japan). Calculations were based on differences in the reactions with and without SOD (100 U/mL) divided by the extinction coefficient for the reduction of ferricytochrome *c* (ε = 21.1/mM/10 mm). Genistein was used as a positive control.

Note Added after ASAP Publication: The version of this paper published on October 28, 2009, had an error on the fourth page, first paragraph, second sentence. The corrected version was published November 3, 2009.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra of **1**−**6** and cif file of X-ray data of **1**. These materials are available free of charge via the Internet at http://pubs.acs.org.

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