Asterolaurins G – J, New Xenicane Diterpenoids from the Taiwanese Soft Coral *Asterospicularia laurae*

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Four new xenicane diterpenoids, asterolaurins G – J (**1** – **4**, resp.) have been isolated from the Taiwanese soft coral *Asterospicularia laurae*. Their structures were determined by extensive spectroscopic analyses (UV, CD, IR, ^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, HMBC, and NOESY). The cytotoxic activities of all compounds were evaluated.

Introduction. – Soft corals belonging to the genus *Xenia* (subclass Octocorallia, order Alcyonacea, family Xeniidae) are rich sources of xenicane-type monocarbocyclic diterpenes with a cyclononane skeleton. *Asterospicularia laurae* is a moderately abundant soft coral species found in southern coast of Taiwan [1]. The similarity in polyp structure is a striking feature among species of the genera *Asterospicularia*, *Xenia*, and *Sympodium*, and several members of these soft corals have been the subject of phytochemical investigation that resulted in the isolation of a number of natural products with interesting antitumor and cytotoxic activities [2][3]. Literature surveys also revealed a few reports concerning the isolation and characterization of marine natural products with the xenicane-diterpenoid skeleton from the soft coral genera *Xenia* [4 – 11], *Anthelia* [12][13], *Alcyonium* [13], and *Capnella* [14], from the blue coral *Heliopora coerulea* [15], and also from gorgonians [16]. Among these, only three reports were on the chemistry of the genus *Asterospicularia*, including the isolation of 24-methyl-5α-cholestane-3β,5,6β,22*R*,24-pentol 6-acetate from *A. randalli* [17], the isolation of 13-*epi*-9-deacetoxyxenicin, 13-*epi*-9-deacetylxenicin, and gorgosterol from *A. laurae* [18], and recently we reported the isolation of six new xenicane-type diterpenoids, asterolaurins A – F, from *A. laurae* [19]. The current work is part of our continuing research program directed towards new metabolites from Taiwanese soft coral *A. laurae*. A search for a second sample collection has led to the isolation of four new xenicanes, designated asterolaurins G – J (**1** – **4**, resp.; *Fig. 1*). Here, we report the structure elucidations of these new marine metabolites, together with the evaluation of their cytotoxic activities.

Results and Discussion. – Compound **1** was obtained as a white solid with the molecular formula $C_{22}H_{30}O_6$ as deduced from its HR-ESI-MS spectrum with a *pseudo*-

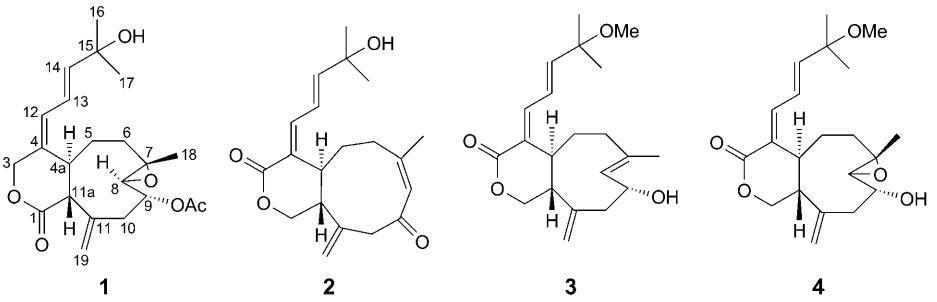

Fig. 1. *Compounds* **1**–**4** *isolated from Taiwanese soft coral* Asterospicularia laurae

molecular-ion peak at *m/z* 413.1937 ($[M+Na]^+$), indicating eight degrees of unsaturation. The IR spectrum revealed absorption bands for a OH group (3429 cm^{-1}) and ester group (1715 cm^{-1}). The ^1H- and ^{13}C-NMR spectra (*Tables 1* and *2*) revealed the presence of one AcO group (δ(C) 170.2 (*s*), 21.1 (*q*); δ(H) 2.09), one OH group at C(15) (δ(C) 70.8 (*s*)), one exocyclic CH_2 group (δ(C) 123.0 (*t*); δ(H) 5.30 br. (*s*)) at C(11) (δ(C) 130.9 (*s*)), a conjugated diene group (δ(C) 128.5 (*d*), 120.8 (*d*), 145.7 (*d*)), a δ-valerolactone ring (δ(C) 172.2 (*s*), 71.3 (*t*), 136.9 (*s*), 35.9 (*d*), 57.1 (*d*)), and an epoxide ring (δ(C) 59.4 (*s*), 64.0 (*d*); δ(H) 3.14 (*d*, (H–C(8))). The COSY spectrum suggested the presence of three spin systems (H–C(4a)/CH_2(5)/CH_2(6); H–C(8)/H–C(9)/CH_2(10); H–C(12)/H–C(13)/H–C(14)) as shown in *Fig. 2*. The HMBCs (*Fig. 2*), CH_2(3)/C(1) and C(4a), and H–C(11a)/C(4), indicated the connections between C(1), C(4), and C(4a). Other HMBCs, CH_2(3)/C(12), H–C(13)/C(4) and C(15), and Me(16)/C(14), C(15) and C(17), established the connection between C(1) and C(3), and the Me groups to the diene. The HMBCs Me(18)/C(7), C(6), and C(8) indicated the linkage of a Me group to the epoxy ring at C(7) and C(8). The correlation H–C(9)/AcO located an AcO group at C(9), and the correlation H–C(19)/C(11a) located an exocyclic CH_2 group at C(11). By deducing the unsaturations of C=C bonds (3), δ-lactone (2), AcO, and epoxide from the total of eight unsaturations, the remaining one unsaturation could be accommodated by a

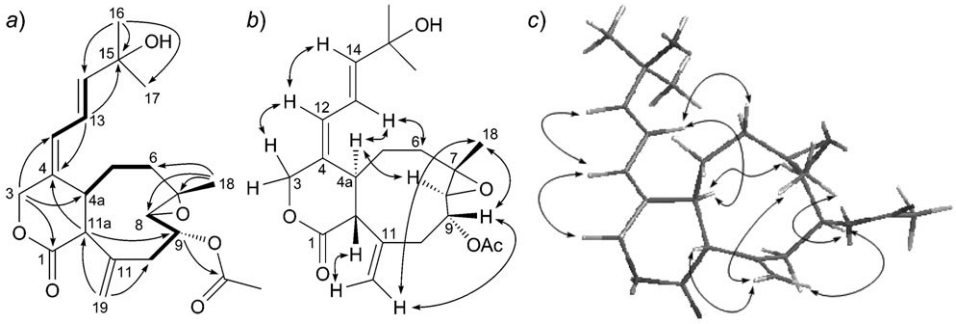

Fig. 2. a) *COSY* (—), *HMBC* (→), *and* b) *NOESY* (↔) *correlations of* **1**. c) *Computer-generated perspectives models of* **1** *using MM2 force-field calculation.*

Table 1. *^{1}H-NMR Data of Compounds* **1**–**4**[a]). δ in ppm, J in Hz.

	1[b])	**2**[b])	**3**[c])	**4**[c])
CH_2(1)	–	4.01 (*t*, *J* = 11.5), 4.19 (*dd*, *J* = 4.5, 11.0)	3.61 (*t*, *J* = 12.0), 4.09 (*dd*, *J* = 4.5, 11.7)	3.68 (*t*, *J* = 11.2), 4.13 (*dd*, *J* = 4.8, 11.2)
CH_2(3)	4.44 (*d*, *J* = 12.0), 4.90 (*d*, *J* = 12.0)			
CH_2(4a)	1.99–2.02 (*m*)	3.08–3.12 (*m*)	3.00–3.04 (*m*)	3.10–3.14 (*m*)
CH_2(5)	1.74–1.80 (*m*)	3.04 (*dt*, *J* = 3.5, 13.5)	1.60–1.65 (*m*)	1.62–1.68 (*m*)
CH_2(6)	1.40–1.46 (*m*), 2.12–2.16 (*m*)	2.19 (*dt*, *J* = 14.5, 4.0)	2.18–2.24 (*m*)	2.14–2.20 (*m*)
H–C(8)	3.14 (*d*, *J* = 9.5)	5.95 (*s*)	5.30 (*d*, *J* = 7.2)	2.97 (*d*, *J* = 8.4)
H–C(9)	4.80 (*dt*, *J* = 4.5, 9.5)	4.80 (*dt*, *J* = 4.5, 10.0)	4.79 (br. *s*)	3.75–3.80 (*m*)
CH_2(10)	2.28–2.34 (*m*)	3.50–3.56 (*m*)	2.45–2.51 (*m*)	2.48–2.54 (*m*)
H–C(11a)	3.20 (*d*, *J* = 10.5)	2.50–2.56 (*m*)	2.08–2.15 (*m*)	2.40–2.46 (*m*)
H–C(12)	6.08 (*dd*, *J* = 11.0)	7.03 (*d*, *J* = 11.5)	6.93 (*d*, *J* = 11.4)	6.99 (*d*, *J* = 11.6)
H–C(13)	6.24 (*dd*, *J* = 11.0, 15.0)	6.42 (*dd*, *J* = 11.5, 15.0)	6.39 (*t*, *J* = 11.4)	6.30 (*dd*, *J* = 11.2, 15.2)
H–C(14)	5.96 (*d*, *J* = 15.0)	6.25 (*d*, *J* = 15.0)	6.12 (*d*, *J* = 15.3)	6.13 (*d*, *J* = 15.6)
Me(16)	1.36 (*s*)	1.38 (*s*)	1.33 (*s*)	1.29 (*s*)
Me(17)	1.38 (*s*)	1.36 (*s*)	1.33 (*s*)	1.29 (*s*)
Me(18)	1.33 (*s*)	1.96 (*s*)	1.71 (*s*)	1.37 (*s*)
CH_2(19)	5.30 (br. *s*)	5.02 (*s*), 5.14 (*s*)	4.91 (*s*), 5.09 (*s*)	5.07 (*s*), 5.26 (*s*)
AcO–C(9)	2.09 (*s*)			
MeO			3.20 (*s*)	3.16 (*s*)

[a]) Data were recorded in $CDCl_3$ on a *Bruker AM-300* MHz apparatus. [b]) Recorded at 500 MHz. [c]) Recorded at 300 MHz.

cyclononane ring. On the basis of these COSY and HMBCs, **1** was assigned as a δ-valerolactone-fused cyclononane skeleton belonging to the xeniolides (lactone derivatives of xenicane-type diterpenoids) [18][20]. Assuming the similar configuration of xeniolides around the ring junction at C(11a) and C(4a), the configurations of all stereogenic centers were elucidated from NOESY experiments of **1** as shown in *Fig. 2*. The NOESY correlations H–C(4a)/H–C(13) and H–C(4a)/H–C(8) suggested that these H-atoms were on the α-face opposite to H–C(11a). The β-configuration of Me(18) was determined by the observation of the series of NOESY correlations, *i.e.*, Me(18)/H_β–C(9)/CH_2(19)/H–C(11a) and H–C(8)/H–C(4a). The (*E*)-configuration of the C(4)=C(12) bond was determined by the large coupling constants between H–C(12), H–C(13), and H–C(14) (J = 15.0, 11.0 Hz), and also supported by the strong NOESY H–C(4a)/H–C(13). The coupling constant (J = 10.5 Hz) between H–C(4a) and H–C(11a) suggested a *trans*-ring junction, which implied that H–C(4a) was α-oriented. A molecular model of structure **1** was generated by CS Chem 3D version 9.0 using MM2 force-field calculation for energy minimization (*ChemBioUltra* calculation program) as shown in *Fig. 2*. The result was consistent with the configuration as established from NOESY experiments. These findings established the structure of **1** as shown in *Fig. 1*, and the name asterolaurin G (**1**) was given.

Table 2. *^{13}C-NMR Data of Compounds* **1**–**4**[a]). δ in ppm.

	1[b])	**2**[b])	**3**[c])	**4**[c])
C(1)	172.2 (*s*)	69.1 (*t*)	70.6 (*t*)	71.0 (*t*)
C(3)	71.3 (*t*)	169.6 (*s*)	170.9 (*s*)	170.0 (*s*)
C(4)	136.9 (*s*)	129.6 (*s*)	133.1 (*s*)	132.1 (*s*)
C(4a)	35.9 (*d*)	40.7 (*d*)	42.9 (*d*)	41.6 (*d*)
C(5)	36.2 (*t*)	35.1 (*t*)	38.0 (*t*)	36.4 (*t*)
C(6)	38.8 (*t*)	31.2 (*t*)	40.1 (*t*)	39.7 (*t*)
C(7)	59.4 (*s*)	148.6 (*d*)	132.8 (*s*)	58.9 (*s*)
C(8)	64.0 (*d*)	130.0 (*d*)	130.5 (*d*)	67.2 (*d*)
C(9)	72.5 (*d*)	200.2 (*s*)	67.4 (*d*)	69.2 (*d*)
C(10)	34.2 (*t*)	52.5 (*t*)	44.9 (*t*)	42.0 (*t*)
C(11)	130.9 (*s*)	143.0 (*s*)	147.5 (*s*)	144.6 (*s*)
C(11a)	57.1 (*d*)	45.5 (*d*)	49.7 (*d*)	49.7 (*d*)
C(12)	128.5 (*d*)	138.5 (*d*)	136.2 (*d*)	137.6 (*d*)
C(13)	120.8 (*d*)	119.5 (*d*)	122.0 (*d*)	121.8 (*d*)
C(14)	145.7 (*d*)	151.3 (*t*)	148.7 (*d*)	149.8 (*d*)
C(15)	70.8 (*s*)	71.1 (*s*)	75.0 (*s*)	75.0 (*s*)
C(16)	29.6 (*q*)	29.7 (*q*)	25.8 (*q*)	25.7 (*q*)
C(17)	29.8 (*q*)	29.7 (*q*)	25.8 (*q*)	25.6 (*q*)
C(18)	19.6 (*q*)	24.2 (*q*)	17.5 (*q*)	17.7 (*q*)
C(19)	123.0 (*t*)	117.1 (*t*)	115.4 (*t*)	117.7 (*t*)
*Ac*O–C(9)	21.1 (*q*), 170.2 (*s*)			
MeO			50.6 (*q*)	50.6 (*q*)

[a]) Data were recorded in $CDCl_3$. [b]) Recorded at 125 MHz. [c]) Recorded at 75 MHz.

Compound **2** was obtained as a white solid with the molecular formula $C_{20}H_{26}O_4$, deduced from a *pseudo*-molecular-ion peak at *m*/*z* 353.1728 ($[M+Na]^+$) in the HR-ESI-MS, indicating eight degrees of unsaturation. The IR spectrum revealed absorptions due to OH (3414 cm^{-1}), diene (2928, 2857 cm^{-1}), and CO groups (1735 cm^{-1}). The ^{1}H- and ^{13}C-NMR, and DEPT spectra (*Tables 1* and *2*) indicated the presence of a diene moiety (δ(C) 138.5 (*d*), 119.5 (*d*), 151.3 (*d*)) at C(4) of a δ-valerolactone ring (δ(C) 169.6 (*s*), 129.6 (*s*), 40.7 (*d*), 45.5 (*d*), 69.1 (*t*)), an exocyclic CH_2 group (δ(C) 117.1; δ(H) 5.02 (*s*), 5.14 (*s*)) at C(11) and a conjugated enone (moiety δ(C) 148.6 (*d*), 130.0 (*d*), 200.2 (*s*)). Furthermore, the presence of two O-bearing C-atoms was deduced from the C-atom signals at δ(C) 69.1 (*t*) and 71.1 (*s*), the former one corresponding to CH_2 (δ(H) 4.01 (*t*), 4.19 (*dd*)) in the lactone ring, and the latter corresponding to two Me groups (δ(C) 29.7 (*q*); δ(H) 1.38 (*s*), 1.36 (*s*)) and linked to the diene as evidenced by respective HMBCs. One more C-atom signal observed at δ(C) 24.2 (*q*) (δ(H) 1.96 (*s*)) was ascribed to the Me group attached to enone moiety. Two spin systems (*a* and *b*; *Fig. 3*) were deduced from combined use of ^{1}H,^{1}H-COSY and HMBC spectra of **2**. The HMBC Me(18)/C(7) and C(6), coupled with the NOESY correlation Me(18)/H–C(8), indicated a conjugated C(9)=O group (δ(C) 202.2 (*s*)); and the correlations Me(16)/C(14), C(15), and C(17) revealed the adjacent diene group. The correlations CH_2(19)/C(11) and C(10), coupled with CH_2(10)/C(9), suggested the adjacent ketone group. The correlations CH_2(5)/C(7) and C(4a) suggested two C-atom linkages between the C=C bond and ring junction. The

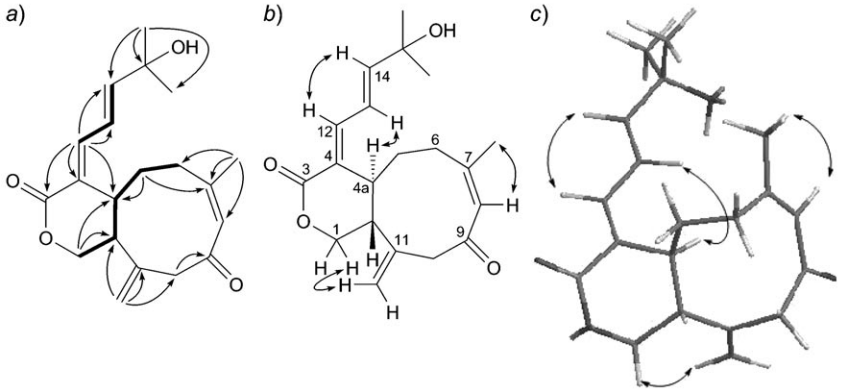

Fig. 3. a) *COSY* (—), *HMBC* (→), *and* b) *NOESY* (↔) *correlations of* **2**. c) *Computer-generated perspectives models of* **2** *using MM2 force field calculation.*

correlations CH_2(1)/C(11a) and C(4a)), and H–C(12)/C(4), C(4a)/C(3), indicated a δ-lactone joined with the ring junction. By deducing the unsaturations of C=C bonds (4), ketone, and δ-lactone (2) from the total of eight degrees of unsaturations allowed us to conclude that compound **2** is another new xeniolide with a nonane ring. The relative configuration of **2** was established from NOESY correlations (*Fig. 3*) and by comparison of its spectroscopic data with those of **1** and other xeniolides [21]. The (*E*)-configuration was assigned to the C(4)=C(12) bond on the basis of the NOESY correlations H–C(4a)/H–C(13) and H–C(12)/H–C(14). The (*E*)-configuration of the C(13)=C(14) bond was established by the large coupling constant observed between H–C(13) and H–C(14) ($J = 15.0$ Hz) as in blumiolide C [22]. The coupling constant ($J = 11.5$ Hz) between Me(18) and H–C(8) suggested the (*Z*)-configuration of the C(7)=C(8) bond [4]. The NOESY correlation between CH_2(19) and H–C(1) (δ(H) 4.01 (*t*)) indicated that the latter H-atom was in α-orientation, which is compatible with its coupling pattern. A molecular model of structure 2 (*Fig. 3*) was consistent with the configurations as established by NOESY experiments. Compound **2** was assigned the name asterolaurin H.

Compound **3** had the molecular formula $C_{21}H_{30}O_4$, as deduced from HR-ESI-MS and NMR data, implying seven degrees of unsaturation. The IR spectrum revealed absorption bands attributed to a OH (3447 cm^{-1}), a diene (2926, 2854 cm^{-1}), and a CO group (1734 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectral features of compound **3** were analogous to those of 9-deoxyxeniolide-A [10]. Three spin systems (*a*–*c*, *Fig. 4*) were observed from COSY and HMBC spectral data (*Fig. 4*). The HMBCs between the H-atoms of Me(18) (δ(H) 1.71 (*s*)) and those of CH_2(6) (δ(C) 40.1 (*t*)), and C=C bond C-atoms C(7) (δ(C) 132.8 (*s*)) and C(8) (δ(C) 130.5 (*d*)), coupled with its NOESY correlation of H–C(8) (δ(H) 5.30 (*d*)) with an O-bearing C-atom C(9) (δ(C) 67.4 (*d*)) suggested an allylic OH partial structure. The HMBCs between the CH_2(19) H-atoms (δ(H) 4.91 (*s*) and 5.09 (*s*)) and the quaternary C-atom C(11) (δ(C) 147.5 (*s*)), the CH C-atom C(11a) (δ(C) 49.7 (*d*)) and the CH_2 C-atom C(10) (δ(C) 44.9 (*t*)) indicated the intermediacy of exocyclic CH_2 group between the allylic OH part and the ring junction.

Aside from these new features, other HMBCs are similar to those of compound **2**, including Me(16)/C(14), C(15), and C(17); H–C(12)/C(3) and C(4); CH_2(1)/C(4a) and C(3); and H–C(4a)/C(11a). Thus, compound **3** is a new xeniolide diterpene containing a nonane ring. The relative configuration of the ring system, which was similar to that of 9-deoxyxeniolide-A, was established by a NOESY experiment as shown in *Fig. 4*. The (*E*)-configuration was assigned to the C(7)=C(8) bond based on the observation of following series of NOESY correlations, Me(18)/H_β–C(9)/H_β–C(10)/H_β–C(11a). The (*E*)-configuration of the C(13)=C(14) bond was established by the large coupling constant observed between H–C(12), H–C(13), and H–C(14), and also the following NOESY correlations, H–C(4a)/H–C(13) and H–C(12)/H–C(14). Therefore, the structure of **3** was assigned to asterolaurin I on the basis of the above results.

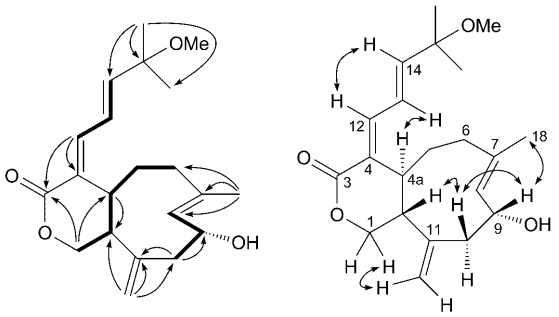

Fig. 4. *COSY* (—), *HMBC* (→), *and NOESY* (↔) *correlations of* **3**

Compound **4** was isolated as an amorphous solid, and combined HR-EI-MS and ^{13}C-NMR data provided the molecular formula $C_{21}H_{30}O_5$, indicating seven degrees of unsaturation. The IR showed absorption bands attributed to a OH (3465 cm^{-1}), a diene (2920, 2852 cm^{-1}), and a lactone group (1703 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectral features of compound **4** were analogous to those of 9-deoxyxeniolide A [10]. The ^{1}H- and ^{13}C-NMR data revealed the presence of an exocyclic CH_2 group (δ(C) 144.6 (*s*), and 117.7 (*t*); δ(H) 5.07 (*s*), 5.26 (*s*), which was linked to the ring-juncture CH groups (δ(C) 49.7 (*d*), and 41.6 (*d*) as verified by HMBCs, and a lactone moiety conjugated with a diene (δ(C) 170.0 (*s*), 132.1 (*s*), 137.6 (*d*), 121.8 (*d*), 149.8 (*d*)) that was connected with a quaternary C-atom (δ(C) 75.0 (*s*)) bearing two Me groups and one MeO group. The new structural feature was an epoxy group located at C(7) (δ(C) 58.9 (*s*) and C(8) (δ(C) 67.2 (*d*)), as revealed by their HMBCs with Me(18) (δ(H) 1.37 (*s*)). Comparison of 1D- and 2D-NMR data of 4 with those of 9-deoxyxeniolide A [10] established 4 to be the 7,8-epoxyxeneolide. The relative configuration of the ring system, which was similar to that of 9-deoxyxeniolide A, was established by a NOESY experiment as shown in *Fig. 5*. The series of NOESY correlations, H_β–C(11a)/H_β–C(10)/H_β–C(9)/Me$_\beta$(18), and H_α–C(8)/H_α–C(4a) established the *trans*-configuration around the epoxy ring. The (*E*)-configuration of the C(13)=C(14) bond was as in compound **3** as established by the large coupling constants observed between H–C(12)/H–C(13)/H–C(14), and the pair of NOESY correlations H–C(4a)/H–C(13) and

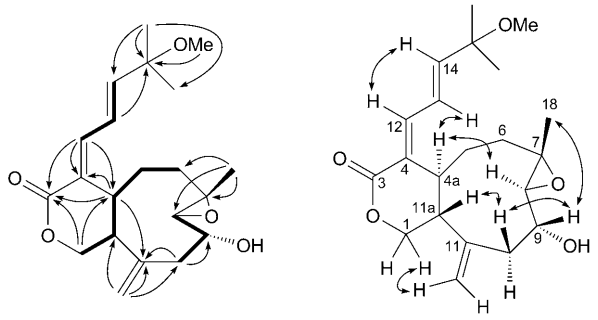

Fig. 5. *COSY* (—), *HMBC* (→), *and NOESY* (↔) *correlations of* **4**

H–C(14)/H–C(12). Therefore the structure of **4**, named asterolaurin J, was determined as shown in *Fig. 1*.

The cytotoxic activities of compounds **1**–**4** were evaluated against four human tumor cell lines. However, no significant activity has been detected for these four new xenicanes against HEp-2 (human laryngeal carcinoma), Daoy (human medulloblastoma), MCF-7 (human breast adenocarcinoma), and WiDr (human colon adenocarcinoma) tumor cells.

Experimental Part

General. Column chromatography (CC): silica gel *60* (SiO_2; *Merck*) or *Sephadex LH-20* (*Amersham Pharmacia Biotech AB*, Uppsala, Sweden). Prep. TLC: precoated silica-gel plates (*Merck*, *Kieselgel 60 F-254*, 1 mm). Optical rotations: *JASCO DIP-1000* polarimeter. IR and UV spectra: *Hitachi T-2001* and *U-3210* spectrophotometers, resp. ^{1}H- and ^{13}C-NMR, COSY, HMQC, HMBC, and NOESY spectra: *Bruker FT-300* spectrometer and a *Varian Unity INOVA 500* FT-NMR at 500 MHz for ^{1}H and at 125 MHz for ^{13}C, resp., TMS as an internal standard; the chemical shifts δ in ppm, and coupling constants in Hz. Low-resolution EI- and FAB-MS: *VG Quattro 5022* mass spectrometer. HR-EI-MS: *JEOL JMS-SX 102* spectrometer.

Animal Material. The soft coral *Asterospicularia laurae* was collected from the southern coast of Taiwan, in September 2008, at a depth of 15 m, and immediately stored in a freezer. This species was identified by one of the authors (*Y.-S. Lin*). A voucher specimen (NTUO-9) was deposited with the School of Pharmacy, National Taiwan University, Taipei, Taiwan.

Extraction and Isolation. The soft coral (wet weight 3.0 kg) was extracted with CH_2Cl_2/MeOH (1:1) at r.t. using a stirrer, and the extract was concentrated under vacuum. The crude extract was partitioned with AcOEt/H_2O 1:1. The AcOEt-soluble portion (42 g) was partitioned with hexane/MeOH/H_2O 4:3:1 to give two layers (hexane layer and MeOH/H_2O layer (30 g)). The MeOH/H_2O layer (30 g) was subjected to *Sephadex LH-20* MeOH chromatography affording two layers, L_1 and L_2. *Fr.* F_2 (850 mg) was subjected to CC (SiO_2; hexane/AcOEt gradient), followed by separation on RP-HPLC (MeOH/H_2O/MeCN 60:35:5) to yield **3** (7 mg). *Fr.* F_3 (360 mg) was separated on RP-HPLC (MeOH/H_2O/MeCN 60:35:65), and further separation by RP-HPLC (MeOH/H_2O/MeCN 55:40:5) furnished **1** (5 mg) and **4** (4 mg). *Fr.* F_5 (1.5 g) was subjected to RP-HPLC (MeOH/H_2O/MeCN 60:35:5) and followed by RP-HPLC (MeOH/H_2O/MeCN 55:40:5) to give **2** (12 mg).

Asterolaurin G (=*(1a*S*,3a*S*,4*E*,7a*R*,10*S*,10a*S*)-4-[(2*E*)-4-Hydroxy-4-methylpent-2-en-1-ylidene]-1a-methyl-8-methylidene-7-oxododecahydrooxireno[5,6]cyclonona[1,2-c]pyran-10-yl Acetate*; **1**). White solid. $[\alpha]_D^{25} = -6.0$ ($c = 0.5$, CH_2Cl_2). UV (MeOH): 214 (3.21). IR (neat): 3429, 2973, 2936, 1715, 1373,

1273. ^{1}H- and ^{13}C-NMR ($CDCl_3$): see *Tables 1* and 2, resp. HR-ESI-MS: 413.1937 ($[M+Na]^+$, $C_{22}H_{30}NaO_6^+$; calc. 413.1940).

Asterolaurin H (=*(4*E*,4a*S*,7*Z*,11a*R*)-4-[(2*E*)-4-Hydroxy-4-methylpent-2-en-1-ylidene]-7-methyl-11-methylidene-1,4,4a,5,6,10,11,11a-octahydrocyclonona[*c*]pyran-3,9-dione*; **2**). White solid. $[\alpha]_D^{25} = +2.4$ ($c = 0.5$, CH_2Cl_2). UV (MeOH): 215.5 (3.86), 239.5 (3.25). IR (neat): 3414, 2928, 2857, 1732, 1235, 1036. ^{1}H- and ^{13}C-NMR ($CDCl_3$): see *Tables 1* and *2*, resp. HR-ESI-MS: 353.1728 ($[M+Na]^+$, $C_{20}H_{26}NaO_4^+$; calc. 353.1729).

Asterolaurin I (=*(4*E*,4a*S*,9*R*,11a*R*)-9-Hydroxy-4-[(2*E*)-4-methoxy-4-methylpent-2-en-1-ylidene]-7-methyl-11-methylidene-4,4a,5,6,9,10,11,11a-octahydrocyclonona[*c*]pyran-3(1*H*)-one*; **3**). White solid. $[\alpha]_D^{25} = +10.5$ ($c = 0.5$, CH_2Cl_2). UV (MeOH): 220 (3.25), 237.5 (3.86). IR (neat): 3447, 2926, 2854, 1734, 1445, 1239. ^{1}H- and ^{13}C-NMR ($CDCl_3$): see *Tables 1* and 2, resp. HR-ESI-MS: 369.2044 ($[M+Na]^+$, $C_{21}H_{30}NaO_4^+$; calc. 369.2042).

Asterolaurin J (=*(1a*S*,3a*S*,4*E*,7a*R*,10*S*,10a*S*)-10-Hydroxy-4-[(2*E*)-4-methoxy-4-methylpent-2-en-1-ylidene]-1a-methyl-8-methylidenedecahydrooxireno[5,6]cyclonona[1,2-*c*]pyran-5(1a*H*)-one*; **4**). White solid. $[\alpha]_D^{25} = +10.5$ ($c = 0.5$, CH_2Cl_2). UV (MeOH): 217 (3.62) 241.5 (3.44). IR (neat): 3465, 2920, 2852, 1703, 1636, 1462. ^{1}H- and ^{13}C-NMR ($CDCl_3$): see *Tables 1* and *2*, resp. HR-ESI-MS: 385.1988 ($[M+Na]^+$, $C_{21}H_{30}NaO_5^+$; calc. 385.1991).

Biological Assay. Cytotoxicity was tested against HEp-2 (human laryngeal carcinoma), Daoy (human medulloblastoma), MCF-7 (human breast adenocarcinoma), and WiDr (human colon adenocarcinoma) tumor cell lines. The assay procedure using MTT (= 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide was carried out as described in [26]. The cells were cultured in *RPMI-1640* medium. After seeding of cells in a 96-well microplate for 4 h, 20 µl of sample was placed in each well and incubated at 37° for 3 d, and then 20 µl of MTT was added for 5 h. After removing the medium and putting DMSO (200 µl/well) into the microplate with shaking for 10 min, the formazan crystals were redissolved, and their absorbance was measured on a microtiter plate reader (*Dynatech*, *MR 7000*) at a wavelength of 550 nm.

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