New Eunicellin Diterpenes and 9,10-Secosteroids from the Gorgonian *Muricella sibogae*

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Bioactivity-guided isolation of the rare gorgonian *Muricella sibogae* (NUTTING) yielded the two new eunicellin diterpenes sibogin A and B (**1** and **2**), the three new 9,10-secosteroids sibogol A–C (**6**–**8**), together with the three known eunicellin diterpenes **3**–**5** and the five known 9,10-secosteroids **9**–**13**. Their structures were established by extensive spectral analysis (1D- and 2D-NMR, IR, and MS). The cytotoxicity of the isolates **1**–**13** was evaluated *in vitro* against the selected tumor cell lines P388 and BEL-7402. All the compounds showed only weak activity against P388 cell lines, with an inhibition rate ranging from 10 to 60% at a concentration of 50 μg/ml, whereas the were inactive against BEL-7402 cell lines.

Introduction. – Both eunicellin diterpenes characterized by an O-bridge between C(2) and C(9) in the 2,11-cyclized cembrane backbone (cembrane = 1,7,11-trimethyl-4-(1-methylethyl)cyclotetradecane), and 9,10-secosteroids featuring a rare 3-hydroxy-10-methyl-substituted aromatic ring and variable side-chain, are two classes of representative natural products which were restricted to marine organism [1]. It should be particularly noticed that, up to now, all the eunicellin diterpenes were almost only found from the class Anthozoa of the orders Gorgonaceae (genus *Briareum*, *Eunicella*, *Solenopodium*, and *Muricella*) and Alcyonaceae (genus *Klyxum*, *Cladiella*, *Litophyton*, and *Alcyonium*) [1a][2]. And the 9,10-secosteroids with only 25 compounds reported so far, were exclusively found from gorgonians (genus *Astrogorgia*, *Calicogorgia*, and *Muricella*) [1b][2]. In addition to unusual structures, these two types of compounds also showed diverse bioactivities such as cytotoxic, antivirus, brine-shrimp lethal, and anti-inflammatory activities, as well as other interesting bioactivities, *e.g.*, inhibiting the expression of the inducible nitric oxide synthase protein, reducing the level of cyclooxygenase-2 protein, stabilizing microtubule, and inhibiting the cell division of the fertilized starfish (*Asterina pectinifera*) eggs [1b][3].

In the course of our searching for bioactive compounds from marine organisms, we encountered the gorgonian *Muricella sibogae* (family Acanthogorgiidae), a rare species collected from the near coast of Weizhou Island in the Guangxi Zhuang Autonomous Region of China. A bioactivity-guided isolation of *M. sibogae* yielded two

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new eunicellin-based diterpenes with new substitution patterns in the ten-membered macrocycle, sibogin A (**1**) and B (**2**)[2]), and three new 9,10-secosteroids with different side chains, sibogols A – C (**6** – **8**), together with the three known eunicellin diterpenes calicophirin C (**3**) [4a], 13-de(acetyloxy)calicophirin B (**4**) [4b], and ophirin (**5**) [4b], and the five known 9,10-secosteroids calicoferol A (**9**) [5], calicoferol D (**10**) [6], calicoferol E (**11**) [6], 24-methylenecalicoferol E (**12**) [7] and 24-methylidenecalicoferol E (**13**; *Fig. 1*) [7]. In this article, we describe the isolation, structure elucidation, and bioactivity of these isolates.

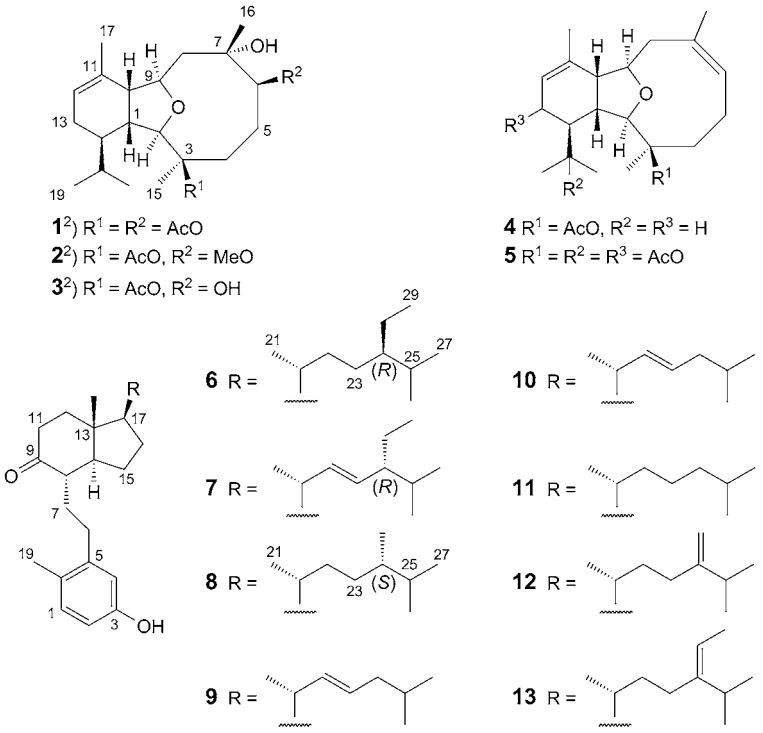

Fig. 1. *Compounds* **1** – **13**, *isolated from* Muricella sibogae

Results and Discussion. – Sibogin A (**1**) was obtained as a colorless oil. The HR-ESI-MS measurement gave a $[M+\mathrm{Na}]^+$ peak at *m*/*z* 445.2555 consistent with the molecular formula $C_{24}H_{38}O_6$ requiring six degrees of unsaturation. Its IR spectrum disclosed the absorptions of ester C=O (1732 cm^{-1}) and a C=C bond (1650 cm^{-1}). The ^{1}H- and ^{13}C-NMR signals (*Table 1*) at δ(H) 2.02 (*s*, 3 H) and 2.06 (*s*, 3 H), and δ(C) 22.4 (*q*), 22.6 (*q*), 169.7 (*s*) and 169.8 (*s*) confirmed the presence of two Ac groups in **1**. The resonances of one trisubstituted C=C bond at δ(H) 5.48 (br. *s*, 1 H) and δ(C) 132.1 (*s*) and 121.6 (*d*) were also observed. Thus, we speculated that compound **1** has a tricyclic skeleton. In addition to these observations, the ^{1}H-NMR spectra of **1** showed

[2]) Trivial atom numbering; for systematic names, see *Exper. Part.*

five Me groups (δ(H) 0.85 (*d*, *J* = 7.8 Hz), 0.98 (*d*, *J* = 7.8 Hz), 1.27 (*s*), 1.58 (*s*) and 1.68 (*s*)), two oxygenated CH groups (δ(H) 3.83–3.90 (*m*, H–C(2)) and 4.29 (*dt*, *J* = 8.8, 3.0 Hz)), and an acyloxygenated CH group (the low-field signal at δ(H) 5.65 (br. *s*)). The ^{13}C-NMR (APT) spectra showed 20 C-atom signals assigned to five Me, four CH_2, and eight CH groups, as well as three quaternary C-atoms (*Table 1*). All these evidences indicated that compound **1** should be a tricyclic diterpene closely similar to the co-isolated calicophirin C (**3**) [4a], with the exception of an additional Ac group. The structure of compound **1** was further identified by 2D-NMR experiments. Its ^{1}H,^{1}H-COSY plot displayed two spin-coupling systems (CH_2(8)/H–C(9)/H–C(10)/H–C(1)/H–C(2) and H–C(14)/CH_2(13)/H–C(12) and H–C(18)/Me(19) and Me(20), and CH_2(4)/CH_2(5)/H–C(6)) (*Fig. 2*). Together with the key HMBCs of the five Me groups and of H–C(2) at δ(H) 3.83–3.90 (*m*) with C(9) (δ(C) 78.1 (*d*)) (*Fig. 2*), a C-atom skeleton of an eunicellin diterpene was deduced for **1**. In particular, the HMBCs Me(16) (δ(H) 1.27)/C(6) (δ(C) 82.5), C(7) (δ(C) 75.0), C(8) (δ(C) 47.3), and both H–C(6) (δ(H) 5.65) and an *Me*CO (δ(H) 2.02)/Me*C*O (δ(C) 169.7) established the presence of an OH group at C(7) and an AcO group at C(6), respectively. The remaining AcO group was located at C(3) by the key HMBC Me(15) (δ(H) 1.58)/C(3) (δ(C) 86.5) and the remarkable downfield shift of Me(15) (δ(H) 1.58) relative to similar 3-OH-substituted structures [4]. The NOESY experiment showed a similar relative configuration of **1** as in the cases of the known co-isolated analogues calicophirin C (= (4*R*,4a*R*,5*R*,6*R*,9*S*,10*S*,12*R*,12a*R*)-3,4,4a,5,6,7,8,9,10,11,12,12a-dodecahydro-1,6,10-trimethyl-4-(1-methylethyl)-5,12-expoxybenzocyclodecene-6,9,10-triol-6-acetate; **3**) [4a] and hirsutalin E [4]. The NOE correlations H–C(1) (δ(H) 2.56–2.60)/H–C(10) (δ(H) 2.41–2.48) and Me(19) (δ(H) 0.85) indicated that H–C(1), H–C(10), and the ^{i}Pr group were on the same β side of the cyclohexene ring, and the cyclohexene ring and the macrocycle were thus *cis*-fused (*Fig. 2*). Also, the NOEs Me(15)/H–C(2) and H–C(6), and H–C(2)/H–C(9), as well as the lack of the correlations H–C(1)/H–C(9), H–C(2)/H–C(10), and H–C(6)/Me(16) suggested that, H–C(2), H–C(6), H–C(9), and Me(15) were α-orientated, while Me(16) was β-orientated (*Fig. 2*). Accordingly, compound **1** was determined to possess the *rel*-(1*R*,2*R*,3*R*,6*S*,7*S*,9*R*,10*R*,14*R*) configuration as shown in *Fig. 1*, and was named sibogin A.

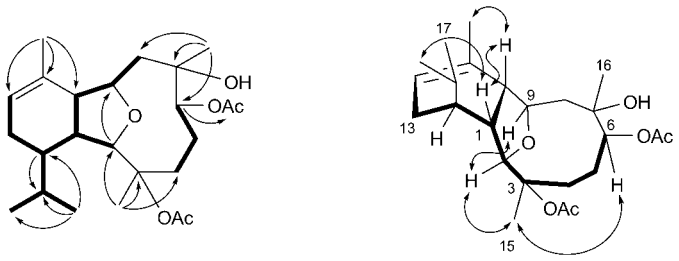

Fig. 2. *Key* ^{1}*H,*^{1}*H-COSY* (—), *HMB* (H → C), *and NOESY correlations* (H ↔ H) *of compound* **1**

Sibogin B (**2**) had the molecular formula $C_{23}H_{38}O_5$ determined by the HR-ESI-MS ([*M* + Na]$^+$ at *m*/*z* 417.2642). Comparison of the ^{1}H- and ^{13}C-NMR data of **2** (*Table 1*) with those of **1** indicated a close similarity of their structure. However, a visible

Table 1. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; $CDCl_3$) *of Compounds* **1** *and* **2**[2]). δ in ppm, J in Hz.

Position	**1**		**2**	
	δ(H)[a])	δ(C)[b])	δ(H)[a])	δ(C)[b])
H–C(1)	2.56–2.60 (*m*)	40.6 (*d*)	2.54–2.60 (*m*)	40.6 (*d*)
H–C(2)	3.83–3.90 (*m*)	88.3 (*d*)	3.89 (*d*, *J* = 5.0)	87.4 (*d*)
C(3)	–	86.5 (*s*)	–	86.4 (*s*)
CH_2(4)	2.56–2.60, 1.30–1.38 (2*m*)	40.0 (*t*)	2.30–2.39, 2.05–2.10 (2*m*)	32.0 (*t*)
CH_2(5)	1.25–1.30, 1.29–1.33 (2*m*)	29.6 (*t*)	1.85–1.92, 1.29–1.34 (2*m*)	26.0 (*t*)
H–C(6)	5.65 (br. *s*)	82.5 (*d*)	4.12 (br. *s*)	87.0 (*d*)
C(7)	–	75.0 (*s*)	–	75.0 (*s*)
CH_2(8)	1.95–2.05, 1.75–1.83 (2*m*)	47.3 (*t*)	1.83–1.90, 1.70–1.78 (2*m*)	46.4 (*t*)
H–C(9)	4.29 (*dt*, *J* = 8.8, 3.0)	78.1 (*d*)	4.34 (*dt*, *J* = 8.8, 3.0)	77.7 (*d*)
H–C(10)	2.41–2.48 (*m*)	49.0 (*d*)	2.30–2.39 (*m*)	49.0 (*d*)
C(11)	–	132.1 (*s*)	–	132.4 (*s*)
H–C(12)	5.48 (br. *s*)	121.6 (*d*)	5.47 (br. *s*)	121.9 (*d*)
CH_2(13)	2.10–2.18, 1.85–2.04 (2*m*)	22.8 (*t*)	2.08–2.13, 1.91–1.96 (2*m*)	22.8 (*t*)
H–C(14)	1.30–1.40 (*m*)	39.4 (*d*)	1.35–1.40 (*m*)	39.4 (*d*)
Me(15)	1.58 (*s*)	23.0 (*q*)	1.63 (*s*)	23.4 (*q*)
Me(16)	1.27 (*s*)	23.3 (*q*)	1.15 (*s*)	23.3 (*q*)
Me(17)	1.68 (*s*)	22.8 (*q*)	1.69 (*s*)	22.1 (*q*)
H–C(18)	1.70–1.78 (*m*)	28.7 (*d*)	1.65–1.73 (*m*)	28.9 (*d*)
Me(19)	0.85 (*d*, *J* = 7.8)	18.4 (*q*)	0.87 (*d*, *J* = 6.7)	19.3 (*q*)
Me(20)	0.98 (*d*, *J* = 7.8)	21.7 (*q*)	0.98 (*d*, *J* = 6.7)	21.7 (*q*)
*Ac*O–C(3)	2.06 (*s*)	22.4 (*q*), 169.8 (*s*)	2.03 (*s*)	22.4 (*q*), 169.8 (*s*)
*Ac*O–C(6)	2.02 (*s*)	22.6 (*q*), 169.7 (*s*)	–	–
*Me*O–C(6)	–	–	3.37 (*s*)	57.3 (*q*)

[a]) By ^{1}H,^{1}H-COSY, and HSQC experiments. [b]) By DEPT, HSQC, and HMBC experiments.

difference in the spectra of **2** was the presence of the signals of only one AcO group (δ(H) 2.03 (*s*, 3 H); δ(C) 22.4 (*q*) and 169.8 (*s*)) and additional MeO signals (δ(H) 3.37 (*s*, 3 H) and δ(C) 57.3(*q*)). Therefore, presumably the AcO group at C(6) of **1** was replaced by a MeO group in **2**. Detailed examinations of a similar downfield shift of Me(15) (δ(H) 1.63 in **2** and 1.58 in **1**) and relative upfield shift of Me(16) (δ(H) 1.15 in **2** and 1.27 in **1**), almost identical positions of the C(3) (δ(C) 86.4 in **2** and 86.5 in **1**) and C(7) signals (δ(C) 75.0 both in **2** and **1**), and the upfield shifted signal of H–C(6) of **2** (δ(H) 4.12 and as compared to **1** (δ(H) 5.65) were compatible with the position of the MeO group at C(6) of **2**. This was further confirmed by the crucial HMBCs Me(16)/C(6) (δ(C) 87.0), C(7), and C(8) (δ(C) 46.4). Furthermore, the analysis of NOE correlations of **2** revealed the same relative configuration at C(1), C(2), C(3), C(6), C(7), C(9), C(10), and C(14) as that of **1**.

Sibogol A (**6**) was obtained as a colorless oil. The HR-ESI-MS gave the molecular formula $C_{29}H_{46}O_2$ (*m*/*z* 427.3555 ($[M+H]^+$)), which accounted for seven degrees of unsaturation. Its IR spectrum showed the C=O absorption at 1739 cm^{-1}. The ^{1}H- and ^{13}C-NMR data of **6** (*Tables 2* and *3*) showed the presence of a trisubstituted benzene

ring by an aromatic *ABX* spin system (δ(H) 6.57 (*dd*, J = 8.1, 2.5 Hz, 1 H), 6.65 (*d*, J = 2.5 Hz, 1 H), and 6.95 (*d*, J = 8.1 Hz, 1 H)) and C-atom resonances at δ(C) 112.5 (*d*), 115.8 (*d*), 127.9 (*s*), 131.0 (*d*), 142.5 (*s*), and 153.8 (*s*), and of one C=O group (δ(C) 213.5 (*s*)), which unambiguously indicated that **6** should possess a bicyclic moiety besides the benzene ring. These analyses, in combination with the presence of six Me groups at δ(H) 0.81 (*d*, J = 6.6 Hz), 0.82 (*d*, J = 6.6 Hz), 0.84 (overlapped), 0.92 (*dd*, J = 6.4, 1.4 Hz), 0.96 (*s*) and 2.23 (*s*, 3 H)), further suggested that **6** featured a 9,10-secosteroid structure closely related to the known co-isolated analogue calicoferol E (= 3-hydroxy-9,10-secocholesta-1,3,5(10)-trien-9-one; **11**) [6]. A careful comparison of the spectral data of **6** and **11** revealed that their δ(C), were almost identical except for those of to the side-chain. The ^{13}C-NMR (APT) spectrum of **6** showed a total of 29 C-atoms s (6 Me, 9 CH_2, 9 CH, and 5 C; *Table 3*), and the presence of an additional CH_2 and Me group as compared to **11**. The cross-peaks between all geminal and vicinal H-atoms observed in the COSY plot of **6**, together with the HMBCs Me(21) (δ(H) 0.92/C(17) (δ(C) 55.0), C(20) (δ(C) 36.0) and C(22) (δ(C) 33.7), Me(29)/C(28) (δ(C) 23.1) and C(24) (δ(C) 45.8) and Me(26) (δ(H) 0.82 and Me(27) (δ(H) 0.81)/C(25) (δ(C) 29.2) and C(24) determined the side chain of **6** (*Fig. 3*). The key NOESY correlations Me(18) (δ(H) 0.96)/H–C(8) (δ(H) 2.31 – 2.42) and H–C(20) (δ(H) 1.38 – 1.44), and H–C(14) (δ(H) 1.68 – 1.74)/H–C(17) (δ(H) 1.20 – 1.30) indicated the same relative configuration of **6** as those in calicoferol E (**11**). Furthermore, since the observed chemical shift difference between C(26) (δ(C) 19.0) and C(27) (δ(C) 19.8) was 0.9 ppm, instead of 0.55 ppm as in the case of (*S*) configuration for a saturated 24-ethyl substituent [8], the absolute configuration at C(24) of **6** was determined as (*R*). Thus, compound **6** was identified as 3-hydroxy-9,10-secostigmasta-1,3,5(10)-trien-9-one and named sibogol A.

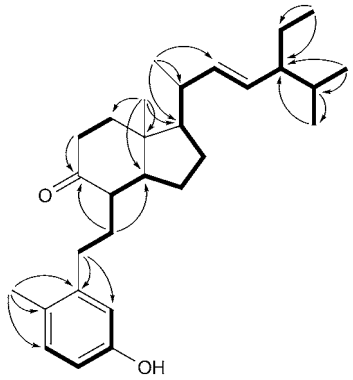

Fig. 3. *Key ^{1}H,^{1}H-COSY* (—) *and HMB* (H → C) *correlations of compound* **6**

The molecular formula of sibogol B (**7**) was determined as $C_{29}H_{44}O_2$ by HR-ESI-MS (*m*/*z* 425.3411 ([M + H]$^+$)). Its ^{1}H- and ^{13}C-NMR data (*Tables 2* and *3*) were very similar to those of **6**. The noticeable differences were the replacement of two CH_2 groups of **6** by a disubstituted C=C bond in **7** (δ(H) 5.13 (*ddd*, J = 15.0, 7.9, 2.2 Hz, CH) and 5.04 (*dd*, J = 15.0, 8.4 Hz, CH); δ(C) 137.5 (*d*) and 131.0 (*d*)). Detailed examination

Table 2. *^{1}H-NMR Data* (500 MHz; $CDCl_3$) *of Compounds* **6**–**8**. δ in ppm, J in Hz.

H-Atom	δ(H)[a])		
	6	**7**	**8**
H–C(1)	6.95 (*d*, *J* = 8.1)	6.95 (*d*, *J* = 8.0)	7.00 (*d*, *J* = 8.1)
H–C(2)	6.57 (*dd*, *J* = 8.1, 2.5)	6.56 (br. *d*, *J* = 8.0)	6.60 (*dd*, *J* = 8.1, 2.5)
H–C(4)	6.65 (*d*, *J* = 2.5)	6.64 (br. *s*)	6.70 (*d*, *J* = 2.5)
CH_2(6)	2.65 (*ddd*, *J*= 12.8, 12.5, 4.9), 2.43–2.52 (*m*)	2.63 (*ddd*, *J* = 12.5, 12.2, 4.5), 2.35–2.43 (*m*)	2.68 (*ddd*, *J* = 12.9, 12.3, 4.7), 2.36–2.45 (*m*)
CH_2(7)	1.68–1.75, 1.50–1.61 (2*m*)	1.70–1.75, 1.55–1.63 (2*m*)	1.75–1.83, 1.58–1.64 (2*m*)
H–C(8)	2.31–2.42 (*m*)	2.30–2.37 (*m*)	2.35–2.42 (*m*)
CH_2(11)	2.48 (*ddd*, *J* = 14.7, 13.8, 6.4), 2.26–2.34 (*m*)	2.49 (*ddd*, *J* = 14.6, 14.0, 6.7), 2.30–2.37 (*m*)	2.52 (*ddd*, *J* = 15.0, 14.0, 6.4), 2.35 (*ddd*, *J* = 15.0, 13.0, 5.3)
CH_2(12)	2.10–2.16, 1.50–1.61 (2*m*)	2.15–2.20, 1.63–1.67 (2*m*)	2.06–2.15, 1.56–1.63 (2*m*)
H–C(14)	1.68–1.74 (*m*)	1.68–1.74 (*m*)	1.67–1.74 (*m*)
CH_2(15)	1.68–1.74, 1.18–1.23 (2*m*)	1.68–1.74, 1.53–1.63 (2*m*)	1.67–1.72, 28–1.33 (2*m*)
CH_2(16)	1.68–1.74, 1.35–1.44 (2*m*)	1.79–1.83, 1.38–1.44 (2*m*)	2.08–2.04, 136–1.44 (2*m*)
H–C(17)	1.20–1.30 (*m*)	1.26–1.34 (*m*)	1.20–1.26 (*m*)
Me(18)	0.96 (*s*)	0.98 (*s*)	1.00 (*s*)
Me(19)	2.23 (*s*)	2.20 (*s*)	2.28 (*s*)
H–C(20)	1.38–1.44 (*m*)	2.05–2.15 (*m*)	1.36–1.44 (*m*)
Me(21)	0.92 (*dd*, *J* = 6.4, 1.4)	1.15 (*d*, *J* = 6.5)	0.95 (*dd*, *J* = 6.4)
CH_2(22) or H–C(22)	1.25–1.34, 0.99–1.06 (2*m*)	5.13 (*ddd*, *J* = 15.0, 7.9, 2.2)	1.26–1.35, 0.88–0.92 (2*m*)
CH_2(23) or H–C(23)	1.08–1.15 (*m*)	5.04 (*dd*, *J* = 15.0, 8.4)	1.26–1.35, 1.00–1.09 (2*m*)
H–C(24)	0.85–0.95 (*m*)	1.48–1.53 (*m*)	1.20–1.29 (*m*)
H–C(25)	1.61–1.69(*m*)	1.51–1.56 (*m*)	1.58–1.63 (*m*)
Me(26)	0.82 (*d*, *J* = 6.6)	0.83 (*d*, *J* = 6.5)	0.89 (*d*, *J* = 6.8)
Me(27)	0.81 (*d*, *J* = 6.6)	0.78 (*d*, *J* = 6.5)	0.84 (*d*, *J* = 6.7)
CH_2(28) or Me(28)	1.25–1.34, 1.08–1.16 (2*m*)	1.26–1.32, 1.19–1.23 (2*m*)	0.81 (*d*, *J* = 6.7)
Me(29)	0.84[b])	0.80[b])	–

[a]) By ^{1}H,^{1}H-COSY and HSQC experiments. [b]) Overlapped.

Table 3. *^{13}C-NMR Data* (125 MHz; $CDCl_3$) *of Compounds* **6**–**8**. δ in ppm.

C-Atom	δ(C)[a])		
	6	**7**	**8**
C(1)	131.0 (*d*)	131.0 (*d*)	131.1 (*d*)
C(2)	112.5 (*d*)	112.5 (*d*)	112.6 (*d*)
C(3)	153.8 (*s*)	153.7 (*s*)	153.8 (*s*)
C(4)	115.8 (*d*)	115.7 (*d*)	115.7 (*d*)
C(5)	142.5 (*s*)	142.5 (*s*)	142.5 (*s*)
C(6)	31.1 (*t*)	31.0 (*t*)	31.5 (*t*)
C(7)	27.6 (*t*)	27.7 (*t*)	27.7 (*t*)
C(8)	50.5 (*d*)	50.5 (*d*)	50.5 (*d*)
C(9)	213.5 (*s*)	213.1 (*s*)	213.7 (*s*)
C(10)	127.9 (*s*)	128.0 (*s*)	128.0 (*s*)
C(11)	38.3 (*t*)	38.4 (*t*)	38.5 (*t*)
C(12)	38.5 (*t*)	38.4 (*t*)	38.3 (*t*)
C(13)	42.8 (*s*)	42.7 (*s*)	42.8 (*s*)
C(14)	55.3 (*d*)	55.3 (*d*)	55.3 (*d*)
C(15)	25.2 (*t*)	25.4 (*t*)	25.2 (*t*)
C(16)	29.1 (*t*)	29.7 (*t*)	29.0 (*t*)
C(17)	55.0 (*d*)	54.9 (*d*)	55.0 (*d*)
C(18)	11.5 (*q*)	11.7 (*q*)	11.5 (*q*)
C(19)	18.4 (*q*)	18.4 (*q*)	18.4 (*q*)
C(20)	36.0 (*d*)	40.3 (*d*)	36.1 (*d*)
C(21)	18.6 (*q*)	21.2 (*q*)	18.6 (*q*)
C(22)	33.7 (*t*)	137.5 (*d*)	33.5 (*t*)
C(23)	26.4 (*t*)	131.0 (*d*)	30.3 (*t*)
C(24)	45.8 (*d*)	51.2 (*d*)	39.1 (*d*)
C(25)	29.2 (*d*)	31.8 (*d*)	31.5 (*d*)
C(26)	19.0 (*q*)	18.4 (*q*)	17.6 (*q*)
C(27)	19.8 (*q*)	21.2 (*q*)	20.2 (*q*)
C(28)	23.1 (*t*)	25.3 (*t*)	15.4 (*q*)
C(29)	11.5 (*q*)	12.2 (*q*)	–

[a]) By DEPT, HSQC, and HMBC experiments.

of the NMR data established the structural changes at the side chain. A combination of COSY, HSQC, and HMBC data further defined the C=C bond at C(22), and its configuration was deduced as (*E*), derived from the large coupling constant value (J(22,23) = 15.0 Hz). The absolute configuration at C(24) of **7** was also determined as (*R*) by the chemical shift differences of Me(26)/Me(29) ($\Delta\delta$(H) 0.03) and Me(27)/Me(29) ($\Delta\delta$(H) 0.02) [8].

HR-ESI-MS Data provided the molecular formula of sibogol C (**8**) as $C_{28}H_{44}O_2$, which was 14 mass units less than that of **6**. Comparison of ^{1}H- and ^{13}C-NMR data (*Tables 2* and *3*) defined its structure as closely similar to **6**. The remarkable difference in the ^{1}H-NMR spectra was the presence of a Me group in **8** (δ(H) 0.81 (*d*, J = 6.7 Hz)) replacing the Et group at C(24) of **6** (δ(H) 1.25–1.34 and 1.08–1.16 (2*m*, CH_2(28)) and 0.84 (Me(29))). The NOESY data indicated that **8** had the same relative configuration as **6**, except for the configuration at C(24) of **8** which was determined as (*S*), based on

the chemical shift difference $\Delta\delta(C) = 2.6$ of C(26)/C(27), whereas (24*R*) configuration for a 24-methyl substituent would have a $\Delta\delta(C) \approx 2$ [8]. Thus, the structure of **8** was determined as 3-hydroxy-9,10-secoergosta-1,3,5(10)-trien-9-one and named sibogol C.

The cytotoxicity of the isolates **1**–**13** was evaluated *in vitro* against the selected tumor cell lines P388 and BEL-7402 by using the MTT (=2-(4,5-dimethylthiazol-2-yl)-3,5-diphenyl-2*H*-tetrazolium bromide) method [9] with adriamycin (ADM) as positive control. However, all the compounds showed only weak activity against P388 cell lines, with an inhibition rate ranging from 10 to 60% at a concentration of 50 μg/ml, whereas they were inactive against BEL-7402 cell lines.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh, *Qingdao Marine Chemical Factory*, Qingdao, P. R. China). TLC: SiO_2 GF_{254} (*Qingdao Marine Chemical Factory*, Qingdao, P. R. China). Semi-prep. HPLC: ODS column (*Kromasil*, 10 mm × 250 mm, 5 μm); flow 4 ml/min. Optical rotation: *Jasco P-1020* digital polarimeter. IR Spectra: *Nicolet-Nexus-470* spectrophotometer with KBr discs; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR: *Bruker-DRX-500* NMR spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *Micromass-Q-Tof-Ultima-Global-GAA076 LC* mass spectrometer; in *m*/*z*.

Animal Material. The *Muricella sibogae* (GXWZ-02) was collected from the coast of Weizhou Island in the Guangxi Zhuang Autonomous Region of China in April 2006 and was frozen immediately after removal from the water. The animal was identified by Prof. *Lin-Ren Zou*, South China Sea Institute of Oceanology, Chinese Academy of Sciences. A voucher specimen has been deposited with the School of Medicine and Pharmacy, Ocean University of China.

Extraction and Isolation. The frozen sample of *M. sibogae* (wet weight *ca.* 7.4 kg) was pulverized and subsequently extracted with MeOH at r.t. The crude extract was desalinated with anh. MeOH to afford a residue (119 g), which was then partitioned with petroleum ether (b.p. 60–90°), AcOEt, and BuOH, resp. Parts soluble in petroleum ether and AcOEt, showing significant cytotoxicities against mouse-leukemia cell line P388 and human hepatoma cell line BEL-7402, were combined and fractionated by CC (SiO_2, petroleum ether/acetone 20:1, 10:1, 5:1, 3:1, and 1:1) and purified by CC (*Sephadex LH-20*): *Fractions A–D*. Subsequently, the most active *Fr. B* was further purified by CC (SiO_2) and reversed-phase semi-prep. HPLC: 13 pure compounds, *i.e.*, **1** (2.0 mg), **2** (2.2 mg), **3** (2.0 mg), **4** (3.7 mg), **5** (3.0 mg), **6** (2.3 mg), **7** (4.2 mg), **8** (4.9 mg), **9** (2.1 mg), **10** (3.0 mg), **11** (4.9 mg), **12** (6.3 mg), and **13** (5.9 mg).

Sibogin A (=rel-(*4*R,*4a*R,*5*R,*6*R,*9*S,*10*S,*12*R,*12a*R)*-3,4,4a,5,6,7,8,9,10,11,12,12a-Dodecahydro-1,6,10-trimethyl-4-(1-methylethyl)-5,12-epoxybenzocyclodecene-6,9,10-triol 6,9-Diacetate*; **1**): Colorless oil. $[\alpha]_D^{20} = +41.5$ ($c = 0.1$, $CHCl_3$). IR (film): 3640, 2959, 2925, 1732, 1650, 1630, 1557, 1539, 1456, 1367, 1243, 1068. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 445.2555 ($[M + Na]^+$, $C_{24}H_{38}NaO_6^+$; calc. 445.2566).

Sibogin B (=rel-(*4*R,*4a*R,*5*R,*6*R,*9*S,*10*S,*12*R,*12a*R)*-3,4,4a,5,6,7,8,9,10,11,12,12a-Dodecahydro-9-methoxy-1,6,10-trimethyl-4-(1-methylethyl)-5,12-epoxybenzocyclodecene-6,10-diol 6-Acetate*; **2**): Colorless oil. $[\alpha]_D^{20} = +28.3$ ($c = 0.1$, $CHCl_3$). IR (film): 3646, 2963, 2931, 1735, 1456, 1370, 1244, 1076. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 417.2642 ($[M + Na]^+$, $C_{23}H_{38}NaO_5^+$; calc. 417.2617).

Sibogol A (= *3-Hydroxy-9,10-secostigmasta-1,3,5(10)-trien-9-one*; **6**): Colorless oil. $[\alpha]_D^{20} = -15.3$ ($c = 0.2$, $CHCl_3$). IR (film): 3486, 2956, 2927, 2868, 1769, 1739, 1698, 1645, 1462, 1117, 991, 867, 809, 741. ^{1}H- and ^{13}C-NMR: *Tables 2* and *3*. HR-ESI-MS: 427.3555 ($[M+H]^+$, $C_{29}H_{47}O_2^+$; calc. 427.3576).

Sibogol B (= *(24*R*)-3-Hydroxy-9,10-secostigmasta-1,3,5(10),22-tetraen-9-one*; **7**): Colorless oil. $[\alpha]_D^{20} = +23.8$ ($c = 0.2$, $CHCl_3$). IR (film): 3448, 2956, 2867, 1740, 1699, 1646, 1559, 1515, 1456, 1259, 1099, 861, 807, 674. ^{1}H- and ^{13}C-NMR: *Tables 2* and *3*. HR-ESI-MS: 425.3411 ($[M+H]^+$, $C_{29}H_{45}O_2^+$; calc. 425.3420).

Sibogol C (= *3-Hydroxy-9,10-secoergosta-1,3,5(10)-trien-9-one*; **8**): Colorless oil. $[\alpha]_D^{20} = -13.2$ ($c = 0.2$, $CHCl_3$). IR (film): 3460, 2956, 2867, 1699, 1646, 1515, 1456, 1099, 858, 674. ^{1}H- and ^{13}C-NMR: *Tables 2* and *3*. HR-ESI-MS: 413.3426 ($[M+H]^+$, $C_{28}H_{45}O_2^+$; calc. 413.3420).

Bioactivity Assay. In vitro cytotoxic activities against P388 leukemia and human hepatoma BEL-7402 cell lines of crude extracts and fractions were carried out according to the MTT method [9].

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