Scalarane Sesterterpenes from the Chinese Sponge *Phyllospongia foliascens*

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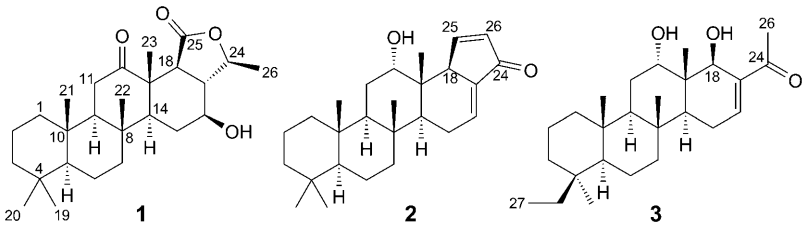
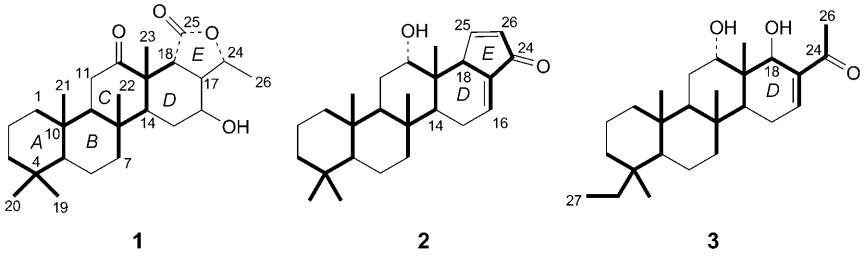
Three new scalarane sesterterpenes, phyllofolactone L (**1**), phyllofenone D (**2**) and phyllofenone E (**3**), were isolated from the acetone extract of the South China Sea sponge *Phyllospongia foliascens*. Their structures were elucidated on the basis of spectroscopic analysis. Phyllofenone D (**2**) was cytotoxic against the P388 leukemia cell line with an IC_{50} value of 6.5 μg/ml.

Introduction. – Marine sponges of the order Dictyoceratida are rich resources of bioactive scalarane-based sesterterpenes [1], and some of them were characterized by a 20(24)-(bis)homoscalarane skeleton and were considered to be ideal chemotaxonomic markers for the foliose sponges of the family Spongiidae [2]. The sponge *Phyllospongia foliascens* has proven to possess novel sesterterpenes with cytotoxic, antimicrobial, anti-inflammatory and anti-HIV activities, such as foliaspongin [3][4], phyllofoliaspongin [5], phyllactones [6], phyllofenones [7], and phyllofolactones [7][8]. In our continuing studies on bioactive constituents of marine sponges collected from the South China Sea, the acetone extract of *P. foliascens* showed significant antineoplastic activity *in vitro*. Bioassay-guided separation led to the isolation of two new 24-homoscalarane sesterterpenes, phyllofolactone L (**1**) and phyllofenone D (**2**), and a new 20,24-bishomo-25-norscalarane sesterterpene, phyllofenone E (**3**), from this sponge. The details of isolation and structure elucidation are reported in this work.



Results and Discussion. – The acetone extract of the marine sponge *P. foliascens* was subjected to solvent partition, CC or vacuum liquid chromatography (VLC) (on SiO_2, *ODS*, and *Sephadex LH-20*), and RP-HPLC to afford three new scalarane sesterterpenes, named phyllofolactone L (**1**), phyllofenone D (**2**), and phyllofenone E (**3**). Their structures were elucidated by high-resolution ESI-MS, and 1D- and 2D-NMR techniques including ^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY.

Phyllofolactone L (**1**) was obtained as colorless needles from $CHCl_3$ and its molecular formula $C_{26}H_{40}O_4$ was deduced from HR-TOF-ESI-MS (*m/z* 439.2822 ($[M+Na]^+$)) and ^{13}C-NMR data. This formula implied seven degrees of unsaturation, which were ascribed to five rings, one ketone CO group (δ(C) 212.7), and one ester CO group (δ(C) 172.6). The ^{1}H-NMR spectrum showed five Me *singlet*s at δ(H) 0.81, 0.84, 0.88, 1.08, and 1.31, and one Me *doublet* at δ(H) 1.48 (*d*, $J=6.0$). The ^{13}C-NMR and DEPT spectra exhibited 26 signals including those of six Me, seven CH_2, and seven CH groups, as well as of six quaternary C-atoms. Two O-bearing CH groups (δ(H) 4.23–4.29 (*m*)/δ(C) 79.7; δ(H) 3.55–3.61 (*m*)/δ(C) 72.2), one CH_2 group (δ(H) 2.80 (*dd*, $J=14.0, 11.8$); 2.45 (*dd*, $J=11.8, 2.4$)/δ(C) 35.4), and one CH group (δ(H) 2.53 (*d*, $J=14.6$)/δ(C) 50.2) connected to CO groups were well resolved, and other C- and H-atoms were also assigned based on ^{1}H- and ^{13}C-NMR, and HMQC spectra analysis (*Table*). A typical sesterterpenoid C-atom system bearing five Me groups along rings *A* to *D* could be established by the HMBC data from the five *singlet*s Me(19–23) to the associated C-atoms (*Fig. 1*). The ^{1}H,^{1}H-COSY correlations of H–C(2) (δ(H) 1.40–1.44 (*m*)) with CH_2(1) and CH_2(3), and of H–C(6) (δ(H) 1.56–1.60 (*m*)) with H–C(5) and CH_2(7), allowed the establishment of rings *A* and *B*. The ^{1}H,^{1}H-COSY correlations between CH_2(11) and H–C(9), and the HMBC correlations from CH_2(11) to C(12) and C(13) confirmed the ring *C*. The ^{1}H,^{1}H-COSY correlations of CH_2(15) with H–C(14) and H–C(16), and of H–C(17) with H–C(16) and H–C(18) permitted the assignment of ring *D*. The ^{1}H,^{1}H-COSY correlations between the H-atom at δ(H) 1.71 (*d*, $J=4.8$) and H–C(16) (δ(H) 3.55–3.61 (*m*)), and the HMBC correlations from this H-atom to C(15) and C(17) indicated that a OH group was connected to C(16). The ^{1}H,^{1}H-COSY correlations of H–C(24) with H–C(17) and H–C(26), and the HMBC correlations from H–C(17) and H–C(18) to C(25) suggested phyllofolactone L (**1**) was a 24-homoscalarane sesterterpene (*Fig. 1*). The chemical shifts of C(24) and C(25) were indicative of an ester linkage. Although the HMBC correlation between H–C(24) and C(25) was not observed, according to the established formula and the chemical shifts of C(24) and C(25), this ester linkage for ring *E* was essential to finally satisfy the degree of unsaturation.



1 **2** **3**

Fig. 1. *Selected HMBC* (▬) *and COSY* (—) *correlations of* **1**–**3**

Table. *Data of ^{1}H-NMR at 600 MHz and ^{13}C-NMR at 150 MHz for* **1**, **2**, *and* **3**. δ in ppm, J in Hz.

Position	**1** (in CD_2Cl_2)		**2** (in C_5D_5N)		**3** (in CD_2Cl_2)	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1α	0.80–0.84 (*m*)	39.8 (*t*)	0.86–0.92 (*m*)	41.6 (*t*)	0.85–0.91 (*m*)	40.0 (*t*)
1β	1.56–1.59 (*m*)		1.50–1.55 (*m*)		1.68–1.72 (*m*)	
2α	1.57–1.62 (*m*)	18.7 (*t*)	1.49–1.54 (*m*)	18.8 (*t*)	1.51–1.55 (*m*)	18.2 (*t*)
2β	1.40–1.44 (*m*)		1.26–1.30 (*m*)		1.36–1.42 (*m*)	
3α	1.09–1.13 (*m*)	42.1 (*t*)	1.04 (br. *t*, J = 13.6)	42.2 (*t*)	0.89–0.93 (*m*)	36.6 (*t*)
3β	1.35 (br. *d*, J = 14.0)		1.25–1.29 (*m*)		1.66–1.70 (*m*)	
4	–	33.5 (*s*)	–	33.3 (*s*)	–	35.9 (*s*)
5	0.79–0.82 (*m*)	56.9 (*d*)	0.74–0.79 (*m*)	56.4 (*d*)	0.97–1.01 (*m*)	58.4 (*d*)
6α	1.56–1.60 (*m*)	18.4 (*t*)	1.41 (br. *d*, J = 13.6)	18.3 (*t*)	1.53–1.57 (*m*)	17.9 (*t*)
6β	1.38–1.44 (*m*)		1.24–1.29 (*m*)		1.40–1.46 (*m*)	
7α	0.91 (*td*, J = 12.8, 3.9)	41.9 (*t*)	0.82–0.86 (*m*)	39.7 (*t*)	0.97–1.03 (*m*)	41.5 (*t*)
7β	1.77 (*dt*, J = 12.8, 3.4)		1.54–1.60 (*m*)		1.70–1.75 (*m*)	
8	–	38.8 (*s*)	–	38.1 (*s*)	–	37.2 (*s*)
9	1.14 (*dd*, J = 14.0, 2.4)	64.3 (*d*)	1.79 (br. *d*, J = 13.0)	51.5 (*d*)	1.39–1.43 (*m*)	51.4 (*d*)
10	–	38.7 (*s*)	–	37.1 (*s*)	–	36.8 (*s*)
11α	2.45 (*dd*, J = 11.8, 2.4)	35.4 (*t*)	1.80 (br. *d*, J = 13.0)	26.0 (*t*)	1.67–1.72 (*m*)	24.5 (*t*)
11β	2.80 (*dd*, J = 14.0, 11.8)		1.70 (br. *t*, J = 13.0)		1.67–1.72 (*m*)	
12	–	212.7 (*s*)	4.06 (br. *s*)	70.8 (*d*)	3.90 (br. *t*, J = 2.8)	70.6 (*d*)
13	–	50.3 (*s*)	–	39.7 (*s*)	–	40.7 (*s*)
14	1.07–1.11 (*m*)	59.6 (*d*)	1.93–1.99 (*m*)	49.5 (*d*)	1.48–1.52 (*m*)	46.8 (*d*)
15α	1.91 (*ddd*, J = 12.6, 4.7, 2.5)	31.2 (*t*)	2.21–2.27 (*m*)	24.7 (*t*)	2.23–2.27 (*m*)	24.4 (*t*)
15β	1.40–1.46 (*m*)		1.96–2.01 (*m*)		2.23–2.27 (*m*)	
16	3.55–3.61 (*m*)	72.2 (*d*)	6.74 (br. *t*, J = 3.6)	129.9 (*d*)	6.92 (br. *t*, J = 4.0)	143.3 (*d*)
17	1.83 (*ddd*, J = 14.6, 10, 9.8)	51.7 (*d*)		138.0 (*s*)		139.2 (*s*)
18	2.53 (*d*, J = 14.6)	50.2 (*d*)	4.12 (br. *s*)	50.0 (*d*)	4.74 (br. *d*, J = 6.0)	71.0 (*d*)
19	0.84 (*s*)	33.2 (*q*)	0.81 (*s*)	33.3 (*q*)	0.82 (*s*)	28.1 (*q*)
20	0.81 (*s*)	21.3 (*q*)	0.78 (*s*)	21.5 (*q*)	1.17–1.24 (*m*), 1.55–1.61 (*m*)	24.3 (*t*)
21	0.88 (*s*)	16.0 (*q*)	0.80 (*s*)	15.9 (*q*)	0.89 (*s*)	16.8 (*q*)
22	1.08 (*s*)	17.3 (*q*)	0.79 (*s*)	16.6 (*q*)	0.95 (*s*)	15.6 (*q*)
23	1.31 (*s*)	14.7 (*q*)	0.51 (*s*)	14.8 (*q*)	0.83 (*s*)	12.8 (*q*)
24	4.23–4.29 (*m*)	79.7 (*d*)	–	196.0 (*s*)	–	202.6 (*s*)
25	–	172.6 (*s*)	7.72 (br. *d*, J = 6.0)	159.7 (*d*)	–	–
26	1.48 (*d*, J = 6.0)	20.2 (*q*)	6.45 (*dd*, J = 6.0, 2.2)	136.6 (*d*)	2.31 (*s*)	25.7 (*q*)
27	–	–	–	–	0.77 (*t*, J = 7.4)	8.3 (*q*)
HO–C(12)			6.16 (*d*, J = 3.0)		1.70 (overlapping)	
HO–C(16)	1.71 (*d*, J = 4.8)					
HO–C(18)					2.24 (br.)	

The NOESY spectrum showed that the rings *A*–*E* were *trans*/*trans*/*trans*/*trans* fused. The β-orientations of Me(26) and HO–C(16) were deduced from the NOSY correlations of H–C(14)/H–C(16), H–C(16)/H–C(18), H–C(18)/H–C(24), and H–C(17)/H–C(26) (*Fig. 2*). Therefore, phyllofolactone L (**1**) was elucidated as 16β-hydroxy-24β-methyl-12-oxoscalarano-25,24-lactone.

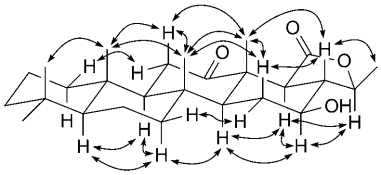


Fig. 2. *Key NOESY correlations of phyllofolactone L* (**1**)

Phyllofenone D (**2**) was isolated as colorless needles from $CHCl_3$, and its molecular formula was established as $C_{26}H_{38}O_2$ from HR-TOF-ESI-MS (*m/z* 405.2773 ($[M+Na]^+$)) and ^{13}C-NMR data. Eight degrees of unsaturation implied by the formula were assigned to five rings, two C=C bonds (δ(C) 129.9, 136.6, 138.0, and 159.7) and one ketone CO group (δ(C) 196.0). The ^{1}H-NMR spectrum showed five Me *singlet*s (δ(H) 0.51, 0.78, 0.79, 0.80, and 0.81), three olefinic H-atoms (δ(H) 6.74 (br. *t*, $J=3.6$), 6.45 (*dd*, $J=6.0, 2.2$), 7.72 (br. *d*, $J=6.0$)), and one O-bearing CH group (δ(H) 4.06 (br. *s*)). The ^{13}C-NMR and DEPT spectra exhibited 26 signals including five Me, seven CH_2, and eight CH groups, as well as six quaternary C-atoms. Inspection of HMBC and ^{1}H,^{1}H-COSY spectra revealed a tetracyclic scalarane framework (*Fig. 1*). The HMBC correlations from the *cis*-coupled olefinic H-atoms H–C(25) and H–C(26) to C(17), C(18), and C(24), and correlation from H–C(16) to C(24) allowed the establishment of ring *E*. The small coupling constants between H–C(12) and CH_2(11) indicated that H–C(12) was equatorial. The NOESY correlations of H–C(5)/H–C(9), H–C(9)/H–C(14), H–C(20)/H–C(21), H–C(21)/H–C(22), H–C(22)/H–C(23), H–C(23)/H–C(12) and H–C(14)/H–C(18) suggested the rings *A* – *D* were *trans*/*trans*/*trans*-fused and H–C(18) was α-orientation. On the basis of the foregoing analysis, phyllofenone D (**2**) was determined as 24-oxo-24-homoscalara-16,25(26)-dien-12α-ol.

Phyllofenone E (**3**) was obtained as colorless needles from $CHCl_3$, and its molecular formula was found to be $C_{26}H_{42}O_3$ from HR-TOF-ESI-MS (*m/z* 425.3034 ($[M+Na]^+$)) and ^{13}C-NMR data. The ^{1}H-NMR spectrum showed six Me groups (δ(H) 0.77, 0.82, 0.83, 0.89, 0.95, and 2.31), one olefinic H-atom (δ(H) 6.92 (br. *t*, $J=4.0$)) and two O-bearing CH groups (δ(H) 3.90 (br. *t*, $J=2.8$), 4.74 (br. *d*, $J=6.0$)). The ^{13}C-NMR and DEPT spectra exhibited 26 signals including six Me, eight CH_2, and six CH groups, as well as six quaternary C-atoms. The C-atoms resonanting at δ(C) 139.2, 143.3, and 202.6 indicated the presence of one C=C bond and one ketone CO group, which accounted for two of six degrees of unsaturation implied by the molecular formula. A tetracyclic 25-norscalarane skeleton could be established from the HMBC and ^{1}H,^{1}H-COSY spectra (*Fig. 1*). The HMBC correlations from H–C(27) to C(4) and C(20), and from H–C(26) to C(17) and C(24) suggested that phyllofenone E (**3**) was a 20,24-bishomoscalarane sesterterpene. The NOESY spectra also indicated that the four rings *A* – *D* were *trans*/*trans*/*trans* fused. The small coupling constants between H–C(12) and CH_2(11) and the NOESY correlations of H–C(12) with H–C(23) suggested that H–C(12) was equatorial. The NOESY correlation of H–C(14) with H–C(18) indicated that H–C(18) had α-orientation. Accordingly, phyllofenone E (**3**) was determined as 20,24-dimethyl-24-oxo-25-norscalar-16-ene-12α,18β-diol.

Phyllofolactone L (**1**) was structurally similar to most scalarane sesterterpenes previously isolated from *P. foliascens* which had a γ-lactone moiety, whereas

phyllofenone E (**3**) possessed a relatively rare 25-norscalarane framework, and phyllofenone D (**2**) had a rare α,β-unsaturated ketone ring *E* [6–10]. Phyllofenone D (**2**) showed cytotoxic activity against the P388 leukemia cell line with an IC_{50} value of 6.5 μg/ml. In contrast, phyllofolactone L (**1**) and phyllofenone E (**3**) were inactive in this assay.

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Experimental Part

General. HPLC: *Waters 1525/2998* liquid chromatograph. CC was performed on *Sephadex LH-20* (*Pharmacia*) and *YMC ODS-A* (50 μm). Vacuum liquid chromatography (VLC) was performed on silica gel (SiO_2; 200–300 mesh, *Yantai*, P. R. China); the fractions were monitored by TLC (*HSGF 254*, *Yantai*, P. R. China) and spots were visualized by heating SiO_2 plates sprayed with 10% H_2SO_4 in H_2O. M.p.: *SGW X-4* melting point apparatus; uncorrected. Optical rotations: *JASCO P-1030* polarimeter. NMR Spectra: *Bruker AVANCE-600* spectrometer. HR-TOF-ESI-MS Spectra: *Q-Tof micro YA019* mass spectrometer.

Animal Material. Specimen of *P. foliascens* was collected around Yongxing island in the South China Sea in June 2007, and was identified by Prof. *Jin-He Li* (Institute of Oceanology, Chinese Academy of Sciences, China). A voucher sample (No. DS-PF 01) was deposited with Laboratory of Marine Drugs, Department of Pharmacy, Changzheng Hospital, Second Military Medical University, P. R. China.

Extraction and Isolation. The fresh sponges (800 g, dry wt.) were extracted with acetone at r.t. The acetone extracts were concentrated under reduced pressure to give 55 g of brown gum, which was partitioned between MeOH/H_2O (9:1) and petroleum ether (PE) to afford 10 g PE phase extract. The MeOH/H_2O phase was diluted to 3:2 with H_2O and extracted with CH_2Cl_2. The CH_2Cl_2 extract (8 g) showed significant cytotoxicity against the HL-60 (IC_{50} 10 μg/ml) and BEL-7402 (IC_{50} 25 μg/ml) cell lines. This extract was subjected to VLC on Si_2O using CH_2Cl_2/MeOH (25:1, 10:1, 5:1, and 2:1) as eluent to afford eight fractions (*Frs. 1–8*). The cytotoxic *Fr. 2* (300 mg) was subjected to chromatography repeatedly on *Sephadex LH-20* and *YMC ODS-A* (50 μm), and further purified by HPLC (*YMC-Pack ODS-A C18*, 5 μm, 10 × 250 mm, 1.5 ml/min, UV detection 210 nm) eluting with MeOH/H_2O (98:2) to yield pure compounds **1** (4.6 mg), **2** (3.3 mg), and **3** (0.9 mg).

Phyllofolactone L (=*16β-Hydroxy-24β-methyl-12-oxoscalarano-25,24-lactone*; *(3*S*,3a*S*,4*S*,5a*S*,5-b*R*,7a*S*,11a*S*,11b*R*,13a*S*,13b*S*)-Octadecahydro-4-hydroxy-3,5b,8,8,11a,13a-hexamethylchryseno[1,2-c]furan-1,13-dione*; **1**). Colorless needles ($CHCl_3$). M.p. 233.0–235.0°. $[\alpha]_D^{20} = +86.8$ ($c = 0.23$, $CHCl_3$). ^{1}H- and ^{13}C-NMR: *Table*. HR-TOF-ESI-MS: 439.2822 ($[M+Na]^+$, $C_{26}H_{40}NaO_4^+$; calc. 439.2824).

Phyllofenone D (=*24-Oxo-24-homoscalara-16,25(26)-dien-12α-ol*; *(5a*S*,5b*R*,7a*S*,11a*S*,11b*R*,13-*S*,13a*S*,13b*R*)-5,5a,5b,6,7,7a,8,9,10,11,11a,11b,12,13,13a,13b-Hexadecahydro-13-hydroxy-5b,8,8,11a,13a-pentamethyl-3*H*-cyclopenta[a]chrysen-3-one*; **2**). Colorless needles ($CHCl_3$). M.p. 280.0–282.0°. $[\alpha]_D^{20} = +113.8$ ($c = 0.18$, $CHCl_3$). ^{1}H- and ^{13}C-NMR: *Table*. HR-TOF-ESI-MS: 405.2773 ($[M+Na]^+$, $C_{26}H_{38}NaO_2^+$; calc. 405.2770).

Phyllofenone E (=*20,24-Dimethyl-24-oxo-25-norscalar-16-ene-12α,18β-diol*; *1-[(1*R*,4a*S*,4b*R*,6a*S*,7-S*,10a*S*,10b*R*,12S*,12aR*)-7-Ethyl-1,4,4a,4b,5,6,6a,7,8,9,10,10a,10b,11,12,12a-hexadecahydro-1,12-dihydroxy-4b,7,10a,12a-tetramethylchrysen-2-yl]ethanone*; **3**). Colorless needles ($CHCl_3$). M.p. 268.5–269.5°. $[\alpha]_D^{20} = -87.5$ ($c = 0.05$, $CHCl_3$). ^{1}H- and ^{13}C-NMR: *Table*. HR-TOF-ESI-MS: 425.3034 ($[M+Na]^+$, $C_{26}H_{42}NaO_3^+$; calc. 425.3032).

Cytotoxicity Assay. Cytotoxicity was evaluated as IC_{50} by using the MTT assay with vincristine as positive control. Extractions and purified compounds were solubilized in DMSO with the working concentration of test substances ranging from 1 to 100 μg/ml. Cells were inoculated into 96-well plates. After incubation for 24 h, the cells were treated with various concentrations of test substances for 48 h

and then were incubated with 1 mg/ml MTT at 37° for 4 h, followed by solubilization in DMSO. The formazan dye product was measured by the absorbance at 470 nm on a microplate reader.

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