Phyllofolactones C and D, Two New Minor Homoscalarane Sesterterpenes from the Chinese Sponge *Phyllospongia foliascens*

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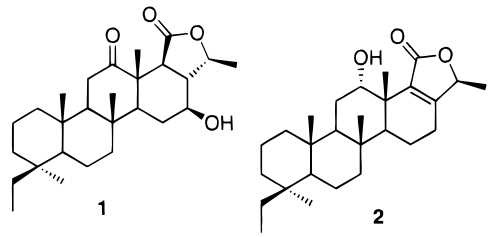
Phyllofolactone C (**3**) and phyllofolactone D (**4**), two new minor homoscalarane sesterterpenes, were isolated from the sponge *Phyllospongia foliascens* collected in the South China Sea. Their structures were assigned by analysis of spectroscopic data.

Sesterterpenes are relatively rare in nature,[1,2] and those possessing the scalarane skeleton occur mainly in marine sponges of the order Dictyoceratida but have been also isolated from nudibranchs that feed on these sponges.[1−3] The Chinese sponge *Phyllospongia foliascens* Pallas (Dictyoceratida, Spongiidae) has proven to be a rich source of scalarane sesterterpenes. Our earlier studies on this sponge collected from the Xisha and Nansha Islands in the South China Sea have led to the isolation of phyllofenones A[4] and B, phyllofolactones A (**1**) and B (**2**),[5] and phyllactones A−G.[6,7] Recently, we reinvestigated a minor fraction remaining from our earlier work, because this fraction was recognized as a mixture of scalarane sesterterpenes. Reversed-phase HPLC separation of this mixture yielded a new bishomoscalarane sesterterpene, phyllofolactone C (**3**), which is the keto analogue of the alcohol **2**, and phyllofolactone D (**4**), a homologue of **3**. In this paper, we report the isolation and structure elucidation of phyllofolactones C (**3**) and D (**4**).



Compound **3** was obtained as an amorphous solid, $[\alpha]_D$ +133.5° (*c* 0.20, CH_2Cl_2). The molecular formula $C_{27}H_{40}O_3$ was established by HRFABMS, *m/z* 413.3056 $[M + H]^+$ (Δ 2.5 mmu) and corroborated by NMR data (Table 1). The latter was assigned unambiguously by COSY, RELAY, HMQC, and HMBC (Figure 1) experiments. The ^1H NMR spectrum of **3** contained four methyl singlets at δ 0.79, 0.91, 1.11, and 1.50 and a methyl triplet at δ 0.75, which was found to be coupled ($^1H-^1H$ COSY) to two multiplets at δ 1.17 and 1.54. All these methyl signals are reminiscent of the spectra of 20,24-bishomoscalarane sesterterpenes.[4−7] Additionally, the LREIMS contained a series of fragment ions [i.e., *m/z* 219, 205 (base peak), 191, 137, 123, 109] typical of this class of sesterterpenes.[5,8] The IR spectrum of **3** showed absorptions consistent with the presence of a 2,3,4-trisubstituted butenolide function (1750 and 1668 cm^{-1}). In support of this, the ^{13}C NMR spectrum of **3** showed signals at δ 170.5 (s), 131.1 (s), 164.1 (s), and 77.5 (d), and the ^1H NMR spectrum revealed a methyl doublet at δ 1.36 (H-26) and a quartet at δ 4.84 (H-24), which were coupled with each other.[5,6] The orientation of the butenolide was determined by the HMBC correlations between one (δ 1.94) of Hs-15, H-26 (δ 1.36) and one of the sp^2 carbons (C-17, δ 164.1). The other sp^2 carbon (C-16, δ 131.2) was correlated to H-23 and H-14. The presence of a ketone was evident from an IR band at 1705 cm^{-1} and a ^{13}C NMR signal at δ 210.8 (s). The ketone was assigned to C-12 in ring C on the basis of the HMBC correlation between the downfield ^{13}C NMR signal (δ 210.8) and H-23 (δ 1.50). The NMR data, in particular the ^{13}C NMR data, of rings A−C and associated substituents of **3** were essentially the same as those for **1**, while the data for the rings D and E in **3** were in good agreement with those for **2**. Therefore, the structure of phyllofolactone C was assigned as **3**.

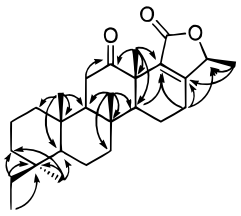


Figure 1. Selected HMBC correlations for **3**.

The second new metabolite, **4**, was also obtained as an amorphous solid, $[\alpha]_D$ +93.8° (*c* 0.24, CH_2Cl_2). The molecular formula $C_{26}H_{38}O_3$ that was established by HRFABMS and NMR data (Table 1) had one CH_2 less than that of **3**, indicating that **4** could be a homologue of **3**. The ^1H and ^{13}C NMR data (Table 1) of **4** were assigned unambiguously by COSY, RELAY, HMQC, and HMBC experiments. These data were virtually identical to those of **3** except for some signals surrounding C-4. The NMR signals [δ_H 0.75 (t), 1.54 (m), and 1.17 (m); δ_C 8.6 (q) and 24.5 (t)] for the ethyl group in compound **3** were missing in the NMR spectra of **4**. However, ^1H and ^{13}C NMR signals for another methyl group appeared at δ 0.82 and 21.2 ppm, respectively. Therefore, the structure of phyllofolactone D was determined as **4**, which has a β-methyl instead of a β-ethyl group at C-4 in **3**. Consistent with this conclusion, the base peak

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Table 1. NMR Data for **3** and **4** in $CDCl_3$

	3		**4**	
position	^{13}C (mult.)[a]	^{1}H (mult., *J* in Hz)[b]	^{13}C (mult.)	^{1}H (mult., *J* in Hz)
1	39.9 (t)	1.57 (m); 0.86 (m)	39.5 (t)	1.57 (m); 0.86 (m)
2	17.9 (t)	1.47 (m); 1.38 (m)	18.0 (t)	1.58 (m); 1.49 (m)
3	36.4 (t)	1.65 (m); 0.82 (m)	41.5 (t)	1.36 (m); 1.13 (m)
4	36.2 (s)		33.3 (s)	
5	58.9 (d)	0.86 (m)	56.8 (d)	0.81 (m)
6	18.1 (t)	1.59 (m); 1.50 (m)	18.3 (t)	1.61 (m); 1.47 (m)
7	41.8 (t)	1.87 (dt, 12.7, 3.2)	41.8 (t)	1.87 (dt, 12.7, 3.2)
		0.96 (m)		0.96 (m)
8	38.4 (s)		38.4 (s)	
9	64.5 (d)	1.25 (dd, 11.7, 2.6)	64.1 (d)	1.26 (dd, 11.7, 2.6)
10	38.7 (s)		38.6 (s)	
11	35.1 (t)	2.83 (dd, 13.8, 11.7)	35.1 (t)	2.83 (dd, 13.8, 11.7)
		2.30 (dd, 13.8, 2.6)		2.30 (dd, 13.8, 2.6)
12	201.8 (s)		201.8 (s)	
13	51.0 (s)		51.0 (s)	
14	58.7 (d)	1.34 (m)	58.7 (d)	1.34 (m)
15	17.3 (t)	1.94 (dd, 13.3, 4.4)	17.2 (t)	1.94 (dd, 13.3, 4.4)
		1.56 (m)		1.56 (m)
16	24.4 (t)	2.34 (dd, 18.6, 4.3)	24.4 (t)	2.34 (dd, 18.6, 4.3)
		2.14 (ddd, 18.6, 11.7, 6.4)		2.14 (ddd, 18.6, 11.7, 6.4)
17	164.1 (s)		164.1 (s)	
18	131.2 (s)		131.2 (s)	
19	28.5 (q)	0.79 (s)	33.2 (q)	0.84 (s)
20	24.5 (t)	1.54 (m); 1.17 (m)	21.2 (q)	0.82 (s)
21	16.7 (q)	0.91 (s)	15.9 (q)	0.89 (s)
22	17.6 (q)	1.11 (s)	17.6 (q)	1.12 (s)
23	19.9 (q)	1.50 (s)	19.8 (q)	1.50 (s)
24	77.5 (d)	4.84 (q, 6.9)	77.5 (d)	4.84 (q, 6.9)
25	170.5 (s)		170.5 (s)	
26	18.4 (q)	1.36 (d, 6.9)	18.4 (q)	1.36 (d, 6.9)
27	8.6 (q)	0.75 (t, 7.4)		

[a] ^{13}C NMR at 125 MHz, referenced to $CDCl_3$ (δ 77), multiplicities determined by DEPT and HMQC experiments. [b] ^{1}H NMR at 500 MHz, referenced to residual solvent $CHCl_3$ (δ 7.26).

in the LREIMS of **4** appeared at *m*/*z* 191, compared to *m*/*z* 205 observed for **3**.[5,8] The chemical shifts of the carbons nearby C-4 were in good agreement with those in the related scalarane sesterterpenes.[1]

The methyl at C-24 was determined to be β-oriented based on comparison of both ^{1}H and ^{13}C NMR chemical shifts [H-26, δ 1.36 (d); C-26, δ 18.4 (q)] of the 24-methyl group in **3** and **4** with those in phyllactone A [H-26, δ 1.51 (d); C-26, δ 19.6 (q)] and phyllactone B [H-26, δ 1.38 (d); C-26, δ 18.5 (q)][6] for which the stereochemistry at C-24 has been established by NOE studies. The relative stereochemistry for the other chiral centers in **3** and **4** is presumed to be the same as in the other bishomoscalarane sesterterpenes isolated from the same sponge[4−7] based on comparison of NMR data and biogenetic grounds. The absolute configurations of all the homoscalarane sesterterpenes[4−7] are assumed to the the same as that of phyllactone B, whose absolute stereochemistry has been determined by a modified Mosher's method,[7] because all exhibit positive optical rotations.

Experimental Section

General Experimental Procedures. Merck Si gel 60 (230−240 mesh) was used for vacuum flash chromatography. HPLC was conducted using a UV detector and a Spherex 5 C_{18} column. IR spectra were obtained on a Bio-Rad 3240-SPC FT instrument. NMR experiments were conducted with a Varian VXR-500 instrument equipped with a 3-mm $^{1}H/^{13}C$ switchable gradient microprobe (MDG-500−3) and a pulsed field gradient driver; signals are reported in parts per million (δ), referenced to the solvent used. FABMS were measured on a VG ZAB-E mass spectrometer, and optical rotations on a Rudolph Autopol III automatic polarimeter.

Animal Material. The sponge *P. foliascens* was collected around Yongxing Island in the South China Sea in April 1988, and was identified by Dr. Jinhe Li, Institute of Oceanology, Qingdao, People's Republic of China. A voucher specimen (no. 881) has been deposited in the Research Center of Organic Natural Products, Zhongshan University.

Extraction and Isolation. The sun-dried specimens of the sponge (3 kg dry wt) were minced and extracted with MeOH (three times). All extracts were combined after removal of MeOH in vacuo, to give a residue (21.2 g) that was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 fraction was separated and the solvent removed in vacuo to yield a viscous oil that was chromatographed on Si gel using a stepwise gradient of EtOAc and light petroleum as eluent. Five fractions were collected. Fraction 4 was rechromatographed on Si gel to give a mixture containing phyllofolactones C and D, which was resolved by reversed-phase HPLC on a C_{18} column using 10% H_2O−MeOH as eluent to give compound **3** (2.7 mg; 9.0 × 10−5% of dry specimen wt) and compound **4** (3.3 mg; 1.1 × 10−4%).

Phyllofolactone C (3): amorphous solid, $[\alpha]_D$ +133.5° (*c* 0.20, CH_2Cl_2); IR (NaCl) ν_{max} 2931, 2857, 1750, 1705, 1668 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1, assignments were made by interpretation of COSY, RELAY, HMQC, HMBC data; EIMS (70 eV) *m*/*z* 412 $[M]^+$ (41), 397 (14), 383 (35), 247 (21), 229 (13), 220 (23), 205 (100), 191 (24), 165 (92), 163 (27), 147 (22), 137 (32), 135 (24), 123 (26), 121 (57), 119 (32), 109 (48), 107 (44), 105 (44), 95 (67), 93 (70), 91 (63); HRFABMS *m*/*z* 413.3056 $[M + H]^+$ (Δ 2.5 mmu).

Phyllofolactone D (4): amorphous solid, $[\alpha]_D$ +93.8° (*c* 0.24, CH_2Cl_2); IR (NaCl) ν_{max} 2931, 2857, 1750, 1705, 1668 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1, assignments were based on COSY, RELAY, HMQC, HMBC data; EIMS (70 eV) *m*/*z* 398 $[M]^+$ (38), 383 (19), 233 (16), 220 (24), 206 (29), 205 (29), 192 (20), 191 (100), 177 (25), 165 (89), 163 (34), 149 (19), 137 (29), 135 (26), 123 (38), 121 (63), 119 (29), 109 (56), 107 (43), 105 (40), 95 (67), 93 (65), 91 (59); HRFABMS *m*/*z* 399.2935 $[M + H]^+$ (Δ −3.6 mmu).

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