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NOVEL BISHOMOSCALARANE SESTERTERPENES FROM THE SPONGE *PHYLLOSPONGIA FOLIASCENS*

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ABSTRACT.—Three new 20,24-bishomoscalarane sesterterpenes, phyllofenone B [2], phyllofolactone A [3], and phyllofolactone B [4], have been isolated from the Pacific sponge *Phyllospongia foliascens*, and their structures determined primarily by a combination of 1D and 2D nmr and mass spectrometry. Phyllofenone B [2] with a previously unknown carbon skeleton shows cytotoxicity against the P-388 cell line ($IC_{50} = 5$ μg/ml).

The sponge genus *Phyllospongia* (syn. *Carteriospongia*) has proven to be a rich source of sesterterpenes (1–11). From this genus of sponges, many 20,24-bishomoscalarane and 20,24-bishomo-25-norscalarane derivatives have been isolated (4–11). A feature common to all these bishomosesterterpenes is oxygenation at C-12. These compounds show characteristic fragment ions at *m/z* 205 and 219 in their mass spectra indicative of non-oxygenated rings A and B. In the course of our systematic search for bioactive substances from marine organisms, we have reported (11) recently the isolation and structure elucidation of phyllofenone A [1], a 20,24-dimethyl-25-norscalarane sesterterpene with antifungal activity from the sponge *Phyllospongia foliascens* (Pallas), Family Spongiidae. We now report the isolation and structure elucidation of three new 20,24-bishomosesterterpenes 2, 3, and 4 from the same sponge.

RESULTS AND DISCUSSION

The MeOH extracts of the sun-dried specimens of the marine sponge *P. foliascens* collected from the South China Sea were partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 fraction was subjected to cc on Si gel (60–100 mesh) followed by flash chromatography and yielded phyllofenone B [2], phyllofolactone A [3], and phyllofolactone B [4]. ¹H-nmr spectra and eims of these compounds indicated that 2, 3, and 4 belong to the 20,24-bishomoscalarane sesterterpene group. The eims of these compounds typically give two fragment ions at *m/z* 205 and 219 due to fragmentation across the C ring, indicating that there is no further substitution on the A and B rings. In the ¹H-nmr spectra, all three compounds showed four methyl singlets and one methyl triplet, which is compatible with 20,24-bishomoscalarane sesterterpenes. This suggestion was also supported by three ¹³C-nmr methine resonances in the region of δ 50–60 (C-5 ~58; C-9 ~52; C-14 ~50) (12). By comparison of the ¹³C data of 2, 3, and 4 with those of 1 (11), and in considering the origin of these compounds, the β configuration is assigned to the C-4 ethyl group in 2–4, since its methylene carbon signal occurs at ~24.5 ppm in good agreement with the shifts determined experimentally for this carbon in 1 (11) and related C-4 β-ethyl homosesterterpenes (9,15).

Phyllofenone B [2] was obtained as needles from EtOH, $[\alpha]^{20}D + 133°$ (CHCl₃, *c* = 0.0047). The ¹³C-nmr spectrum showed 26 resolved peaks, comprising (from DEPT) five methyl, eight methylene, seven methine, and six quaternary carbons. However, one methine carbon resonating at δ 49.4 was much more intense than all other peaks in the spectrum and was assigned to two unresolved methine signals. From the ¹³C-nmr data and the molecular ion peak at *m/z* 396 in eims, a molecular formula of $C_{27}H_{40}O_2$ was established. Absorption maxima in the ir spectrum of 2 at 3300–3500,

1

2

3

4

5
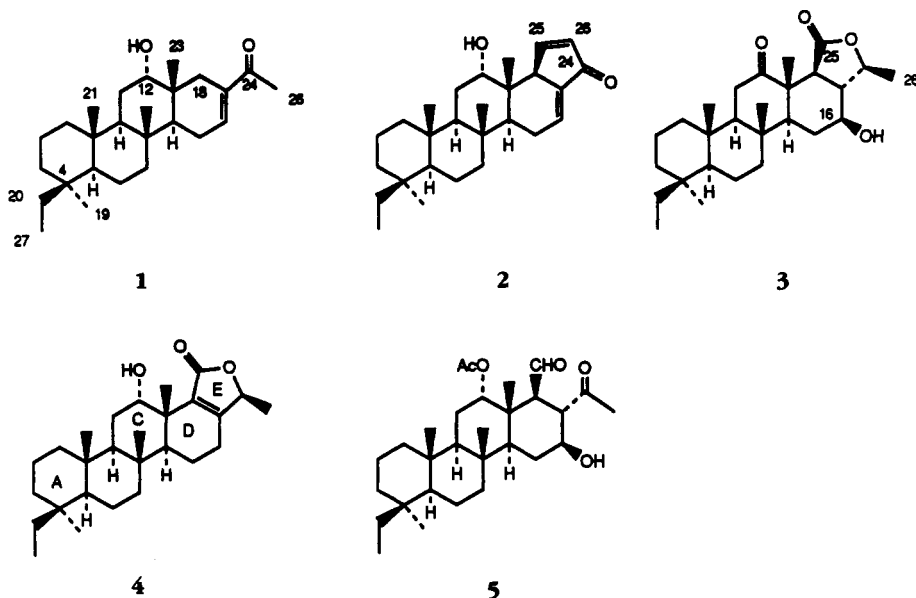
1694, 1662 cm^{-1}, together with signals in the ^{13}C-nmr spectrum (Table 2) at δ 130.2 (d), 136.8 (d), 137.0 (s), 158.6 (d), 196.5 (s), and 74.5 (d), indicated the presence of a hydroxy group, two double bonds, and an α,β-unsaturated ketone carbonyl. Uv absorption at 248 nm (ε = 21879) was consistent with the presence of an α,β-unsaturated ketone. Taking into account the above data for three degrees of unsaturation, phyllofenone B [**2**] was deduced to be pentacyclic. The ^{1}H-nmr spectrum (Table 1) con-

TABLE 1. ^{1}H-nmr Data of Compounds **2**, **3**, and **4**.

Proton	Compound		
	2	**3**	**4**
H-9	1.38 (1H)	1.10 (1H)	
H-11	1.83 (1H), 1.68 (1H)	2.75 (dd, 11.5, 12.5, 1H), 2.27 (dd, 11.5, sm, 1H)	1.72 (dd, 7.5, 3.8, 1H), 1.56 (dd, 7.5, 1.9, 1H)
H-12	3.93 (br t, ~3, 1H)		4.64 (br t, 1H)
H-14	1.78 (dd, 1H)	1.07 (dd, 1H)	
H-15	2.43 (br d, 20, sm, 1H) 2.09 (br dd, 20, 10, sm, 1H)	1.90 (ddd, 1H), 1.53 (m, 1H)	
H-16	6.63 (br t, 1H)	3.56 (m, 1H)	
H-17		1.82 (ddd, 14.5, 10, 10, 1H)	
H-18	3.68 (br s, 1H)	2.53 (d, 14.5, 1H)	
H-19 . . .	0.80 (s, 3H)	0.75 (s, 3H)	0.78 (s, 3H)
H-20	1.51 (dq, 1H), 1.18 (dq, 1H)	1.60 (dq, 14, 7, 1H), 1.13 (dq, 14, 7, 1H)	1.37 (1H), 1.14 (1H)
H-21	0.87 (s, 3H)	1.04 (s, 3H)	0.85 (s, 3H)
H-22	0.88 (s, 3H)	0.86 (s, 3H)	0.87 (s, 3H)
H-23	0.53 (s, 3H)	1.31 (s, 3H)	1.11 (s, 3H)
H-24		4.25 (m, 10, 6, 1H)	4.78 (q, 6.7, 1H)
H-25	7.55 (dd, 6.0, sm, 1H)		
H-26	6.34 (dd, 6.0, 2.4, 1H)	1.47 (d, 6, 3H)	1.35 (d, 6.7, 1H)
H-27	0.75 (t, 7.5, 3H)	0.70 (t, 7, 3H)	0.74 (t, 7.5, 3H)

tained four methyl singlets at δ 0.80, 0.87, 0.88, 0.53. A methyl triplet at δ 0.75 was found to be coupled (^{1}H-^{1}H COSY spectrum) to two doublets of quartets at δ 1.18 and 1.51, which was consistent with an ethyl group attached to an asymmetric center. All these methyl signals were reminiscent of the spectra of 20,24-bishomoscalarane sesterterpenes. Other prominent features in the ^{1}H-nmr spectrum were two one-proton doublets of doublets at δ 7.55 and 6.34 assigned as olefinic protons in the E ring, a one-proton multiplet at δ 6.63 assigned to the olefinic proton on the D ring, a broadened one-proton triplet at δ 3.93 assigned to an equatorial proton at a carbon bearing a hydroxy group, and a broadened one-proton singlet at δ 3.68 assigned as H-18 which was shifted downfield due to being flanked by two double bonds. The hydroxyl group was assigned to C-12 in ring C on the basis of nmr data. ^{1}H-^{1}H COSY showed correlation between the resonance at δ 3.93 (H-12) and signals at δ 1.83 (H-11β) and 1.68 (H-11α). The latter signals were correlated to each other and in turn correlated to a signal at δ 1.39 (H-9α). This position of oxygenation agrees with that of all previously reported scalarane derivatives. The small coupling constant for H-12 (ca. 3 Hz) indicated a 12α-OH stereochemistry, and this was also confirmed by nOe difference spectroscopy. On irradiation of the methyl signal at δ 0.53 (Me-23), a 4.1% nOe enhancement was observed for H-12 (δ 3.93). A 4.5% nOe was also observed for this signal upon irradiation of H-25 (δ 7.55).

TABLE 2. ^{13}C-nmr Data of Bishomoscalarane Sesterterpenes from *Phyllospongia foliascens*.[a]

Carbon	Multiplicity	Compound				
		1	2	3	4	Acetate of 5
C-1 . . .	t	40.1	40.3	39.9	39.9	40.3
C-2 . . .	t	18.0	18.0	18.0	18.1	18.1
C-3 . . .	t	36.6	36.6	36.4	36.6	36.7
C-4 . . .	s	36.1	36.1	36.2	36.0	36.2
C-5 . . .	d	58.5	58.5	58.7	58.4	58.6
C-6 . . .	t	18.3	18.3	18.1	18.3	18.6
C-7 . . .	t	41.7	41.8	41.9	41.7	41.8
C-8 . . .	s	37.5	38.1	38.6	37.6	38.2
C-9 . . .	d	51.9	52.3	51.5	52.4	52.9
C-10 . . .	s	37.0	37.0	38.5	37.0	37.0
C-11 . . .	t	25.1	25.8	35.0	24.3	21.8
C-12 . . .	d	74.5	71.8	212.4 (s)	70.1	76.0
C-13 . . .	s	36.8	39.3	50.0	40.3	40.6
C-14 . . .	d	46.8	49.4	50.0	49.9	50.9
C-15 . . .	t	24.0	24.5	31.0	24.0[b]	26.0
C-16 . . .	d	139.8	130.2	72.0	16.6 (t)[b]	74.9
C-17 . . .	s	137.9	137.0	59.2 (d)	165.3	49.3 (d)
C-18 . . .	d	35.6 (t)	49.4	64.2	133.4 (s)	58.7
C-19 . . .	q	28.5	28.5	28.4	28.5	28.5
C-20 . . .	t	24.5	24.6	24.4	24.4	24.6
C-21 . . .	q	15.9	15.7	16.7	16.8	17.2
C-22 . . .	q	17.3	17.2	17.2	16.9	16.9
C-23 . . .	q	20.1	14.7	14.7	21.7	16.8
C-24 . . .	s	199.4	196.5	79.4 (d)	78.6 (d)	211.1
C-25 . . .	s		158.6 (d)	172.4	172.6	202.4 (d)
C-26 . . .	q	25.2	136.8 (d)	20.1	18.4	32.9
C-27 . . .	q	8.7	8.6	8.6	8.6	8.6

[a]Some assignments of like multiplicity and very similar chemical shifts may be reversed.
[b]Assigned by analogy to the 12-OAc analogue of **4** (15).

The structure of the D and E rings was deduced from the ^{1}H-^{1}H COSY and differ-ence decoupling experiments. The olefinic protons at δ 6.34 and 7.55 were coupled to each other ($J = 6$ Hz, cis coupling) and were both coupled to the proton resonating at δ 3.68. This δ 3.68 signal showed slight coupling to the remaining olefinic signal at δ 6.63. The latter was coupled to a pair of geminally coupled protons (δ 2.43 and 2.09) which were in turn coupled to a proton absorbing at δ 1.78. These data support the D/E ring formulation of structure **2**. The relative location of the double bond and carbonyl group in ring E was confirmed by the fact that H-25 (δ 7.55) induced an nOe on H-12. The α configuration assigned to H-14 and H-18 is based on an nOe observed between these protons.

The ^{13}C data of phyllofenone B [**2**] were assigned from the ^{1}H-^{13}C COSY spectrum and by analogy to phyllofenone A [**1**] (11) and related sesterterpenes (8,9). Some as-signments were confirmed by selective INEPT experiments reported in Table 3.

TABLE 3.   Results of Selective INEPT Experiments of Compound **2**.

Irradiated Proton	Observed Carbon
7.55 (H-25)	49.4 (C-18), 136.8 (C-26), 137.0 (C-17)
6.63 (H-16)	24.5 (C-15), 49.4 (C-18, C-14), 196.5 (C-24)
6.34 (H-26)	137.0 (C-17), 158.6 (C-25), 196.5 (C-24)
3.93 (H-12)	14.7 (C-23), 49.4 (C-18, C-14), 52.3 (C-9)
3.68 (H-18)	14.7 (C-23), 39.3 (C-13), 130.2 (C-16)
	136.8 (C-26), 137.0 (C-17), 158.6 (C-25)
0.53 (H-23)	39.3 (C-13), 49.4 (C-18, C-14)

Phyllofolactone A [**3**] crystallized as needles from Me$_2$CO, [α]^{20}D +78.4° ($c = 0.051$, CHCl$_3$). The molecular formula C$_{27}$H$_{42}$O$_4$ was deduced from ^{13}C-nmr data that revealed 27 carbons (six methyl, eight methylene, seven methine, and six quater-nary carbons) and the eims which showed a molecular ion peak at m/z 430. ^{13}C-nmr res-onances at δ 212.4 (s), 172.4 (s), 79.4 (d), and 72.0 (d) and ir absorption maxima at 3520, 1765, and 1715 cm^{-1} indicated the presence of a ketone, an ester carbonyl group, and a hydroxyl group in the molecule. No multiple-bonded carbons were appar-ent other than the ester and ketone carbons; thus a pentacyclic system with a secondary alcohol group was indicated. The fifth ring was formulated as a γ-lactone to account for the ir absorption maximum at 1765 cm^{-1} and the ^{13}C-nmr signals at δ 172.4 (s) and 79.4 (d). Like that of other 20,24-bishomoscalarane sesterterpenes (6,8,9,11), the ^{1}H-nmr spectrum of **3** showed four methyl singlets at δ 0.75, 0.86, 1.04, and 1.31 and one methyl triplet at δ 0.70 ($J = 7$). A sixth methyl signal, a doublet at δ 1.47 ($J = 6$) was assigned to a methyl group on the oxygen-bearing carbon of the γ-lactone (C-24). This methyl signal was correlated to a multiplet at δ 4.25 that showed further correla-tion to a methine proton signal at δ 1.82 (ddd, 14.5, 10, 10). The latter was further correlated on the one hand to a methine proton signal at δ 2.53 (d, 14.5), and on the other hand to a multiplet at δ 3.56 which showed correlation to a pair of geminal proton signals at δ 1.90 and 1.53. These geminal proton signals were both correlated to a sig-nal at δ 1.07. This spin system traces out rings D and E of structure **3**.

H-17 exhibits large couplings to H-18 and H-16 on the six-membered ring, and this is consistent with trans diaxial relationships between these three protons. Hence a trans fusion for ring D/E and an equatorial orientation for the C-16 hydroxyl group are confirmed. The 10 Hz coupling between H-17 and H-24 (δ 4.25) is consistent with the ca. 170' dihedral angle predicted from Dreiding models for a trans H-17/H-24 ar-

rangement with a concomitant 24β-methyl configuration. This stereochemistry was confirmed by a nOe experiment in which the H-16 signal (δ 3.56) was irradiated and enhancements were observed for both H-18 and H-24, but not H-26. These data support the stereochemistry shown for rings D and E in **3**. The remaining ketone group in **3** was assigned to C-12 in ring C on the basis of ms data (intense *m/z* 205 peak typical of non-oxygenated rings A and B) and ^{1}H nmr data: geminally coupled signals at δ 2.75 (H-11β, nOe with Me-23) and 2.27 (H-11α) which were coupled (COSY) to a signal at δ 1.10 (H-9α). Hence the structure of phyllofolactone A is confirmed to be that shown for **3**. The ^{13}C-nmr data of **3** were assigned by H/C correlations and analogy to related compounds (8–11).

Phyllofolactone B [**4**] was obtained as fine needles, $[\alpha]^{20}$D +61.9° (CHCl$_3$, *c* = 0.0042). The molecular formula $C_{27}H_{42}O_3$ was established from ^{13}C-nmr (27 carbons) and eims ([M]$^+$ 414) data. The ir spectrum indicated the presence of a hydroxyl group (3468 cm^{-1}) and an α,β-unsaturated γ-lactone (1728, 1675 cm^{-1}). The resonances at δ 172.6 (s), 165.1 (s), 133.3 (s), and 78.6 (d) in the ^{13}C-nmr spectrum supported the presence of an α,β-unsaturated γ-lactone and a tetrasubstituted double bond. The downfield quartet (J = 6.7 Hz) at δ 4.78 was attributed to a proton on the γ-carbon of the γ-lactone, and hence there was also a methyl group at this position.

The remaining downfield ^{1}H-nmr signal, a broad triplet (small J) at δ 4.64 was assigned to a proton on a hydroxylated carbon (C-12). The small coupling constant of this proton and the chemical shift of the associated carbon, δ 70.1, both indicate that the OH at this position is α-oriented (14). Prominent peaks in the mass spectrum also supported a C-12 location for the hydroxyl group (Figure 1). Selective INEPT results that support structure **4** are recorded in the experimental section. The remaining assignments are by analogy to **2**, **3**, and literature data (8–11, 15). In particular, the ^{1}H and ^{13}C data for the 24-methyl group and the remainder of ring E are the same as that of the 12-OAc analogue of **4** for which the 24β configuration has been established by an exhaustive nmr study (15).
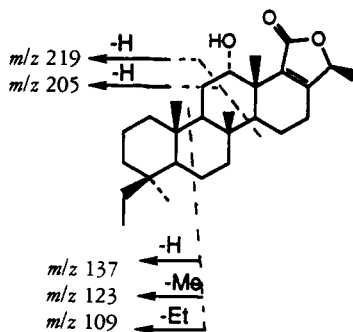

FIGURE 1.   Mass spectral fragmentation pathway for compound **4**.

Phyllofenone B [**2**] is especially interesting because it has a previously unknown carbon skeleton. It is possible that **2** was formed from compound **5**, isolated from the same sponge (8,9), by aldol condensation, dehydration, and deacetylation. Sesterterpenes **3** and **4** are related to **5** as well via oxidations, reductions, lactonization, and dehydration followed by double bond migration. Phyllofenone B [**2**] shows cytotoxicity against the P-388 murine leukemia cell line (IC$_{50}$ = 5 μg/ml), but phyllofolactones A and B were inactive in this assay.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on an X4 micro-melting point apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ with a Rudolph Research Autopol III automatic polarimeter. Ir spectra were recorded on KBr plates as films using an FT ir spectrophotometer. Uv spectra were taken in MeOH on a Perkin-Elmer Lambda 3 UV/VIS spectrophotometer. Eims were run on a VG ZAB-E mass spectrometer, and ^1H- and ^{13}C-nmr spectra were recorded on Varian XL-300 or VXR-500 spectrometers using $CHCl_3$ (referenced to TMS) as internal reference. ^{13}C multiplicities were assigned from DEPT experiments. All solvents used were analytical grade. Light petroleum refers to the 60–90° fraction. Si gel, 60–100 mesh, was used for cc, and tlc grade Si gel H was used for flash cc.

BIOLOGICAL 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.

ISOLATION OF SESTERTERPENES.—The sun-dried specimens (3 kg) were extracted with MeOH, and the solvent was removed 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 give a viscous oil that was chromatographed on Si gel, eluting with light petroleum/EtOAc using a step gradient of increasing EtOAc to afford five fractions. Fractions 3–5 were subjected to flash chromatography and yielded, in order of increasing polarity, **4** (6.6 mg, 0.00022%), **2** (15 mg, 0.0005%), and **3** (45.3 mg, 0.0015%).

PHYLLOFENONE B [**2**].—Needles from EtOH: mp 241–243°; $[\alpha]^{20}D$ 133.0° ($c = 0.004$, $CHCl_3$); uv λ max 248 nm (ε = 21879, MeOH); ir (KBr) ν max 3270–3540 (br, OH), 1694, 1662 (α,β-unsaturated ketone) cm^{-1}; eims m/z (70 eV) $[M]^+$ 396 (11), 378 (11), 349 (24), 219 (17), 213 (40), 205 (90), 160 (100); ^1H nmr see Table 1; ^{13}C nmr see Table 2. NOe measurements: irradiated signal, assignment (enhanced signal, assignment, % enhancement): irradiation of the methyl signal δ 0.53, H-13, (δ 3.94, H-12, 4.1%); 3.68, H-18, (1.78, H-14, 6%, 7.55, H-25, 4.1%); 6.34, H-26, (7.55, H-25); 7.55, H-25, (3.68, H-18, 3.6%; 3.94, H-12, 4.5%, 6.34, H-26, 9.6%).

PHYLLOFOLACTONE A [**3**].—Needles from Me_2CO: mp 332–334°; $[\alpha]^{20}D$ +78.4° ($c = 0.051$, $CHCl_3$); ir (KBr) ν max 3520 (OH), 1765 (γ-lactone), 1715 (ketone) cm^{-1}; eims m/z 430 (31), 415 (34), 401 (46), 331 (20), 219 (7), 205 (27), 137 (31), 123 (33), 109 (52), 95 (58), 81 (62), 69 (49), 55 (100); ^1H nmr see Table 1; ^{13}C nmr see Table 2.

PHYLLOFOLACTONE B [**4**].—Needles from Me_2CO: mp 232–234°; $[\alpha]^{20}D$ +61.9 ($c = 0.0042$, $CHCl_3$); uv λ max 214 nm (ε 15663, MeOH); ir (KBr) ν max 3468 (OH), 1728, 1675 (α,β-unsaturated γ-lactone) cm^{-1}; eims m/z $[M]^+$ 414 (21), 399 (48), 396 (20), 385 (4), 381 (10), 367 (6), 231 (22), 219 (5), 206 (15), 205 (80), 191 (15), 177 (23), 166 (100), 165 (64), 151 (31), 137 (18), 123 (30), 121 (34), 109 (33), 95 (43), 81 (44), 69 (35), 55 (56); ^1H nmr see Table 1; ^{13}C nmr see Table 2. Selective INEPT experiment results: irradiated proton signal, assignment (observed carbon signal, assignment) δ 4.64, H-12 (21.7, C-23; 40.3, C-13; 49.9, C-14; 52.4, C-9); δ 1.11, H-23 (40.3, C-13; 49.9, C-14); 4.78, H-24 (18.4, C-26; 133.4, C-18; 165.3, C-17; 172.6, C-25).

ACKNOWLEDGMENTS

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