# New Sesterterpenoids from the Marine Sponge *Phyllospongia papyracea*

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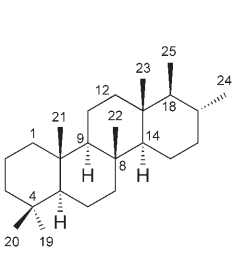
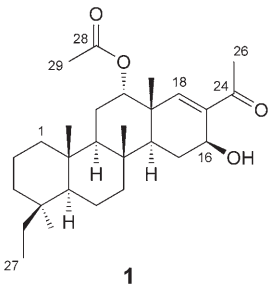
Chemical investigation of an extract of the marine sponge *Phyllospongia papyracea*, collected from the South China Sea, led to the isolation and identification of three new scalarane-type sesterterpenoids, compounds **1**–**3**. Their structures were elucidated by spectroscopic methods, including 1D- and 2D-NMR as well as high-resolution ESI-MS experiments. (12*α*,16*β*)-12-Acetoxy-16-hydroxy-20,24-dimethyl-25-norscalar-17-en-24-one (**1**) was cytotoxic against the leukemia P388 cancer cell line, with an $IC_{50}$ value of 5 μg/ml.

**Introduction.** – Typical metabolites of sponges of the order Dictyoceratida are scalarane-type sesterterpenoids, which possess distinct pharmacological properties such as cytotoxicity, antimicrobial, antifouling, antifeedant, ichthyotoxic, anti-inflammatory, anti-HIV, erythroid-differentiation, vasodilatory, as well as antithrombocyte activities [1]. With their unique skeleton and broad biological activities, scalarane-type sesterterpenoids have attracted much attention in (bio)synthetic chemistry [2–4]. In addition, scalarane sesterterpenoids are considered to represent useful chemotaxonomic markers within sponges of the order Dictyoceratida [5].

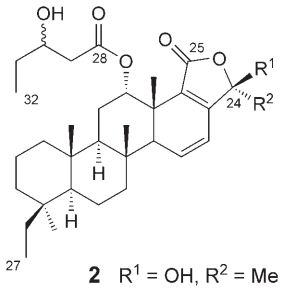
Recently, we investigated the chemical constituents of the sponge *Phyllospongia papyracea* collected from Hainan Island in the South China Sea. As a result, we obtained three new scalarane-type sesterterpenoids, compounds **1**–**3**. Herein, we describe the structures and cytotoxicities of these compounds.




Scalarane




**1**




**2** $R^1$ = OH, $R^2$ = Me
**3** $R^1$ = Me, $R^2$ = OH

**Results and Discussion. –** 1. *Structure Elucidation*. The molecular formula of compound **1** was deduced as $C_{28}H_{44}O_4$ by HR-ESI-TOF-MS (*m/z* 427.3165 ($[M + H - H_2O]^+$)) and $^{13}$C-NMR (*Table*). The molecular formula was consistent with seven degrees of unsaturation. The $^{13}$C-NMR spectrum of **1** showed seven Me, eight $CH_2$, six CH, and seven quaternary C-atoms. The $^1$H-NMR spectrum (*Table*) showed a Me *triplet* at $\delta$(H) 0.76, four Me *singlet*s at $\delta$(H) 0.81, 0.83, 0.87, and 1.18, and two additional Me resonances at $\delta$(H) 2.25 and 2.04 attributed to a methyl ketone and an AcO group, respectively. These data suggested that **1** was a 20,24-dimethylscalarane[1]) derivative [6]. The trisubstituted C=C bond between C(17) and C(18) was confirmed by a *singlet* for H–C(18) at $\delta$(H) 6.58, and by ROESY correlations for H–C(18)/Me(12) and H–C(18)/Me(23). The methyl ketone unit was connected to C(17), forming an $\alpha,\beta$-unsaturated C=O system, as supported by UV absorption maxima at 252 nm, and by HMBC correlations for H–C(18)/C(24) and H–C(26)/C(17). The OH group was placed at C(16) [$\delta$(C) 68.1 (*d*); $\delta$(H) 4.59 (*dd*, $J$ = 9.0, 7.0 Hz)], and its $\beta$-orientation was established by the ROESY correlation H–C(14)/H–C(16). The AcO group was connected to C(12), based on HMBC cross-peak between H–C(12) [$\delta$(H) 5.02 (*dd*, $J$ = 3.0, 2.5 Hz)] and the C=O group at $\delta$(C) 170.8. The small coupling constants between H–C(12) and the $CH_2$(11) indicated that H–C(12) was equatorial. Considering that H–C(1) at $\delta$(H) 0.66 (*ddd*, $J$ = 13.0, 13.0, 3.5 Hz) showed two identical $J$ values of 13.0 Hz attributed to $^2J_{gem}$ and $^3J_{ax,ax}$, in combination with ROESY correlations with H–C(3), H–C(5), and H–C(9) (*Figure*), we confidently placed this H-atom at the axial position. Its resonance at high magnetic field could be accounted to stronger shielding effects due to the higher electron density at the axial than the equatorial positions of the six-membered ring. Likewise, by comparing their coupling constants and by interpreting ROESY data, we assigned all chemical shifts and relative configurations to the H-atoms in positions 2, 3, 6, 7, 11, and 15. Comprehensive analysis of the 2D-NMR (HMQC, HMBC, $^1$H,$^1$H-COSY, ROESY) data finally allowed us to assemble the structure of **1**, which was identified as (12$\alpha$,16$\beta$)-12-acetoxy-16-hydroxy-20,24-dimethyl-25-norscalar-17-en-24-one.

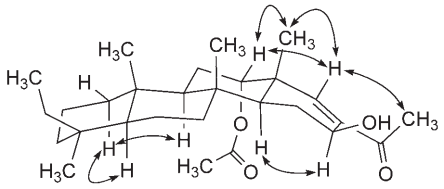



Figure. *Selected ROESY correlations of* **1**

Compounds **2** and **3** were obtained as a mixture after purification by flash chromatography on silica gel, followed by repeated reverse-phase HPLC. They always exhibited a single HPLC peak for different solvent systems (MeOH/$H_2O$, MeCN/$H_2O$,

[1]) Scalarane = (4a*S*,4b*R*,6a*R*,7*S*,8*R*,10a*R*,10b*S*,12a*S*)-1,1,4a,6a,7,8,10b-heptamethyloctadecahydrochrysene.

Table. *[1]H- and [13]C-NMR Data of* **1** *and* **2**/**3**. At 500/125 MHz, resp., in $CDCl_3$; $\delta$ in ppm, $J$ in Hz. Asterisks (*) mark doubled signals due to the epimeric mixture, and 'ax' and 'eq' refer to axial and equatorial positions, resp. Arbitrary atom numbering.

| Position | **1** | | | **2**/**3** | | |
|---|---|---|---|---|---|---|
| | $\delta$(H) | $\delta$(C) | ROESY | $\delta$(H) | $\delta$(C) | ROESY |
| 1 | ax: 0.66 (*ddd*, $J$ = 13.0, 13.0, 3.5) eq: 1.53–1.57 (*m*) | 40.2 (*t*) | 3-ax, 5, 9 | ax: 0.77 (*ddd*, $J$ = 12.5, 12.5, 4.0) eq: 1.54–1.58 (*m*) | 40.2 (*t*) | 3-ax, 5 |
| 2 | ax: 1.34–1.40 (*m*) eq: 1.49–1.55 (*m*) | 18.3 (*t*) | | ax: 1.34–1.40 (*m*) eq: 1.46–1.51 (*m*) | 18.1 (*t*) | |
| 3 | ax: 0.84–0.90 (*m*) eq: 1.65–1.69 (*m*) | 36.7 (*t*) | 1-ax | ax: 0.81–0.86 (*m*) eq: 1.64–1.68 (*m*) | 36.8 (*t*) | 1-ax |
| 4 | | 36.2 (*s*) | | | 36.1 (*s*) | |
| 5 | 0.90 (*dd*, $J$ = 12.0, 2.0) | 58.9 (*d*) | 1-ax | 0.88–0.90 (*m*) | 58.6 (*d*) | 1-ax |
| 6 | ax: 1.41–1.49 (*m*) eq: 1.56–1.60 (*m*) | 18.1 (*t*) | | ax: 1.35–1.43 (*m*) eq: 1.48–1.52 (*m*) | 17.7 (*t*) | |
| 7 | ax: 0.97 (*ddd*, $J$ = 12.0, 12.0, 4.0) eq: 1.85 (*ddd*, $J$ = 12.0, 3.0, 3.0) | 41.3 (*t*) | 15 | ax: 0.86–0.91 (*m*) eq: 1.62–1.65 (*m*) | 40.0 (*t*) | |
| 8 | | 37.3 (*s*) | | | 37.3 (*s*) | |
| 9 | 1.24 (*dd*, $J$ = 12.0, 3.5) | 53.5 (*d*) | 1-ax, 11-eq | 1.05–1.08 (*m*) | 57.0 (*d*) | 11-eq |
| 10 | | 37.1 (*s*) | | | 36.6 (*s*) | |
| 11 | ax: 1.75 (*ddd*, $J$ = 14.0, 12.0, 3.0) eq: 1.84 (*ddd*, $J$ = 14.0, 3.5, 2.5) | 22.2 (*t*) | 12 9, 12 | ax: 1.65–1.71 (*m*) eq: 1.94 (*ddd*, $J$ = 13.5, 3.0, 2.5) | 24.1 (*t*) | 12, 21 9, 12 |
| 12 | 5.02 (*dd*, $J$ = 3.0, 2.5) | 76.7 (*d*) | 11-ax, 11-eq, 18, 23 | 5.19 (*dd*, $J$ = 3.0, 2.5) | 75.3 (*d*) | 11-ax, 11-eq, 23 |
| 13 | | 41.6 (*s*) | | | 42.2/42.0 (*s*)* | |
| 14 | 1.46–1.49 (*m*) | 47.4 (*d*) | 15, 16 | 2.19 (*d*, $J$ = 3.5) | 58.6/58.5 (*d*)* | |
| 15 | 2.10–2.15 (*m*) | 25.7 (*t*) | 7-eq, 14, 16 | 6.44 (*dd*, $J$ = 9.5, 3.0) | 139.7/139.4 (*d*)* | 16 |
| 16 | 4.59 (*dd*, $J$ = 9.0, 7.0) | 68.1 (*d*) | 14, 15 | 6.36 (*d*, $J$ = 9.5) | 119.4/119.2 (*d*)* | 15 |
| 17 | | 138.9 (*s*) | | | 158.2/158.0 (*s*)* | |
| 18 | 6.58 (*s*) | 152.3 (*d*) | 12, 23 | | 132.2/131.9 (*s*)* | |
| 19 | 0.81 (*s*) | 28.6 (*q*) | | 0.82 (*s*) | 28.5 (*q*) | |
| 20 | 1.17 (*dq*, $J$ = 15.0, 7.5) 1.51 (*dq*, $J$ = 15.0, 7.5) | 24.6 (*t*) | | 1.16 (*dq*, $J$ = 15.0, 7.5) 1.57 (*dq*, $J$ = 15.0, 7.5) | 24.5 (*t*) | |
| 21 | 0.83 (*s*) | 16.9 (q) | | 1.06 (*s*) | 18.8 (*q*) | 11-ax |
| 22 | 0.87 (*s*) | 17.1 (*q*) | | 0.85 (*s*) | 16.6 (*q*) | |
| 23 | 1.18 (*s*) | 21.2 (*q*) | 12, 18 | 1.12/1.11 (*s*)* | 12.9/12.7 (*q*)* | 12 |
| 24 | | 202.2 (*s*) | | | 102.7/102.6 (*s*)* | |
| 25 | | | | | 168.2/167.7 (*s*)* | |
| 26 | 2.25 (*s*) | 25.8 (*q*) | 18 | 1.58/1.70 (*s*)* | 24.5/23.9 (*q*)* | |
| 27 | 0.76 (*t*, $J$ = 7.5) | 8.7 (*q*) | | 0.74 (*t*, $J$ = 7.5) | 8.6 (*q*) | |
| 28 | | 170.8 (*s*) | | | 172.8 (*s*) | |
| 29 | 2.04 (*s*) | 21.4 (*q*) | | 2.51–2.53 (*m*) | 42.0 (*t*) | |
| 30 | | | | 4.02–4.06 (*m*) | 69.7 (*d*) | |
| 31 | | | | 1.26–1.28 (*m*) | 29.6 (*t*) | |
| 32 | | | | 0.99 (*t*, $J$ = 7.0) | 10.0 (*q*) | |

and i-PrOH/$H_2O$) and different gradients. The ratio **2**/**3** was estimated to be *ca.* 1:1 by NMR analysis.

The molecular formula of **2**/**3** was deduced as $C_{32}H_{48}O_6$ by HR-ESI-TOF-MS (*m*/*z* *529.3534 ([M + H]$^+$)*). The characteristic NMR peaks (*Table*) for the *trans*-configured rings *A*–*C* of **1** were also found in the spectrum of **2**/**3**, however, some carbon resonances for the *D*- and *E*-rings were doubled. Two C=O groups [$\delta$(C) 172.8, 168.2/167.7], two C=C bonds [$\delta$(C) 158.2/158.0 (*s*); 132.2/131.9 (*s*); 139.7/139.4 (*d*); 119.4/119.2 (*d*)] and five rings represented nine degrees of unsaturation. The $^{13}$C-NMR resonances at $\delta$(C) 168.2/167.7 (*s*), 158.2/158.0 (*s*), 132.2/131.9 (*s*), 102.7/102.6 (*s*), and 24.5/23.9 (*q*) suggested the presence of a cyclic butenolide functional group in ring *E*. A hemiacetal quaternary C-atom [$\delta$(C)102.7/102.6] was located at C(24), forming an epimeric center, as confirmed by HMBC correlations for H–C(26)/C(17) and H–C(26)/C(24). The $\Delta^{15,16}$ unsaturation was confirmed by the HMBC correlations for both H–C(15) and H–C(16) with C(17), and by a $^1$H,$^1$H-COSY correlation for H–C(14)/H–C(15). The cross-peaks for Me(32)/$CH_2$(31), H–C(30)/$CH_2$(29) and H–C(30)/$CH_2$(31) in the $^1$H,$^1$H-COSY spectrum, as well as the HMBC correlations for Me(32)/C(31), $CH_2$(31)/C(30), $CH_2$(29)/C(28), and $CH_2$(29)/C(30) established a 3-hydroxypentanoyl moiety. The long-range correlation H–C(12)/C(28) showed that the 3-hydroxypentanoyl group was placed at the O-atom at C(12). By detailed comparison with the NMR data of previously reported scalarane sesterterpenes, we confirmed that **2** and **3** are isomers of phyllactones D and E [7], which had previously been obtained from *Phyllospongia foliascens* collected at Nansha Island (South China Sea). Based on the absence of a large coupling constant (>10 Hz) and the presence of a ROESY correlation for H–C(12)/Me(23), we assigned the $\beta$-orientation to H–C(12).

From the above data, the structures of compounds **2** and **3** were, thus, determined as (12$\alpha$,24*R*)- and (12$\alpha$,24*S*)-12-[(3-hydroxypentanoyl)oxy]-20,24-dimethyl-25-oxoscalar-15,17-dien-25,24-olide, respectively.

2. *Biosynthetic Aspects and Biological Properties.* So far, a variety of scalarane-type sesterterpenoids have been isolated from *Dictyoceratida* sponges, but norscalarane sesterterpenes are relatively rare. Compound **1** represents a new variant of a 20,24-dimethyl-25-norscalarane sesterterpene, possessing an OH group at C(16) and a C=C bond in position 17 in ring *D*. This indicates that this type of norscalarane is derived by a unique biosynthetic pathway. The isolation of **1**–**3** from *Phyllospongia papyracea* also provides additional supporting information in terms of chemotaxonomy. However, the reason why scalarane-type sesterterpenoids exist commonly in this order of sponges remains unknown.

Compound **1** showed cytotoxic activity towards the leukemia P388 tumor cell line, with an $IC_{50}$ value of 5 μg/ml. In contrast, compounds **2** and **3** were apparently inactive in this assay.

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## Experimental Part

*General.* HPLC was performed on a *Phenomenex C18* reverse-phase column (250 × 10 mm), with a single-wavelength (230 nm) UV detector and an ELS detector. UV Spectra: *Perkin-Elmer Lambda-40*

UV spectrophotometer; $\lambda_{max}$ ($\varepsilon$) in nm. Optical rotations: *Jasco DPI-370* digital polarimeter. IR Spectra: *Perkin-Elmer 337* spectrophotometer, in $CH_2Cl_2$ soln.; in $cm^{-1}$. $^1H$- and $^{13}C$-NMR Spectra: *Varian Inova-500* spectrometer, at 500/125 MHz, resp. in $CDCl_3$ soln.; $\delta$ in ppm rel. to $Me_4Si$, *J* in Hz. ESI- and HR-ESI-TOF-MS: *Mariner 5200* mass spectrometer; in *m*/*z*.

*Sponge Material*. The marine sponge *Phyllospongia papyracea* was collected in July 2005 by scuba diving at a depth of 10–15 m along the coast of Hainan Island in the South China Sea. A voucher specimen (No. 2005H76) was deposited at the Research Center of Organic Natural Products, Sun Yat-sen University, Guangzhou, P. R. China.

*Extraction and Isolation*. The dry sponge (116 g) was soaked in MeOH ($3 \times 1000$ ml). Then, the solvent was evaporated under reduced pressure, and the resulting residue (5.7 g) was further extracted with AcOEt/$H_2O$. The org. layer was concentrated under reduced pressure, and the resulting AcOEt-soluble material (2.1 g) was subjected to flash chromatography (FC) ($SiO_2$; hexane/AcOEt/MeOH gradient) to afford nine fractions (*Fr. 1–Fr. 9*). *Fr. 6* (236 mg) was further separated by prep. RP-HPLC ($H_2O$/MeOH 40:60 → 0:100) to afford **1** (19 mg). *Fr. 7* (53 mg) was further purified by repeated RP-HPLC ($H_2O$/MeOH 60:40 → 0:100) to yield **2**/**3** (4 mg) as an inseparable mixture.

*(12α,16β)-12-Acetoxy-16-hydroxy-20,24-dimethyl-25-norscalar-17-en-24-one* (**1**). Colorless solid. UV (MeOH): 252 (3534). $[\alpha]_D^{24} = -95$ ($c = 0.33$, MeOH). IR ($CH_2Cl_2$): 3497, 2957, 2927, 2874, 2847, 1735, 1648, 1460, 1423, 1379, 1245, 1200, 1186, 1034, 1013, 975, 956, 764, 749. $^1H$- and $^{13}C$-NMR: see *Table*. ESI-MS: 467.3 ($[M + Na]^+$), 445.3 ($[M + H]^+$), 427.3 ($[M + H - H_2O]^+$), 385.8 ($[M + H - AcOH]^+$), 367.3 ($[M + H - H_2O - AcOH]^+$). HR-ESI-TOF-MS: 427.3165 ($[M + H - H_2O]^+$, $C_{28}H_{43}O_3^+$; calc. 427.3212).

*(12α,24*R*)-* (**2**) *and (12α,24*S*)-12-[(3-Hydroxypentanoyl)oxy]-20,24-dimethyl-25-oxoscalar-15,17-dien-25,24-olide* (**3**). Colorless solid (*ca.* 1:1 mixture). UV (MeOH): 258 (4600). $[\alpha]_D^{24} = +68$ ($c = 0.67$, MeOH). IR ($CH_2Cl_2$): 3483, 2960, 2932, 2877, 2848, 1718, 1689, 1649, 1587, 1461, 1391, 1374, 1356, 1266, 1246, 1180, 1168, 1100, 1037, 1012, 981, 735. $^1H$- and $^{13}C$-NMR: see *Table*. HR-ESI-TOF-MS: 529.3534 ($[M + H]^+$, $C_{32}H_{49}O_6^+$; calc. 529.3529).

*Cytotoxicity Assay*. The *in vitro* cytotoxicities of **1** and **2**/**3** were determined by means of the colorimetric MTT[2]) assay. Leukemia P388 cells were seeded in 96-well plates at a density of $3 \times 10^7$ cells/l, and the compounds were added at various concentrations (0.1–50 mg/l). After 48 h, MTT was added to the culture medium at a final concentration of 0.5 mg/ml, and the plates were incubated for 4 h at 37°. The insoluble formazan product was then precipitated by centrifugation, and the supernatant was removed. The formazan crystals were dissolved in DMSO (100 µl) with gentle shaking at r.t. The UV/VIS absorbance at 570 nm was recorded with a *Bio-Rad* (Hercules, CA) microplate reader, and the data were analyzed in the usual way.

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[2]) MTT = 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide.