Lobophytones H—N, Biscembranoids from the Chinese Soft Coral Lobophytum pauciflorum

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Chemical examination of a Chinese soft coral Lobophytum pauciflorum resulted in the isolation of seven new biscembranoids, named lobophytones H—N (1—7). Their structures were determined by interpretation of 1D- and 2D-NMR (correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond connectivity (HMBC), and nuclear Overhauser effect spectroscopy (NOESY)) spectroscopic data in association with MS and IR data. All compounds were tested for the inhibition of lipopolysaccharide (LPS)-induced nitric oxide (NO) production, cytotoxicity of mouse peritoneal macrophage, and antibacterial activities.

Key words soft coral; Lobophytum pauciflorum; lobophytone; structure; elucidation

Results and Discussion

Repeated column chromatography including reversed-phase HPLC separation of EtOAc-soluble fraction obtained from the EtOH extract of this specimen yielded seven biscembranoids (1—7) (Fig. 1). All of the isolated biscembranoids shared the partial structure closely related to cembranoid-dienophile methyl tortuosoate.

Lobophytone H (1) had a molecular formula of C41H62O8 as determined by high resolution-electrospray ionization-mass spectrum (HR-ESI-MS) (m/z 705.4309 [M+Na]+, Calcd for C41H62O8Na, 705.4336) and NMR data, requiring 11 degrees of unsaturation. The 1H-NMR spectrum exhibited proton resonances for nine methyls including three olefinic methyls (δH 1.59, 1.67, 1.71) and a OMe (δH 3.42), while the 13C-NMR spectrum present 41 carbon resonances including three ketones out of four carbonyl resonances, six olefinic carbons, and four oxygen-bearing sp3 carbons (Tables 1, 2). As part of our continuing interest in the chemical diversity from soft corals of South China Sea, soft coral L. pauciflorum was collected from Sanya Bay, Hainan Island of China. In this paper, we report seven new biscembranoids isolated from the same specimen and their structural elucidation.
The noticeable difference was found by \( J = 4.5, 8.0 \) Hz and chemical shift of C-33 (\( \delta_C 66.0 \)) in 1 to replace the corresponding proton (\( \delta_H 4.83, d, d = 9.5 \) Hz) and carbon (\( \delta_C 70.1, s \)) of the latter compound. These findings suggested the chiral configuration of C-33 in 1 to be in opposite to that of methyl tortuaote A. The nuclear Overhauser effect (NOE) correlations of H-33/H11005 (\( \delta_H 6.97, s \)) and an olefin conversion from C-27/C-28 to C-27/C-39. Thus, lobophytone I (\( \delta_H 3.49, d, d = 9.5 \) Hz), and the absence of NOE correlation between H-33/H-21 as observed in methyl tortuaote A, suggested H-33 being \( \beta \)-oriented. Thus, 1 is a C-33 epimer of methyl tortuaote A. In addition, the NOE correlations of the J values of the protons locating at rings C and D were close to those of lobophytone A, whose absolute configurations were determined by X-ray diffraction. Since lobophytone A was isolated from the same specimen as 1, the configurations in rings C and D of 1 were biogenetically assumed to be the same as those of lobophytone A.

Spectroscopic analysis and comparison of NMR and MS data revealed that the gross structure of lobophytone I (2) was identical to ximaolide E, a bisemibranched from S. tortuosum. However, nuclear Overhauser enhancement spectroscopy (NOESY) relationships between H-33/H11005 and \( \delta_H 3.38, m \) along with the absence of NOE correlation between H-33/H-21 as observed in ximaolide E, indicated that H-33 (\( \delta_H 4.83, d, d = 9.5 \) Hz) of 2 was \( \beta \)-oriented as the case of 1. The similar NOE relationships of 2 in comparison with that of 1 indicated that 2 maintained the same configurations in respect to rings C and D of 1. Biogenetically, the structure of 2 was considered to be derived from 1 through an olefin conversion from C-27/C-28 to C-27/C-39. Thus, lobophytone I (2) was determined to be a C-33 epimer of ximaolide E.

The molecular formula of lobophytone J (3) was determined to be C_{32}H_{56}O_{10} on the basis of HR-ESI-MS data (m/z 765.4540 [M+Na]^+). Analysis of 1D- and 2D-NMR spectro-
scopic data revealed that the gross structure of 3 was closely related to that of nyalolide, a biscembrane analogue also isolated from the same specimen. The presence of an acetyl group was observed from the NMR spectra at $\delta_{\text{H}}$ 1.94 (d, $J=11.2$ Hz) and acetyl carbonyl carbon ($\delta_{\text{C}}$ 169.9) ascertained the acetoxy group to be substituted at C-38.

Table 2. $^1$H-NMR Spectroscopic Data of Lobophytones H—N (1—7)$^a$

<table>
<thead>
<tr>
<th>Position</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3.65 dd (6.1, 8.3)</td>
<td>3.68 dd (6.4, 9.0)</td>
<td>3.40 m</td>
<td>3.40 m</td>
<td>3.39 m</td>
<td>3.46 m (8.6)</td>
<td>3.40 m (8.4)</td>
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<tr>
<td>4</td>
<td>2.88 dd (9.5, 19.3)</td>
<td>2.93 dd (9.8, 19.8)</td>
<td>3.14 m</td>
<td>3.17 dd (10.3, 19.6)</td>
<td>3.16 dd (10.3, 19.6)</td>
<td>3.15 dd (10.3, 19.6)</td>
<td>3.17 dd (10.5, 19.3)</td>
</tr>
<tr>
<td>5</td>
<td>2.40 d (19.3)</td>
<td>2.42 d (22.1, 19.8)</td>
<td>2.34 m</td>
<td>2.40 d (20.0)</td>
<td>2.43 d (19.6)</td>
<td>2.44 d (19.6)</td>
<td>2.45 d (29.2, 19.3)</td>
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<tr>
<td>7</td>
<td>1.94 m</td>
<td>1.92 m</td>
<td>1.02 m</td>
<td>1.02 m</td>
<td>1.05 m</td>
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<td>1.06 m</td>
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<tr>
<td>8</td>
<td>0.89 m</td>
<td>0.95 m</td>
<td>0.90 m</td>
<td>0.90 m</td>
<td>0.95 m</td>
<td>0.92 m</td>
<td>1.02 m</td>
</tr>
<tr>
<td>9</td>
<td>0.90 m</td>
<td>1.74 m</td>
<td>1.73 m</td>
<td>1.73 m</td>
<td>1.68 m</td>
<td>1.67 m</td>
<td>1.69 m</td>
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<td>11</td>
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<td>1.95 m</td>
<td>1.86 m</td>
<td>1.86 m</td>
<td>1.86 m</td>
<td>1.86 m</td>
<td>1.69 m</td>
</tr>
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</table>

$^a$ Spectra were measured in DMSO-$d_6$. *Overlapped with H$_2$O signal.

was indicative of 22E geometry. The NOESY cross-peaks from H-26 ($\delta_{\text{H}}$ 3.38, dd) to H$_{-40}^1$ ($\delta_{\text{H}}$ 1.06, s) and H-24a ($\delta_{\text{H}}$ 2.04, m) and between H-22 ($\delta_{\text{H}}$ 5.16, d, $J=11.0$ Hz) and H-24a assigned to the same face of H-26, H$_{-40}$, and H-22, which were in opposite to that of H$_{38}$. Additional NOE interactions between H-30 ($\delta_{\text{H}}$ 3.30, dd, $J=3.8, 10.3$ Hz)/H$_{33}$, H-30/H$_{28a}$ ($\delta_{\text{H}}$ 1.53, m), and H$_{39}$ ($\delta_{\text{H}}$ 1.01, s)/H$_{28a}$ suggested the same orientation of H-30, H-33, and H-39. Moreover, the strong NOE cross-peaks between H-21/H$_{38}$, H-21/H$_{33}$, and H-33/H$_{38}$ indicated the same face of H-21 and H-33. It is noted that all known biscembranoids exclusively showed $\beta$-orientation of MeO group, while $\alpha$-oriented H-21 resulted in NOE relationship between MeO and H-22. In compound 3, the presence of NOE relationship between...
MeO/H-21 but absence of MeO/H-22 relationship suggested β-face of H-21. Thus, the relative configurations of 3 were determined to be the same as those of lobophytone B,10 in which β-orientations of H-21, H-30, H-33, and H3-39 and α-orientations of H-26 and H3-40 were assigned.

The graphic structure of 3 was almost identical to ximaoilides F18 except for the different orientation of AcO at C-33. However, comparison of NMR data revealed downfield shifted C-26 (δ 83.2) and upfield shifted C-27 (δ 68.6) and C-30 (δ 71.2) of 3 to replace those (δ 73.8, 83.6, 88.9, respectively) of ximaoilide F. These findings suggested ximaoilides F (also G18) containing an ether bridge across C-27 and C-30 as in the case of bizgaucumlides G and H19 rather than the ether bridge between C-26/C-30 as reported in literature.

The 1D- and 2D-NMR spectroscopic analysis in association with HR-ESI-MS data resulted in the gross structure of lobophytone K (4) to be close similar to that of ximaoilide C,30 except for the presence of an additional hydroxy group and the absence of a chlorine atom. The replacement of a hydroxy group of 4 to a chlorine atom at C-31 of ximaoilide C was evident from OH-31 resonance (J 1, 2) of lobophytone N (5) had the same molecular formula as 4, as determined by HR-ESI-MS and NMR data. The 1H- and 13C-NMR spectra were recorded on an Avance-500 FT 500 MHz NMR spectrometer (Bruker) using tetramethyl silane (TMS) as an internal standard. Chemical shifts (δ) are expressed in parts per million (ppm), and coupling constants (J) are reported in Hertz (Hz). HR-ESI-MS spectra were obtained from the Bruker APEX IV instruments. Column chromatography was carried out with Si gel (160—200 mesh and 200—300 mesh), while GF254 Si gel for TLC was provided by Qingdao Marine Chemistry Co., Ltd. Optical rotations were measured on a Perkin-Elmer 243B polarimeter. IR spectra were determined on a Thermo Nicolet Nexus 470 Fourier transform (FT)IR spectrometer. 'H- and 13C-NMR spectra were recorded on an Avance-500 FT 500 MHz NMR spectrometer (Bruker) using tetramethyl silane (TMS) as an internal standard. Chemical shifts (δ) are expressed in parts per million (ppm), and coupling constants (J) are reported in Hertz (Hz). HR-ESI-MS spectra were obtained from the Bruker APEX IV instruments. Column chromatography was carried out with Si gel (160—200 mesh and 200—300 mesh), while GF254 Si gel for TLC was provided by Qingdao Marine Chemistry Co., Ltd. HPLC chromatography was performed on an Alttech instrument (426-HPLC pump, Alttech UV–vis-200 detector) equipped with Kromasil semipreparative (10 μm, ODS, 10 mm×250 mm) columns.

**Experimental**

**General Experimental Procedures** Optical rotations were measured on a Perkin-Elmer 243B polarimeter. IR spectra were determined on a Thermo Nicolet Nexus 470 Fourier transform (FT) IR spectrometer. 'H- and 13C-NMR spectra were recorded on an Avance-500 FT 500 MHz NMR spectrometer (Bruker) using tetramethyl silane (TMS) as an internal standard. Chemical shifts (δ) are expressed in parts per million (ppm), and coupling constants (J) are reported in Hertz (Hz). HR-ESI-MS spectra were obtained from the Bruker APEX IV instruments. Column chromatography was carried out with Si gel (160—200 mesh and 200—300 mesh), while GF254 Si gel for TLC was provided by Qingdao Marine Chemistry Co., Ltd. HPLC chromatography was performed on an Alttech instrument (426-HPLC pump, Alttech UV–vis-200 detector) equipped with Kromasil semipreparative (10 μm, ODS, 10 mm×250 mm) columns.

**Animal Material** Soft coral Lobophytum pacificorum was collected from the inner coral reef at a depth of 10 m in Sanya Bay, Hainan Island of China, in 2008. The fresh samples (2.3 kg, wet weight) were frozen immediately. The specimen was identified by Leen van Ofwegen (National Museum of National History Naturalis). The coral specimen (HSF-6) was deposited

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**Table 3. Inhibition of LPS-Induced NO Production and Cytotoxicity against PEMΦ**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration (μg)</th>
<th>Level of NO (μg)</th>
<th>IR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td></td>
<td>1.21</td>
<td>25.13</td>
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<tr>
<td>LPS</td>
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<td>1.26</td>
<td>95.00</td>
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<tr>
<td>1</td>
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<td>15.85</td>
<td>22.84</td>
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</tr>
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<td>1.0</td>
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<td>7.93</td>
</tr>
<tr>
<td>7</td>
<td>1.0</td>
<td>20.46</td>
<td>18.58</td>
</tr>
</tbody>
</table>

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*DMSO = dimethyl sulfoxide; LPS = lipopolysaccharide; Dex = dexamethasone.*


at the State Key Laboratory of Natural and Biomimetic Drugs, Peking University, P.R. China.

**Extraction and Isolation** The frozen soft coral *L. pauciforum* was homogenized and then extracted with EtOH. The concentrated extract was de-salted by dissolving in MeOH to yield a residue (92.7 g). This residue was partitioned between H$_2$O and EtOAc, and then n-BuOH. The EtOAc fraction (12.1 g) was subjected to Si gel column chromatography with a gradient (petroleum ether–acetone, 20:1, 10:1, 3:1, 1:1) to obtain seven fractions (F1–F7). F1–F4 mainly contained lipids and steroids, as detected by H-NMR, while F5 and F6 showed the spectral features of biscembranes. Thus, F5 and F6 were combined (1.69 g) and subsequently subjected to Sephadex LH-20 eluting with CH$_3$Cl–MeOH–H$_2$O (1:2) to afford 25 mg. SF2 (180 mg) showed blue-green spots after spraying with anisaldehyde, and was then separated on reversed-phase semipreparative HPLC with CH$_3$CN–H$_2$O (61%) as a mobile phase to obtain 5 (2.8 mg). SF3 (90 mg) was followed by the same protocol as that of SF2 on ODS HPLC eluting with MeOH–H$_2$O (80%) to obtain 4 (9.3 mg), 3 (5.4 mg), and 6 (5.2 mg). SF4 (49.2 mg) was separated on ODS HPLC with MeOH–H$_2$O (82%) as a mobile phase to afford 5 (5.2 mg). SF5 (4.6 mg) was obtained from SF5 (40.5 mg).

Lobophytone H (1): Amorphous powder; $\delta^1$H 253.1, 13C 251.0 (C=O); IR (KBr) 1612, 1590, 1455, 1380, 1222 cm$^{-1}$. MS $m/z$ 175.0580 (M$^+$).

Lobophytone I (2): Amorphous powder; $\delta^1$H 253.1, 13C 251.0 (C=O); IR (KBr) 1612, 1590, 1455, 1380, 1222 cm$^{-1}$. MS $m/z$ 175.0580 (M$^+$).

Lobophytone J (3): Amorphous powder; $\delta^1$H 253.1, 13C 251.0 (C=O); IR (KBr) 1612, 1590, 1455, 1380, 1222 cm$^{-1}$. MS $m/z$ 175.0580 (M$^+$).

Lobophytone K (4): Amorphous powder; $\delta^1$H 253.1, 13C 251.0 (C=O); IR (KBr) 1612, 1590, 1455, 1380, 1222 cm$^{-1}$. MS $m/z$ 175.0580 (M$^+$).

Lobophytone L (5): Amorphous powder; $\delta^1$H 253.1, 13C 251.0 (C=O); IR (KBr) 1612, 1590, 1455, 1380, 1222 cm$^{-1}$. MS $m/z$ 175.0580 (M$^+$).

Lobophytone M (6): Amorphous powder; $\delta^1$H 253.1, 13C 251.0 (C=O); IR (KBr) 1612, 1590, 1455, 1380, 1222 cm$^{-1}$. MS $m/z$ 175.0580 (M$^+$).

**Assay for Inhibition of Lipopolysaccharide (LPS)-Induced Nitric Oxide Production**

**Production and Cytotoxicity of Mouse Peritoneal Macrophage (PEMΦ)** Dexamethasone (DEX, positive control, 20 mM in dimethyl sulfoxide (DMSO)) and each compound (20 mM in DMSO) were diluted to 1–20 μM range at r.t. before experiment. The final percentage of DMSO in the reaction mixture was less than 0.5% (v/v). LPS (1 μg/ml), 4% sodium thioglycollate, RPMI1640, fetal bovine serum (FBS), phosphate buffered saline (PBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Griess reagents were purchased from Sigma (St. Louis, MO, U.S.A.). Mouse peritoneal macrophages (PEMΦ) were obtained from C57BL/6J male mice, and then plated onto 48 well plates and cultured for 2 h in Dulbecco’s modified Eagle’s medium (DMEM) containing 5% FBS at 37 °C. Mouse PEMΦ were incubated with test compounds for 1 h at 37 °C before stimulation with 1 μg/ml of lipopolysaccharide (LPS) for 24 h. In primary test, blank control (enchylema) and LPS were added with compound (1 μM), and DEX (1 μM) was prepared. Cells (5×10$^4$ cells) were pre-incubated at 37 °C for 24 h in serum-free medium, and NO production was monitored by measuring nitrite levels in culture media using Griess reagent. Absorbance was measured at 548 nm in incubated media with Griess reagent for 10 min. Viable adherent cells were stained with MTT (2 μg/ml) for 4 h. Media was then removed and the formazan crystals produced were dissolved in DMSO (200 μl). Absorbance was tested at 540 nm. The cytotoxicity of PEMΦ was tested by MTT colorimetry. One-way analysis of variance was applied for all statistical analyses by independent experiments. Individual values comparing by t-test and a p-value <0.01 were considered as significant.

**Antibiotic Assay** Antimicrobial and antifungal bioassays were conducted in triplicate by following the method in document and the National Center for Clinical Laboratory Standards (NCCLS) recommendations.19

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**References**