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Lobophytones A−G, New Isobiscembranoids from the Soft Coral *Lobophytum pauciflorum*

Pengcheng Yan,[†] Yang Lv,[‡] Leen van Ofwegen,[§] Peter Proksch,[||] and Wenhan Lin*,[†]

State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing 100083, China, Analytical and Testing Center, Institute of Materia Medica, CAMS, Beijing, 100050, China, National Museum of Natural History Naturalis, 2300 RA Leiden, The Netherlands, and Institute of Pharmaceutical Biology and Biotechnology, Heinrich-Heine University, 40225 Duesseldorf, Germany

whlin@bjmu.edu.cn

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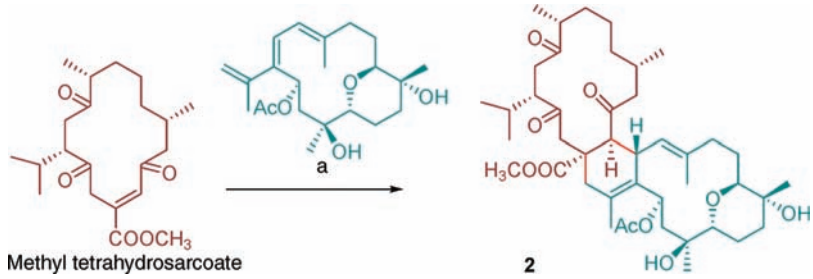
ABSTRACT



Seven new biscembranoids, lobophytones A−G (1−7), together with three known biscembranes were isolated from the Chinese soft coral *Lobophytum pauciflorum*. Their structures were elucidated by the analysis of 1D and 2D NMR (COSY, HSQC, HMBC, and NOESY) data in association with IR and MS experiments. The absolute configurations of compound 1 were determined by X-ray diffraction using the Flack parameter. Lobophytones A−G differ from the "normal" biscembranoids due to the antipodal Diels−Alder cycloaddition between cembranoid−diene and cembranoid−dienophile. The biogenetic pathway of the isolated compounds is depicted. Compound 4 showed significant inhibition toward LPS-induced nitric oxide (NO) in mouse peritoneal macrophage with IC_{50} = 4.70 μM.

Biscembranoids are a family of unusual marine tetraterpenoids derived from the Diels−Alder cycloaddition between cembranoid−diene and cembranoid−dienophile.[1] Since the first biscembranoid named methyl sartortuoate was reported from the Chinese soft coral *Sarcophyton tortuosum*,[2] more than 31 biscembranoids have been isolated to date, which are exclusively obtained from the genus *Sarcophyton* (*S. tortuosum*, *S. glaucum*, *S. latum*, and *S. elegans*). It is noted that the diverse biscembranoids vary mainly in the oxygenation and cyclization of ring C but maintain the basic structure of ring A. Soft corals of the genus *Lobophytum* are a family with more than 20 species present in tropical and subtropical waters. Previous chemical investigation of this genus afforded numerous structurally diverse cembranoid diterpenes. However, no biscembranoids have been found from this genus. As part of our study on the chemical diversity from soft corals in the South China Sea, the species *Lobophytum pauciflorum*, collected from Sanya Bay of Hainan Island, was examined chemically.

[†] Peking University.
[‡] Institute of Materia Medica.
[§] National Museum of Natural History Naturalis.
[||] Heinrich-Heine University.

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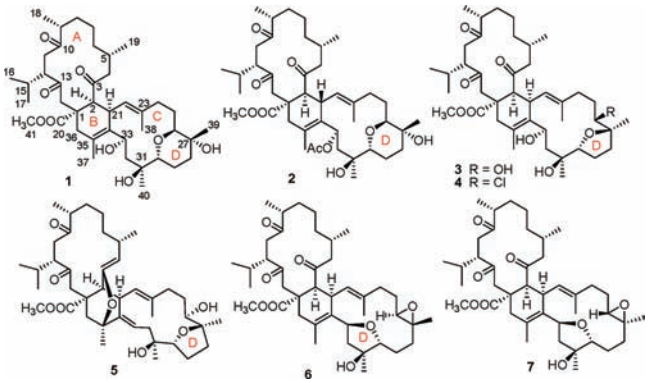
Repeated column chromatography followed by reversed-phase HPLC of the EtOAc-soluble fraction from the ethanol extract of this specimen led to the isolation of compounds **1**−**7**.



Lobophytone A (**1**), obtained as colorless crystals, had a molecular formula of $C_{41}H_{64}O_9$ as determined by HRESIMS *m*/*z* 723.4462 $[M + Na]^+$ and NMR data, indicating 10 degrees of unsaturation. The ^{13}C NMR and DEPT spectra of **1** presented a total of 41 carbon resonances, including a carbonyl carbon (δ_C 175.1), three ketones (δ_C 211.1, 211.9, and 214.8), four olefinic carbons (δ_C 125.0 (CH), 126.4 (qC), 130.5 (qC), and 138.6 (qC), and six oxygenated carbons (δ_C 68.8 (qC), 69.9 (CH), 70.0 (CH), 74.2 (qC), and 84.2 (CH)). The ^1H NMR spectrum showed eight methyl signals that were attributed to two vinyl methyl singlets at δ_H 1.87 (s, H_3-37) and 1.72 (s, H3−38) and four methyl doublets at δ_H 0.82 (d, J = 6.9 Hz, H_3-17), 0.80 (d, J = 6.9 Hz, H_3-16), 1.13 (d, J = 6.9 Hz, H_3-18), and 0.81 (d, J = 6.9 Hz, H_3-19), in addition to two methyl singlets at δ_H 1.00 (s, H_3-39) and 0.98 (s, H_3-40). The HMQC spectrum assigned all protons and the corresponding carbons in the molecule. These NMR data (Supporting Information, Table 1) are characteristic of a biscembranoid, closely related to those reported in the literature.[4,5] Analysis of 2D NMR spectroscopic data established a structure of the 14,6,14-membered tricyclic backbone, and ring A contained three ketone groups that were located at C-3, C-10, and C-13, while C-5, C-9, and C-12 were positioned by methyl groups and an isopropyl group, respectively. The COSY correlations established the segments from C-24 to C-26, C-28 to C-30, and C-32 to C-33 in ring C, and C-2 to C-22 via C-21. A tetrahydropyran ring located between C-26 and C-30 was evident from the HMBC interactions from H-26 (δ_H 3.49, brd, J = 7.3 H_Z) to C-30 (δ_C 70.0) and, in turn, from H-30 (δ_H 3.44, brd, J = 11.0 HZ) to C-26 (δ_C 84.2). In addition, C-27, C-31, and C-33 were hydroxylated on the basis of the HMBC cross-peaks observed from the methyl protons H_3-39 and H_3-40 and the hydroxy protons OH-27 (δ_H 4.00, s), OH-31 (δ_H 5.09, s), and OH-33 (δ_H 5.55, br) through two to three bonds to the carbons in the backbone. The substructures of rings A and C were closely related to that of desacetylnyalolide.[1] However, the COSY cross-peaks revealed that H-21 (δ_H 3.29, dd, J = 7.3, 10.9 H_Z) correlated to H-22 (δ_H 4.75, d, J = 10.9 H_Z) and H-2 (δ_H 3.42, d, J = 7.3 H_Z), indicating that C-2 is a methine group rather than a quaternary carbon as assigned to methyl sartortuoate, which co-occurred in the specimen. Thus, C-2 may not be substituted by a methyl ester. The presence of the HMBC correlations from H-2 and the methoxy protons (δ_H 3.53, s) to the carbonyl carbon C-20 (δ_C 175.1) and absence of the HMBC relationship between H-21 and C-20 confirmed the methyl ester to be positioned at C-1 instead of C-2. The HMBC correlations from the methylene protons H_2-14 (δ_H 2.65, 2.93) to C-1 (δ_C 44.6, qC), C-2 (δ_C 52.1, CH), and C-20 and from H_3-37 (δ_H 1.87) to C-34 (δ_C 130.5), C-35 (δ_C 126.4), and C-36 (δ_C 40.0) provided the additional data to support the location of the methyl ester and a double bond at C-34/C-35.

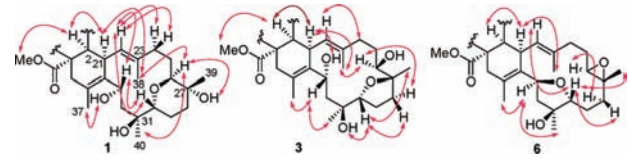


Figure 1. Key NOE correlations of compounds **1**, **3**, and **6**.

The relative stereochemistry of **1** was established by NOESY spectroscopic analysis (Figure 1), while the absolute stereochemistry was determined by the X-ray diffraction of the single crystal using the Flack parameter (Figure 2).[6] Thus, the absolute configurations of **1** were unambiguously determined as 1*R*,2*R*,5*R*,9*R*,12*R*,21*S*,26*S*,27*R*,30*R*,31*S*,33*R*.

The NMR data of lobophytone B (**2**) (Supporting Information, Table 2) were compatible with those of **1**, indicating an analogue. The difference was found by the presence of an additional acetyl group, as evident from its molecular weight being 42 amu higher than that of **1** as determined by HRESIMS (*m*/*z* 765.4540 $[M + Na]^+$) and the NMR data for an acetyl group (δ_H 1.87, s; δ_C 21.3, 170.5). Interpretation of 1D and 2D NMR spectroscopic data confirmed that **2** shared the main structural part of **1**, with the exception of C-33 which was substituted by an acetyl group, as evident

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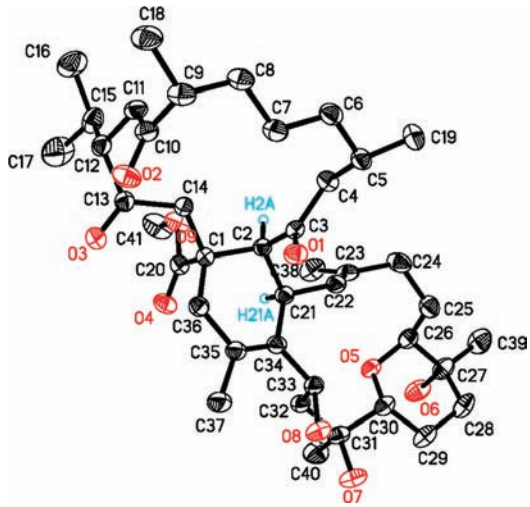

Figure 2. Single-crystal X-ray structure of **1**.

from the HMBC correlation between H-33 (δ_H 4.76, dd, J = 2.0, 10.3 Hz) and the acetyl carbonyl carbon. In addition, the coupling constant $J_{H\text{-}21/H\text{-}2}$ (10.0 Hz) of **2** was 2.7 Hz larger than that of **1** (7.3 Hz), suggesting H-21 (δ_H 3.59) and H-2 (δ_H 2.69) of **2** were oriented in the *trans*-axial configuration. The obvious NOE relationships between H-21/H-14b (δ_H 2.32), H-21/H_3-38 (δ_H 1.69), and H-2/H-22 (δ_H 4.62) led to the assignment of H-21β, which induced the rotation of **1** to the negative phase ($[\alpha]^{25}_D$ −90.0) in contrast to that of **1**. The configurations of the remaining stereogenic centers in ring C of **2** were the same as that of **1** based on the similar NOE relationships.

Lobophytone C (**3**) had the same molecular formula as that of **1**, as determined by HRESIMS and NMR data. The NMR spectroscopic data of rings A and B were closely similar to those of **1**, and those of ring C were related to the data reported for ximaolide C,[3] a biscembrane isolated from *Sarcophyton tortuosum*. A tertahydrofuran ring bonded to C-27 and C-30 was recognized from the significant downfield shifted C-27 (δ_C 85.1) and C-30 (δ_C 89.1) in addition to the HMBC correlation between H-30 (δ_H 3.74, dd, J = 5.4, 11.0 Hz) and C-27. The COSY correlations between H-33 (δ_H 4.68)/OH-33 (δ_H 3.97) and H-26 (δ_H 3.02)/OH-26 (δ_H 4.50), as well as the HMBC correlations from OH-31 (δ_H 4.00) to C-30, C-31 (δ_C 73.7), and C-32 (δ_C 42.0), thus assigned C-26, C-31, and C-33 of **3** to be hydroxylated. Accordingly, the structure of **3** differed from that of ximaolide C due to a hydroxyl substitution at C-31 to replace a chlorine atom of the latter compound.

The relative configurations of rings A and B were identical to those of **1** as determined by the similar NOE interactions and the NMR data. The NOE cross-peaks between H-2 (δ_H 4.04)/H-21 (δ_H 3.79), H-21/H_3-38 (δ_H 1.72), H_3-38/H-26, and H-26/H-28a (δ_H 2.21) suggested α-orientation of H-2, H-21, and H-26, whereas the correlations of H-33 (δ_H 4.68)/H_3-37 (δ_H 1.63), H-33/H_3-40 (δ_H 0.95), H-30/OH-31, H-30/H_3-39 (δ_H 0.99), H-30/H-28b (δ_H 1.36), and OH-31/H-21 (δ_H 3.79) indicated H-30, H-33, and OH-31 to be β-oriented. Compound **3** is assumed to be derived from **1** through the rearrangement of the epoxy position from C-26 to C-27.

Lobophytone D (**4**) had the molecular formula of $C_{41}H_{63}O_8Cl$ as determined by the HRESIMS ion at m/z 741.4112 $[M + Na]^+$, indicating the presence of a chlorine atom and 10 degrees of unsaturation. The NMR spectroscopic data (Supporting Information, Table 4) revealed that the structure of **4** was closely related to **3**, except for the presence of a chlorine atom and absence of a hydroxy group. The NMR data of **4** differed from those of **3** by C-26, which shifted to the upfield region at δ_C 65.4 instead of δ_C 70.8 of the latter compound, thus indicating the location of the chlorine atom at C-26. The presence of COSY correlation between H-33 (δ_H 4.65)/OH-33 (δ_H 4.22) and the HMBC correlations from OH-31 (δ_H 4.20) to C-30 (δ_C 89.9), C-31 (δ_C 73.7), and C-40 (δ_C 23.7) confirmed the assignment. The relative configurations of **4** were determined to be the same as that of **3** based on their similar NMR data and NOE relationships.

The molecular formula of lobophytone E (**5**) was determined to be $C_{41}H_{62}O_8$ by HRESIMS (m/z 683.4516 $[M + H]^+$), indicating 11 degrees of unsaturation. Its ^1H and ^{13}C NMR data were almost identical to those of ximaolide D,[3] implying that both compounds should be identical. However, the COSY spectrum showed that H-21 (δ_H 2.82, dd, J = 2.7, 10.3 H_Z) correlated to H-22 (δ_H 4.81, d, J = 10.3 H_Z) and H-2 (δ_H 1.95, d, J = 2.7 H_Z), and the HMBC relationship between H-22 and C-2 (δ_C 47.8, CH) indicated C-2 to be a methine group rather than a quaternary carbon as described in ximaolide D. Thus, compound **5** must belong to an isobiscembrane skeleton related to **1**. Additional evidence was found by the presence of the isolated geminal protons H_2-36 (δ_H 2.48, d, J = 14.4 H_Z; 1.23, d, J = 14.4 H_Z) and their HMBC interactions with C-20 (δ_C 175.2), C-14 (δ_C 48.2, CH_2), C-2, C-37 (δ_C 22.9, CH_3), and C-34 (δ_C 140.1, qC). Moreover, the HMBC relationships from H-21 to C-3 (δ_C 148.8, qC) and C-35 (δ_C 76.8, qC) and from H-4 (δ_H 3.86, brd, J = 10.0 Hz) to C-2 indicated an ether bridge crossed from C-3 to C-35. The rest of the structure in **5** was in agreement with that of ximaolide D, as evident from the 1D and 2D NMR spectroscopic data analysis. The relative configurations of rings A and C were the same as those of ximaolide D due to the similar NOE relationship and NMR data for the corresponding moieties. The NOE cross-peak between MeO and H-2 confirmed a *cis*-junction of rings A and B. Since the methyl ester at C-1 was biogenetically considered to be α-oriented, the C-3/C-35 ether bond was assumed to be β-oriented. Finally, the α-orientation of H-21 was determined on the basis of the NOE correlation between H-2 and H-21.

The molecular formula of lobophytone F (**6**) was determined as $C_{41}H_{62}O_8$ by the HRESIMS ion at m/z 705.4365 $[M + Na]^+$, implying 11 degrees of unsaturation. The NMR data of **6** indicated that it has the same partial structure in regard to rings A and B as that of **1**, while rings C and D

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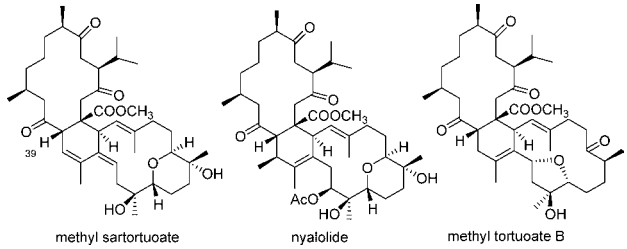
were related to that of methyl isosartortuoate.[5h] Analysis of 2D NMR data revealed that **6** has an epoxy group to be located at C-26/C-27, but it lacked a ketone group at C-26 as in the case of the known analogue. This was evident from the HMBC correlations from H_3-39 (δ_H 1.13, s) to C-26 (δ_C 61.5, CH) and C-27 (δ_C 59.0, qC). Ring C containing a tetrahydrofuran ring which was located between C-30/C-33 was based on the HMBC correlation from H-30 (δ_H 3.22, brd, J = 11.7 Hz) to C-33 (δ_C 74.8). The relative configurations were determined by NOESY analysis. The presence of NOE cross-peaks between H-2 (δ_H 3.65)/H-21 (δ_H 3.55) and H-21/H_3-38 (δ_H 1.72) was in agreement with an α-orientation of H-2 and H-21 and 22*E* geometry. Additional NOE relationships between H-33 (δ_H 4.80)/H_3-40 (δ_H 1.09) and H-33/H-21 were assigned to the α-orientation for H-33 and H_3-40, while the NOE correlations of H-30 (δ_H 3.22)/H_3-39 (δ_H 1.13) and H-30/H-22 (δ_H 4.84) led to the assignment of H-26β and H_3-39β. The presence of NOE cross-peaks between H-26/H-28b (δ_H 0.93) and H_3-39/H-25b (δ_H 1.45), in association with the absence of NOE relationship of H_3-39/H-26, indicated *E*-geometry of the epoxy group.



Lobophytone G (**7**) had the same molecular formula as that of **6** as determined by HRESIMS and NMR data. It was determined to be a stereoisomer of **6** based on the extensive 2D NMR spectroscopic analysis and by comparison of its NMR data with those of **6**. The difference was found by the ^{13}C NMR values of C-26 (δ_C 64.5, CH) and C-27 (δ_C 61.1, qC) that were slightly downfield shifted in comparison with those of **6**. The NOE relationships of H-2 (δ_H 3.59, d, J = 7.0 Hz), H-21 (δ_H 3.57, dd, J = 7.0, 8.9 Hz), and H-33 (δ_H 4.60, dd, J = 7.3, 11.3 Hz) of **7** were similar to those of **6**, indicating the same orientations of these protons. In addition, the presence of NOE cross-peaks between H-30 (δ_H 3.96)/H-22 (δ_H 5.15), H-30/H-26 (δ_H 2.81), and H_3-39 (δ_H 1.19)/H_3-38 (δ_H 1.63) and absence of the H-30/H_3-39 relationship suggested the *E*-geometry of the epoxy group with β-oriented H-26.

Thus far, all biscembranoids reported in the literature featured a methyl ester at C-2. The present study reports a new Diels−Alder reaction between cembranoid−diene and cembranoid−dienophile. Since the diene **a** in Figure 3 was also isolated from this specimen, the biogenetic pathway to derive **2** is depicted. It is noted that the "normal" biscembranoids, methyl sartortuoate, nyalolide, and methyl tortuoate B, were also isolated from the same fraction; thus, the pathways of the "normal" and "iso" Diels−Alder cycloaddition co-occurred in this species. The reported biscembranes were exclusively found as the COMe *endo* adducts, and the mechanism has been discussed in the literature.[7] However, the "iso" biscembranes were dominated by the CO *endo* approach with the exception of compound **2** (Figure 4).

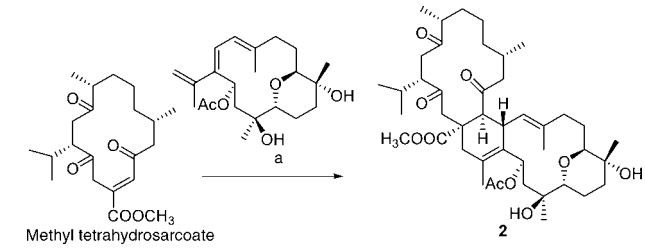


Figure 3. Probable Diels−Alder cycloaddition leading to **1** and **2**.

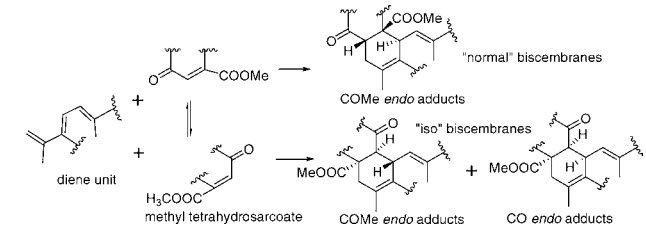


Figure 4. Possible Diels−Alder reaction to derive "normal" and "iso" biscembranes.

Compound **4** showed significantly inhibition toward lipopolysaccharide (LPS)-induced nitric oxide (NO) in mouse peritoneal macrophage with IC_{50} = 4.70 μM, while the rest of the compounds showed weak inhibition (IC_{50} > 10 μM).

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Supporting Information Available: Experimental details; ^{1}H and ^{13}C NMR data, 1D and 2D NMR, EIMS, and IR spectra of **1**−**7**; X-ray crystallographic data for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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