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Two new biscembranes with unprecedented carbon skeleton and their probable biogenetic precursor from the Hainan soft coral *Sarcophyton latum*

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Abstract—A new cembranolide diterpene, isosarcophytonolide D (**1**), and two biscembranes with an unprecedented fused carbon skeleton, bislatumlides A (**3**) and B (**4**), were isolated from the Hainan soft coral *Sarcophyton latum*. Their structures and relative stereochemistry were established by 1D and 2D NMR spectroscopic techniques. Compounds **3** and **4** should be obtained by Diels–Alder addition of two cembranoid units. Compound **1** could be one of the biosynthetic precursors. Both **3** and **4** showed mild cytotoxicity toward A549, HT-29, and P388 cell lines.

Marine organisms, comprising more than half of the total global biodiversity, offer an enormous source of novel and biologically active compounds. The soft corals (phylum Cnidaria, class Anthozoa, subclass Octocorallia, order Alcyonacea) have been proven to be a storehouse of terpenoids. Nearly 30 species of soft corals of the genus *Sarcophyton*, from different geographical areas, have been chemically examined. They contain an impressive series of cembranoid diterpenes.[1] In particular, the rare and unusual biscembranoids were only isolated from this genus. The first biscembrane, methyl sartortuoate, was reported from the Chinese soft coral *Sarcophyton tortuosum*. by Su.[2] To date, 12 biscembranoids have been discovered from two species of genus *Sarcophyton* (*S. tortuosum* and *S. glaucum*).[3–8] The occurrence of biscembranoids can be justified by Diels–Alder addition of two different cembranoid units. However, until now, all reported biscembranoids exhibited the coupling between the 1(2) double bond activated by a 20-carboxymethyl group and a conjugated trisubstituted 21(34), 35(36)-butadiene moiety (according to the numbering assigned to compounds **5** and **6**).

The soft corals of genus *Sarcophyton* are prolific in the South China Sea. In the course of our search for biologically active substances from Chinese marine organisms, we chemically examined *Sarcophyton latum* collected off Ximao Island, Hainan Province, China. Four new cembranolides, named sarcophytonolides E–H,[9] were isolated. Recently, this animal was re-collected off the coast of Sanya, China. Chemical investigation of the ethyl ether-soluble fraction from the acetone extract of this organism led to the isolation of a new cembranolide diterpene, isosarcophytonolide D (**1**), which was isomeric with the previously reported sarcophytonolide D (**2**),[10] along with two biscembrane-type tetraterpenoids, bislatumlides A (**3**) and B (**4**), exhibiting an unprecedented fused carbon skeleton. The present Letter deals with the isolation and structural elucidation of these new compounds.

The workup for the extraction and isolation of cembranolides was basically performed as previously reported.[9] This common procedure yielded three new compounds named isosarcophytonolide D (**1**, 38 mg), bislatumlides A (**3**, 10.2 mg), and B (**4**, 9.8 mg), respectively.

Isosarcophytonolide-D (**1**)[11] was obtained as an UV-absorbing [λ_{max} 231 nm, log ε 4.11] colorless oil. Its molecular formula, $C_{22}H_{30}O_5$, was deduced from its

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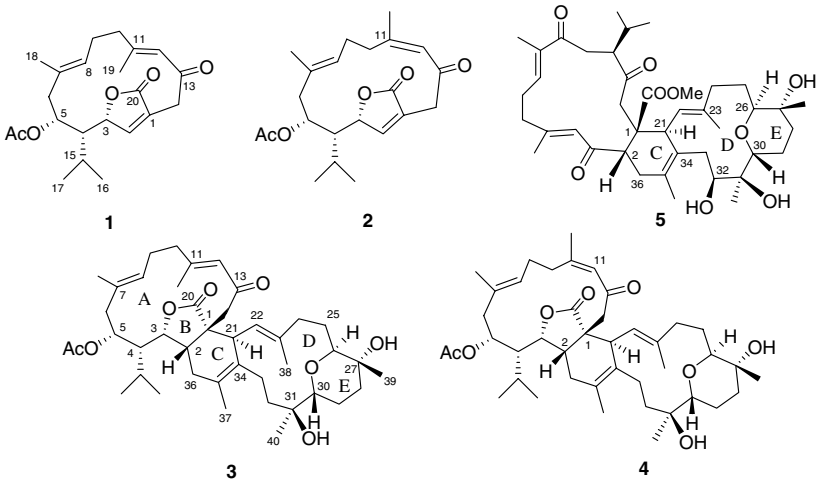


HREIMS {*m*/*z* 374.2080 $[M]^+$, calcd for $C_{22}H_{30}O_5$, 374.2094}. A comparison of overall ^1H and ^{13}C NMR data[10,11] revealed great similarities between compound **1** and sarcophytonolide-D (**2**), which was previously isolated from Hainan soft coral *S. tortuosum*.[10] Both **1** and **2** possess the same α,β-unsaturated γ-lactone system, an acetoxyl group at C-5 and an isopropyl group at C-4. In fact, the ^1H and ^{13}C NMR data[10,11] of **1** and **2** were almost identical except for those of C-10, C-12, and C-19. These differences could be easily justified by the different geometry of the double bond at $\Delta^{11(12)}$ (*E* for **1** and *Z* for **2**). The Δ^{11} *E* configuration in **1** was confirmed by the upfield shifted ^{13}C NMR resonances of C-19 (from δ_C 24.8 in **2** to δ_C 18.8 in **1**) and supported by the strong NOE correlations of H-12/H_2-10. The relative stereochemistries at C-3, C-4, and C-5 were assigned the same as those of **2** through both comparison of ^{13}C NMR data of compound **1** with those of **2**,[10,11] showing almost identical chemical shift values for C-1–C-5, and interpretation of its NOESY spectrum. Compound **1** was therefore established as Δ^{11} isomer of **2** and named isosarcophytonolide-D.

Bislatumlide A (**3**)[12] was isolated as an UV-absorbing [λ_{max} 242 nm, log ε 4.17] amorphous white powder and exhibited the $[M+H]^+$ ion at *m*/*z* 695.4581 (calcd 695.4523), corresponding to the molecular formula $C_{42}H_{63}O_8$. The IR spectrum showed the absorptions indicative of hydroxyl groups (3565 cm^{-1}), ester carbonyls (1763 and 1736 cm^{-1}), and conjugated carbonyl (1678 cm^{-1}). In the ^1H NMR spectrum, resonances due to eight methyls, including two methyls of an isopropyl group (δ 1.08, 1.09, each 3H, d, $J = 7.2$ Hz, δ 2.14, 1H, m), four vinyl methyls (δ 1.69, 2.12, 1.70, 1.76, each 3H, br s), and two methyls linked to hydroxyl-bearing carbons (δ 1.12, 1.13 each 3H, s), were observed as well as those of three carbonyls (δ 198.2, 178.1, 170.5) in the ^{13}C NMR spectrum (Table 1). These data implied that bislatumlide A was a biscembranoid, such as bisglaucumlides A–D,[8] which have been recently isolated from the same genus, *Sarcophyton*. The gross structure of **3** was deduced to be as shown in Figure 1 by detailed interpretation of the NMR spectra, including ^1H–^1H COSY, HMQC, and HMBC spectra (Table 1) and by comparison of the NMR data with those of related compounds. In particular, the resonances in the NMR spectrum due to the rings A and B (left half moiety of the molecule, **X**) were very similar to those of the co-occurring **1**, while the right half moiety **Y** (rings C–E) was reminiscent of those of bisglaucumlide A (**5**).

For the partial structure **X** (Fig. 1), HMBC correlations clearly suggested the presence of an α,β-unsaturated γ-lactone moiety at C-1–C-3, C-20, an isopropyl at C-4, an acetoxyl at C-5, a conjugated enone moiety at C-11–C-13, and two trisubstituted double bonds at Δ^7 and Δ^{11}, respectively, which were the same as that of co-occurring isosarcophytonolide A (**1**).

For the partial structure **Y** (Fig. 1), the segment from C-21 to C-31 could be readily identified by both the extensive interpretation of its ^1H–^1H COSY, HMQC, HMBC spectra and by the comparison of ^1H and ^{13}C NMR data of C-21–C-31 with those of the corresponding part of model compound **5**.[8] In addition, in a similar manner, the fragment C-34–C-36/C-37, the same as those of **5**, was also easily recognized. In fact, **3** differs from **5** only by the lack of hydroxyl group at C-32. Due to the dehydroxyl effect, the ^{13}C NMR signal of C-32 was apparently upfield shifted (from δ 70.8 in **5** to 36.9 in **3**). Moreover, due to the absence of γ-gauche effect caused by the C-32 hydroxyl group, the ^{13}C NMR signals of C-34 and C-40 of **3** were reasonably downfield shifted with respect to those of **5** (from δ 125.2 and 18.8 in **5** to 135.6 and 23.1 in **3**, respectively) giving further support for the lack of C-32 hydroxyl group in **3**.

Finally, the partial structures **X** and **Y** were connected together to form ring C by the linkages from C-1 to C-21 and C-2 to C-36 by the HMBC correlations of H_2-14/C-21, H-3/C-36, H-2/C-35, and H-21/C-1 and C-20, and ^1H–^1H COSY correlations of H-2 and H_2-36 according to the planar structure of **3**.

The relative stereochemistry for both olefins and chiral carbons of **3** was established by extensive interpretation of NOESY spectrum (Fig. 2), as well as by comparison of ^1H and ^{13}C NMR data of **3** with those of model compounds **1** and **5**. In fact, a substantial similarity in both

Table 1. NMR data of compounds **3** and **4** in $CDCl_3$ recorded at 400/100 MHz

No.	**3** δ_H (mult., *J*, Hz)	δ_C	**4** δ_H (mult., *J*, Hz)	δ_C
1	—	51.9, s	—	50.9, s
2	2.39 (m)	44.2, d	2.13 (m)	43.7, d
3	3.68 (dd, 11.2, 4.8)	81.7, d	3.72 (dd, 11.2, 4.7)	82.1, d
4	1.45 (br d, 11.2)	48.7, d	1.89 (m)	46.1, d
5	4.86 (dd, 10.4, 3.6)	73.3, d	4.86 (dd, 10.4, 3.6)	72.1, d
6α	2.30 (m)	41.1, t	2.30 (m)	41.5, t
6β	2.30 (m)		2.13 (m)	
7	—	132.5, s	—	131.8, s
8	5.10 (t, 6.8)	126.7, d	5.16 (t, 7.6)	127.8, d
9	2.34 (m)	24.7, t	2.30 (m)	25.3, t
10	2.30 (m)	40.2, t	3.10 (m)	31.1, t
11	—	160.7, s	—	161.9, s
12	5.91 (s)	126.3, d	6.00 (s)	124.9, d
13	—	198.2, s	—	198.7, s
14α	2.58 (d, 12.6)	48.9, t	2.38 (d, 15.1)	52.4, t
14β	2.68 (d, 12.6)		3.04 (d, 15.1)	
15	2.19 (m)	25.9, d	2.20 (m)	25.7, d
16	1.09 (d, 7.2)	18.0, q	1.09 (d, 7.2)	18.3, q
17	1.08 (d. 7.2)	25.1, q	1.17 (d. 7.2)	24.7, q
18	1.69 (s)	17.9, q	1.67 (s)	17.1, q
19	2.12 (s)	19.4, q	1.89 (s)	24.9, q
20	—	178.1, s	—	178.9, s
21	3.09 (br d, 10.3)	43.9, d	2.85 (d, 10.3)	45.6, d
22	6.15 (d, 10.3)	121.3, d	5.96 (d, 10.3)	121.1, d
23	—	140.3, s	—	139.6, s
24α	2.69 (br d, 13.4)	38.4, t	2.65 (d, 13.4),	38.3, t
24β	1.78 (dd, 13.4, 2.8)		1.76 (m)	
25α	1.56 (m)	26.1, t	1.55 (m)	26.0, t
25β	2.17 (m)		2.20 (m)	
26	3.57 (d, 10.0)	85.6, d	3.55 (d, 10.0)	85.6, d
27	—	69.9, s	—	69.8, s
28α	1.66 (dd, 12.1, 2.9)	31.5, t	1.64 (m)	31.5, t
28β	1.56 (m)		1.53 (m)	
29α	1.59 (m)	20.0, t	1.55 (m)	20.0, t
29β	1.76 (m)		1.67 (m)	
30	3.67 (br d, 11.2)	68.1, d	3.63 (br d, 10.8)	68.8, d
31	—	73.8, s	—	73.8, s
32α	1.11 (m)	36.9, t	1.13 (m)	37.1, t
32β	1.60 (m)		1.59 (m)	
33α	2.19 (m)	22.6, t	2.21 (m)	22.6, t
33β	1.92 (m)		1.89 (m)	
34	—	135.6, s	—	134.5, s
35	—	127.8, s	—	128.9, s
36α	2.20 (m)	34.3, t	2.19 (m)	33.9, t
36β	1.84 (m)		1.78 (m)	
37	1.70 (s)	20.2, q	1.72 (s)	20.4, q
38	1.76 (s)	19.6, q	1.70 (s)	19.5, q
39	1.12 (s)	25.4, q	1.11 (s)	25.4, q
40	1.13 (s)	23.1, q	1.13 (s)	23.1, q
41	—	170.5, s	—	170.5, s
42	2.08 (s)	21.3, q	2.06 (s)	21.2, q

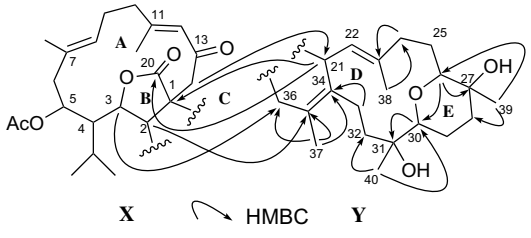


Figure 1. Partial structures **X**, **Y** of bislatumlide A (**3**).

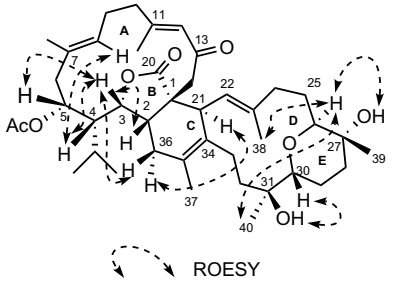
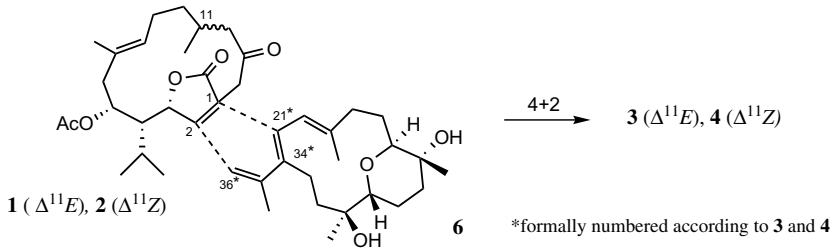


Figure 2. Selected key NOESY correlations for **3**.

carbon and proton values of segments C-1–C-14, C-20 between **3** and **1**; C-23–C31 between **3** and **5** was observed, whereas significant differences were detected for C-21 and C-22 according to the different orientation of the carboxyl group at C-1. Thus the structure of bislatumlide A was proposed as depicted in **3**.

Bislatumlide B (**4**)[13] was also isolated as an UV-absorbing [λ_{max} 240 nm, log ε 3.84] white powder. Its molecular formula, $C_{42}H_{62}O_8$, deduced from its HRESIMS {*m*/*z* 695.4553 $[M+H]^+$} was the same as **3**. Detailed analysis of 1D and 2D NMR spectra revealed that the gross structure of **4** was identical with that of **3**. However, careful comparison of their ^{13}C NMR data revealed that the chemical shifts of C-19 and C-10 of **4** were significantly different from those of **3**. In particular, the ^{13}C NMR signal of C-19 was downfield shifted (from δ 24.9 to 19.4) indicating the *Z* nature of Δ^{11} olefin in **4**. The 11*Z* geometry was further confirmed by strong NOE correlations of H-12/H_3-19, and the smaller ε value of unsaturated ketone UV-absorbing of **4** than that of **3**. Thus, compound **4** was the 11*Z* isomer of **3** and should be formally obtained by coupling compound **2** with an undetected cembranoid displaying a diene moiety as that depicted in formula **6**.

A plausible biosynthetic pathway for compounds **3** and **4** is proposed in Scheme 1. It is obvious that **1** was one of the two precursors of **3**. Another precursor should be compound **6**. Diels–Alder coupling of **1** and **6** should lead to biscembrane **3**. The possible coupling should occur between 1*Z* double bond of **1** and $\Delta^{21(34),35(36)}$ diene of **6**. Therefore, the carbon framework of **3** is different from that of all previously described biscembranes. In fact, until now, no paper has reported cembranoid dimers involving the coupling with a lactone ring. In addition, almost all the previous cembrane dimers exhibited the reactive olefinic double bond at positions 1,14 (according to the numbering assigned to compound **1**), whereas the conjugated double bond in the lactone ring of compound **1** was between the carbons C-1 and C-2. The Diels–Alder addition which arises from supra–supra transition state explains the trans stereochemistry of H-2 and lactone as well as the cis geometry of lactone and H-21. Analogously, the formation of biscembrane **4** by Diels–Alder coupling of the 11*Z* isomer of **1**, sarcophytonolide A (**2**),[10] with compound **6** could be hypothesized. However, an acid-catalyzed isomerization of **3** into **4** should also be taken into consideration. In fact, we did observe that compound **3**



Scheme 1. Plausible Diels–Alder reaction leading to compounds **3** and **4**.

could slowly transform into **4** by isomerization of the Δ^{11} double bond when it was stored in $CDCl_3$.

Bislatumlides A (**3**) and B (**4**) exhibited mild cytotoxicity toward several cell lines. IC_{50} values of 7 μg/mL were determined in assays against A-549 lung carcinoma and HT-29 colon adenocarcinoma human tumor cell lines, and 5.8 μg/mL against the P388 murine lymphocytic leukemia cell line.

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11. Isosarcophytonolide A (**1**): colorless oil, $[\alpha]_D^{20}$ −66 (*c* 0.67, $CHCl_3$); IR ν_{max} (film) cm^{-1}: 2958.3, 1760.7, 1737.6, 1686.0, 1615.0, 1443.0, 1379.4, 1241.1, 1080.4, 1020.6, 970.0, 729.0; LC-ESIMS: 397 $[M+Na]^+$, LREIMS, *m/z*: 374 (M^+), 314, 296, 271, 218, 175, 161, 135; HREIMS *m/z*: 374.2080 ($C_{22}H_{30}O_5$, calcd 374.2094); ^1H NMR ($CDCl_3$, 500 MHz): 7.49 (s, H-2), 5.02 (d, $J = 10.7$ Hz, H-3), 1.47 (d, $J = 10.7$ Hz, H-4), 4.95 (m, H-5), 2.30 (m, H-6β), 2.14 (m, H-6α), 4.95 (m, H-8), 2.29 (m, H-9), 2.29 (m, H-10α), 2.06 (m, H-10β), 5.67 (s, H-12), 3.49 (d, $J = 16.1$ Hz, H-14α), 3.13 (d, $J = 16.1$ Hz, H-14β), 2.20 (m, H-15), 1.13 (d, $J = 7.1$ Hz, H_3-16), 1.12 (d, $J = 7.1$ Hz, H_3-17), 1.58 (s, H_3-18), 2.14 (s, H_3-19), 2.08 (s, $COCH_3$); ^{13}C NMR ($CDCl_3$, 125 MHz): 129.3 (s, C-1), 152.3 (d, C-2), 81.3 (d, C-3), 51.1 (d, C-4), 72.9 (d, C-5), 42.3 (t, C-6), 132.4 (s, C-7), 126.1 (d, C-8), 24.4 (t, C-9), 40.6 (t, C-10), 159.4 (s, C-11), 122.6 (d, C-12), 195.7 (s, C-13), 40.5 (t, C-14), 25.8 (d, C-15), 18.8 (q, C-16), 25.1 (q, C-17), 17.8 (q, C-18), 18.8 (q, C-19), 172.5 (s, C-20), 21.0 (q, $COCH_3$), 170.8 (s, $COCH_3$).
12. Bislatumlide A (**3**): white powder, $[\alpha]_D^{20}$ −15 (*c* 1.06, $CHCl_3$); IR ν_{max} (KBr) cm^{-1}: 3465.5, 2937.1, 1762.6, 1735.6, 1677.8, 1608.4, 1436.7, 1373.1, 1242.0, 1180.2, 1041.4, 754.0; UV λ_{max} (MeOH): 246 nm (ε 14,626); LC–MS: *m/z* 695 $[M+H]^+$, HRESIMS *m/z*: 695.4581 ($C_{22}H_{33}O_5$, calcd 695.4523); ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz): see Table 1.
13. Bislatumlide B (**4**): white powder, $[\alpha]_D^{20}$ −15 (*c* 1.34, $CHCl_3$); IR ν_{max} (KBr) cm^{-1}: 3465.5, 2937.1, 1762.0, 1735.6, 1673.9, 1606.4, 1446.4, 1375.0, 1242.0, 1022.1, 756.0; UV λ_{max} (MeOH): 240 nm (ε 6931); LC–MS: *m/z* 695 $[M+H]^+$, HRESIMS *m/z*: 695.4553 ($C_{22}H_{33}O_5$, calcd 695.4523); ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz): see Table 1.