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Lobophytones U–Z_1, Biscembranoids from the Chinese Soft Coral *Lobophytum pauciflorum*

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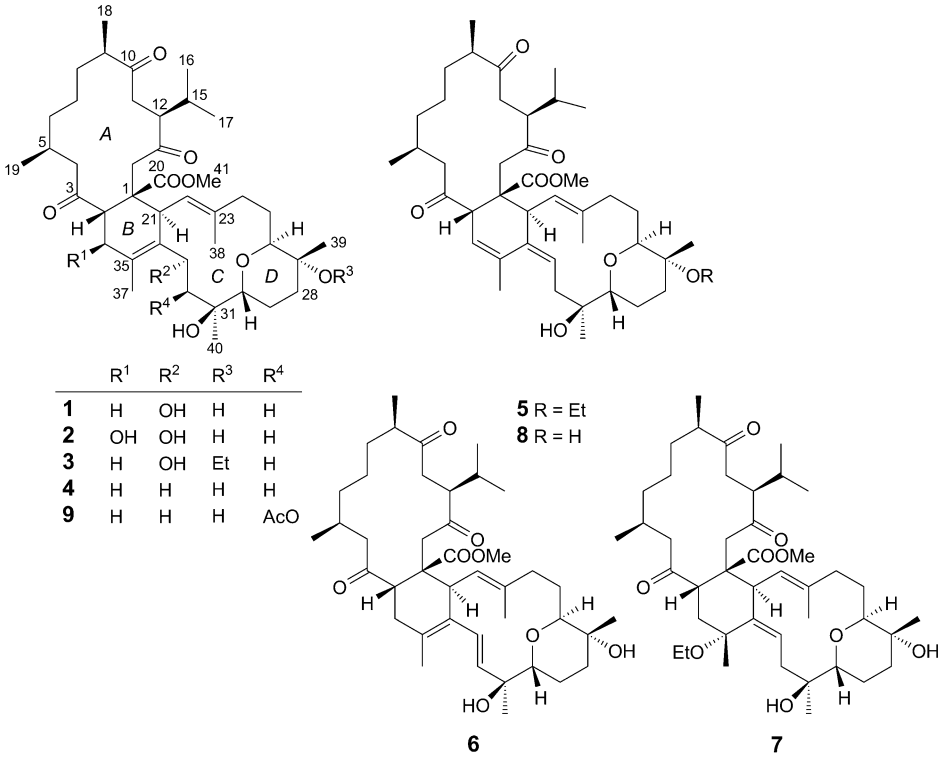
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Chemical examination of a Chinese soft coral *Lobophytum pauciflorum* resulted in the isolation of seven new biscembranoids named lobophytones U–Z_1 (**1**–**7**, resp.), together with methyl sartortuoate (**8**) and nyalolide (**9**). The structures of the new compounds were elucidated by 1D- and 2D-NMR (COSY, HSQC, HMBC, and NOESY) spectroscopic analysis in association with MS and IR data. All compounds were tested against lipopolysaccharide (LPS)-induced nitric oxide (NO) release in mouse peritoneal macrophage. Lobophytone Z (**6**) inhibited NO production with an IC_{50} value of 2.6 μM. Lobophytone H (**1**) showed inhibitory activities against the bacteria *S. aureus* and *S. pneumoniae*.

Introduction. – Biscembranoids are a family of marine natural products with an unusual structure pattern, which is featured by the tetraterpenoids with a 14–6–14-membered tricyclic backbone. The structural variation of biscembranes is frequently found in ring *C*, where the high oxygenation, and tri-, penta-, and hexaepoxy cyclization occurred. The plausible biogenetic pathway is assumed to involve a *Diels–Alder* cycloaddition of two cembranes, cembranoid diene and cembranoid dienophile [1–8]. Methyl sarcoate [3] and methyl tetrahydrosarcoate [7] (methyl tortuosoate) [6] are supposed to be the precursors (dienophiles) to form the left part of biscembranoids. Most biscembranoids have been isolated from coral genus *Sarcophyton* (*S. tortuosum*, *S. glaucum*, *S. latum*, and *S. elegans*), except for a number of isobiscembranoids [9] being isolated from soft coral *Lobophytum pauciflorum* (*Ehrenberg*, 1834; Alcyoniidae). Soft corals of genus *Lobophytum* mainly contain various cembranoids and steroids. Some compounds possess HIV-inhibitory [10], cytotoxic [11–14], and anti-inflammatory activities [15]. In the course of our ongoing investigation of chemical diversity of *L. pauciflorum* from Hainan Island of China, we isolated seven new biscembranoids, **1**–**7**, and two known biscembranes, **8** and **9**, from the AcOEt-soluble fraction of the specimen. Here, we report the structure elucidation of the new compounds and their bioactivities.

Results and Discussion. – Repeated column chromatography, followed by reversed-phase HPLC separation of the AcOEt-soluble fraction of *L. pauciflorum*, yielded nine

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	R^1	R^2	R^3	R^4
1	H	OH	H	H
2	OH	OH	H	H
3	H	OH	Et	H
4	H	H	H	H
9	H	H	H	AcO

biscembranoids. Methyl sartortuoate (**8**) [4] and nyalolide (**9**) [7] were identified on the basis of spectroscopic analysis and comparison with the reported data in literature.

Lobophytone U (**1**) has the molecular formula $C_{41}H_{64}O_9$ as determined by HR-ESI-MS (*m/z* 723.4462 ($[M+Na]^+$)) and NMR data, indicating ten degrees of unsaturation. The ^{1}H-NMR spectrum exhibited resonances for nine Me groups including two olefinic Me (δ(H) 1.58, 1.69) and a MeO group (δ(H) 3.41), while ^{13}C-NMR spectrum presented 41 C-atom resonances including those of three ketones out of four CO C-atoms, four C=C C-atoms, and five O-bearing C-atoms (*Tables 1* and *2*). The ^{1}H- and ^{13}C-NMR data of **1** featured a biscembrane skeleton, closely related to desacetylnyalolide isolated formerly from *Sarcophyton elegans* [7]. Interpretation of COSY and HMBC spectra revealed that **1** differed from desacetylnyalolide due to a OH group at C(33) of **1**, rather than at C(32) of the latter. This assignment was evident from the HMBCs of H–C(33) (δ(H) 4.78, *dd*, $J=3.0$, 9.5) to C(34) (δ(C) 132.8), C(35) (δ(C) 126.2), and C(31) (δ(C) 73.0). A COOMe group at C(1) was determined on the basis of the HMBCs from H-atoms of Me(41) and H–C(21) to CO(20). This group was biogenetically assumed to be β-oriented, as evident from the same group of known biscembranes determined as β exclusively. The NOE relationship between H–C(2) (δ(H) 3.57) and MeO suggested a *cis*-fusion of rings *A* and *B*. The closely similar NMR data of ring *A* of **1** and desacetylnyalolide indicated the same configurations of the stereogenic centers, with respect to C(5), C(9), and C(12). The NOE correlation

Table 1. *^{1}H-NMR Data of Lobophytones U–Z_1* (**1**–**7**, resp.; δ in ppm, (D_6)DMSO)

	1	**2**	**3**	**4**	**5**	**6**	**7**
H–C(2)	3.57 (*t*, *J* = 8.0)	3.44 (*d*, *J* = 7.0)	3.63 (*t*, *J* = 7.8)	3.78–3.64 (*m*)	3.95 (br. *s*)	3.36–3.22 (*m*)	3.53 (*dd*, *J* = 2.7, 13.2)
H_a–C(4)	2.97 (*dd*, *J* = 10.0, 19.6)	3.15 (*dd*, *J* = 10.5, 19.6)	3.00 (*dd*, *J* = 10.3, 19.0)	2.29–2.19 (*m*)	3.02–2.93 (*m*)	2.95 (*dd*, *J* = 9.5, 19.0)	3.02 (*dd*, *J* = 10.5, 19.6)
H_b–C(4)	2.41 (*d*, *J* = 19.6)	2.34 (*d*, *J* = 19.6)	2.42 (*d*, *J* = 19.0)	2.29–2.19 (*m*)	2.53–2.40 (*m*)	2.50 (*d*, *J* = 19.0)	2.38 (*d*, *J* = 19.6)
H–C(5)	1.71–1.59 (*m*)	1.73–1.61 (*m*)	1.69–1.58 (*m*)	1.95–1.80 (*m*)	1.69–1.59 (*m*)	1.75–1.61 (*m*)	1.66–1.52 (*m*)
H_a–C(6)	1.00–0.87 (*m*)	1.00–0.88 (*m*)	0.96–0.77 (*m*)	1.13–0.94 (*m*)	1.00–0.84 (*m*)	1.03–0.91 (*m*)	1.00–0.85 (*m*)
H_b–C(6)	1.10–0.98 (*m*)	1.12–0.95 (*m*)	1.10–0.92 (*m*)	0.84–0.68 (*m*)	0.90–0.76 (*m*)	0.97–0.83 (*m*)	0.99–0.86 (*m*)
H_a–C(7)	1.02–0.88 (*m*)	1.16–0.98 (*m*)	0.94–0.82 (*m*)	0.81–0.67 (*m*)	1.12–0.97 (*m*)	1.05–0.91 (*m*)	0.86–0.67 (*m*)
H_b–C(7)	0.96–0.76 (*m*)	1.08–0.85 (*m*)	1.07–0.90 (*m*)	1.04–0.83 (*m*)	1.14–1.00 (*m*)	1.15–1.10 (*m*)	1.00–0.87 (*m*)
H_a–C(8)	1.38–1.22 (*m*)	1.36–1.21 (*m*)	1.38–1.21 (*m*)	1.80–1.66 (*m*)	1.38–1.21 (*m*)	1.42–1.29 (*m*)	1.48–1.30 (*m*)
H_b–C(8)	1.44–1.28 (*m*)	1.48–1.32 (*m*)	1.44–1.31 (*m*)	1.82–1.67 (*m*)	1.40–1.28 (*m*)	1.40–1.27 (*m*)	1.33–1.25 (*m*)
H–C(9)	2.31–2.17 (*m*)	2.43–2.26 (*m*)	2.30–2.19 (*m*)	2.30–2.18 (*m*)	2.26–2.14 (*m*)	2.40–2,24 (*m*)	2.45–2.28 (*m*)
H_a–C(11)	2.82 (*dd*, *J* = 10.5, 16.6)	2.77 (*dd*, *J* = 10.2, 17.0)	2.82 (*dd*, *J* = 10.3, 16.6)	2.70 (*dd*, *J* = 11.0, 17.6)	2.86–2.70 (*m*)	2.75 (*dd*, *J* = 10.3, 17.1)	2.77 (*dd*, *J* = 9.8, 17.4)
H_b–C(11)	1.91 (*d*, *J* = 16.6)	1.86 (*d*, *J* = 17.0)	1.88 (*d*, *J* = 16.6)	1.88 (*d*, *J* = 17.6)	1.88–1.72 (*m*)	1.98 (*d*, *J* = 17.1)	1.88 (*d*, *J* = 17.4)
H–C(12)	2.88 (br. *d*, *J* = 10.5)	2.90–2.77 (*m*)	2.89–2.76 (*m*)	2.82–2.68 (*m*)	2.86–2.72 (*m*)	2.36–2.15 (*m*)	2.92–2.81 (*m*)
H_a–C(14)	2.96 (*d*, *J* = 19.8)	2.84 (*d*, *J* = 19.5)	2.98 (*d*, *J* = 19.6)	3.18 (*d*, *J* = 19.4)	2.76 (*d*, *J* = 19.2)	2.98 (*d*, *J* = 19.8)	3.19 (*d*, *J* = 18.8)
H_b–C(14)	2.76 (*d*, *J* = 19.8)	2.86 (*d*, *J* = 19.5)	2.74 (*d*, *J* = 19.6)	2.90 (*d*, *J* = 19.4)	2.78 (*d*, *J* = 19.2)	2.88 (*d*, *J* = 19.8)	2.70 (*d*, *J* = 18.8)
H–C(15)	2.20–2.06 (*m*)	2.33–2.16 (*m*)	2.19–2.13 (*m*)	1.85–1.68 (*m*)	2.21–2.07 (*m*)	2.25–2.11 (*m*)	2.38–2.20 (*m*)
H–C(16)	0.95 (*d*, *J* = 6.7)	0.95 (*d*, *J* = 6.9)	0.93 (*d*, *J* = 6.9)	0.87 (*d*, *J* = 6.6)	0.93 (*d*, *J* = 6.9)	0.94 (*d*, *J* = 6.9)	0.95 (*d*, *J* = 6.9)
H–C(17)	0.61 (*d*, *J* = 6.7)	0.61 (*d*, *J* = 6.9)	0.64 (*d*, *J* = 6.9)	0.77 (*d*, *J* = 6.6)	0.63 (*d*, *J* = 6.9)	0.67 (*d*, *J* = 6.9)	0.63 (*d*, *J* = 6.9)
H–C(18)	1.06 (*d*, *J* = 7.0)	1.05 (*d*, *J* = 7.0)	1.05 (*d*, *J* = 6.9)	1.09 (*d*, *J* = 7.0)	1.04 (*d*, *J* = 6.6)	0.95 (*d*, *J* = 6.9)	1.04 (*d*, *J* = 6.9)
H–C(19)	0.81 (*d*, *J* = 6.9)	0.83 (*d*, *J* = 6.9)	0.81 (*d*, *J* = 6.9)	0.82 (*d*, *J* = 6.6)	0.82 (*d*, *J* = 6.9)	0.81 (*d*, *J* = 6.9)	0.81 (*d*, *J* = 6.9)
H–C(21)	3.40 (*d*, *J* = 10.6)	3.31 (*d*, *J* = 10.5)	3.39 (*d*, *J* = 10.8)	3.72 (*d*, *J* = 11.4)	3.38 (*d*, *J* = 10.8)	3.31 (*d*, *J* = 10.8)	3.37 (*d*, *J* = 11.0)
H–C(22)	4.96 (*d*, *J* = 10.6)	5.20 (*d*, *J* = 10.5)	4.94 (*d*, *J* = 10.8)	4.80 (*d*, *J* = 11.4)	4.68 (*d*, *J* = 10.8)	4.59 (*d*, *J* = 10.8)	5.23 (*d*, *J* = 11.0)
H_a–C(24)	2.30–2.17 (*m*)	2.28–2.15 (*m*)	2.30–2.18 (*m*)	2.35–2.19 (*m*)	2.20–2.04 (*m*)	2.18–2.02 (*m*)	2.19–2.04 (*m*)
H_b–C(24)	1.76–1.60 (*m*)	1.75–1.59 (*m*)	1.68–1.51 (*m*)	1.82–1.68 (*m*)	1.61–1.47 (*m*)	1.70–1.60 (*m*)	1.65–1.50 (*m*)
H_a–C(25)	1.73–1.58 (*m*)	1.74–1.57 (*m*)	1.79–1.60 (*m*)	1.83–1.68 (*m*)	1.56–1.40 (*m*)	1.63–1.48 (*m*)	1.64–1.50 (*m*)
H_b–C(25)	1.49–1.38 (*m*)	1.47–1.36 (*m*)	1.37–1.22 (*m*)	1.47–1.30 (*m*)	1.45–1.31 (*m*)	1.34–1.20 (*m*)	1.45–1.30 (*m*)
H–C(26)	3.30–2.17 (*m*)[a])	3.28–2.16 (*m*)	3.47 (*d*, *J* = 11.1)	3.56 (*d*, *J* = 9.8)	3.56 (*d*, *J* = 9.0)	3.51 (*d*, *J* = 9.8)	3.41 (br. *d*, *J* = 8.8)
H_a–C(28)	1.51–1.34 (*m*)	1.48–1.32 (*m*)	1.63–1.48 (*m*)	1.51–1.35 (*m*)	1.65–1.47 (*m*)	1.50–1.34 (*m*)	1.46–1.30 (*m*)

Table 1 (cont.)

	1	**2**	**3**	**4**	**5**	**6**	**7**
H_b–C(28)	1.38–1.20 (*m*)	1.36–1.19 (*m*)	1.27–1.19 (*m*)	1.37–1.28 (*m*)	1.20–1.08 (*m*)	1.30–1.15 (*m*)	1.15–1.00 (*m*)
H_a–C(29)	1.65–1.47 (*m*)	1.63–1.45 (*m*)	1.56–1.40 (*m*)	1.65–1.47 (*m*)	1.60–1.42 (*m*)	1.60–1.43 (*m*)	1.31–1.16 (*m*)
H_b–C(29)	1.28–1.18 (*m*)	1.26–1.13 (*m*)	1.24–1.10 (*m*)	1.35–1.25 (*m*)	1.24–1.08 (*m*)	1.38–1.29 (*m*)	1.58–1.40 (*m*)
H–C(30)	3.19 (*dd*, *J* = 3.6, 11.7)	3.20–3.02 (*m*)	3.20 (*dd*, *J* = 3.2, 11.7)	3.41–3.27 (*m*)[a])	3.07–2.96 (*m*)	2.96–2.80 (*m*)	3.21–3.09 (*m*)
H_a–C(32)	1.99–1.80 (*m*)	2.05–1.90 (*m*)	1.89–1.72 (*m*)	1.61–1.44 (*m*)	2.42 (*dd*, *J* = 11.5, 14.2	5.65 (*d*, *J* = 16.1)	2.47–2.30 (*m*)
H_b–C(32)	1.25–1.15 (*m*)	1.26–1.12 (*m*)	1.26–1.14 (*m*)	0.80–0.69 (*m*)	1.95 (*d*, *J* = 14.2)		1.87–1.72 (*m*)
H_a–C(33)				2.30–2.15 (*m*)	5.78 (*d*, *J* = 11.5)	6.32 (*d*, *J* = 16.1)	5.76 (*d*, *J* = 11.3)
H_b–C(33)	4.78 (*dd*, *J* = 3.0, 9.5)	4.77 (br. *d*, *J* = 10.5)	4.81–4.64 (*m*)	1.73–1.60 (*m*)			
H_a–C(36)	2.24–2.10 (*m*)	4.02 (*d*, *J* = 7.0)	2.21–2.14 (*m*)	2.85–2.70 (*m*)	5.09 (br. *s*)	2.32–2.17 (*m*)	1.83–1.66 (*m*)
H_b–C(36)	2.05–1.90 (*m*)		2.09 (*dd*, *J* = 6.9, 19.1)	1.83–1.68 (*m*)		2.21–2.08 (*m*)	1.42–1.30 (*m*)
Me(37)	1.69 (*s*)	1.71 (*s*)	1.68 (*s*)	1.56 (*s*)	1.81 (*s*)	1.73 (*s*)	1.23 (*s*)
Me(38)	1.58 (*s*)	1.55 (*s*)	1.59 (*s*)	1.87 (*s*)	1.71 (*s*)	1.76 (*s*)	1.74 (*s*)
Me(39)	0.96 (*s*)	0.96 (*s*)	0.95 (*s*)	1.01 (*s*)	0.92 (*s*)	0.94 (*s*)	0.96 (*s*)
Me(40)	1.01 (*s*)	1.01 (*s*)	0.98 (*s*)	1.01 (*s*)	1.07 (*s*)	1.10 (*s*)	1.09 (*s*)
MeO	3.41 (*s*)	3.40 (*s*)	3.40 (*s*)	3.43 (*s*)	3.45 (*s*)	3.40 (*s*)	3.36 (*s*)
MeCH_2			3.31 (*dq*, *J* = 7.0, 12.0), 3.25 (*dq*, *J* = 7.0, 12.0)		3.38 (*dq*, *J* = 7.0, 12.0), 3.24 (*dq*, *J* = 7.0, 12.0)		3.36 (*dq*, *J* = 7.0, 12.0), 3.23 (*dq*, *J* = 7.0, 12.0)
MeCH_2			1.04 (*t*, *J* = 7.0)		1.06 (*t*, *J* = 7.0)		1.09 (*t*, *J* = 7.0)

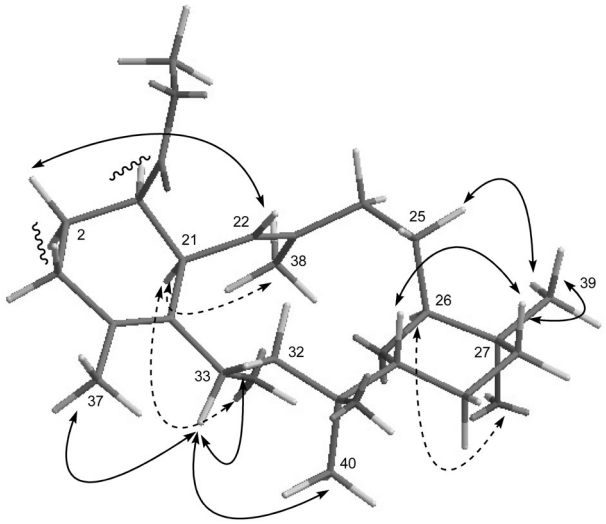
[a]) Overlapped with H_2O signal.

Table 2. *^{13}C-NMR Data of Lobophytones U–Z_1* (**1**–**7**, resp.; δ in ppm, (D_6)DMSO)

	1	**2**	**3**	**4**	**5**	**6**	**7**
C(1)	49.2	52.2	49.3	47.9	49.6	49.7	50.8
C(2)	44.2	54.3	44.2	46.8	48.2	44.4	42.1
C(3)	213.7	214.1	213.8	211.5	212.9	212.5	213.0
C(4)	53.4	55.5	53.6	48.9	53.8	52.1	53.5
C(5)	27.2	27.1	27.2	26.7	27.4	27.1	27.1
C(6)	37.8	37.4	37.9	36.4	38.1	37.2	37.5
C(7)	25.4	25.7	25.8	23.9	26.1	25.2	25.5
C(8)	33.9	33.7	34.0	32.4	34.4	33.4	33.7
C(9)	47.6	46.9	47.7	46.4	48.3	46.8	46.7
C(10)	213.4	213.2	213.3	213.7	212.9	213.4	213.5
C(11)	31.9	31.3	31.6	37.4	30.7	32.9	31.3
C(12)	51.4	52.1	51.3	51.3	51.0	52.2	52.1
C(13)	210.6	209.8	210.7	212.9	209.8	210.2	209.1
C(14)	46.2	45.9	46.3	48.3	45.7	45.6	45.2
C(15)	28.9	28.6	28.9	29.3	28.8	28.7	28.7
Me(16)	21.4	21.5	21.4	20.8	21.5	21.4	21.5
Me(17)	17.8	17.5	17.8	19.1	17.6	18.0	17.6
Me(18)	17.5	17.6	17.6	17.0	17.7	17.4	17.6
Me(19)	22.8	22.6	22.8	21.8	22.9	22.5	22.6
C(20)	174.7	174.4	174.8	173.4	174.2	174.1	174.3
C(21)	42.3	42.5	42.4	40.6	46.5	45.1	47.8
C(22)	125.2	126.8	124.0	124.5	122.0	125.6	123.9
C(23)	135.7	135.3	135.3	139.3	138.2	139.1	136.9
C(24)	35.8	35.4	35.9	39.1	38.5	39.1	39.5
C(25)	25.7	25.0	25.3	27.6	28.6	27.2	27.1
C(26)	82.1	81.9	79.4	84.4	80.1	84.4	84.4
C(27)	69.0	69.2	73.7	68.9	73.3	69.0	68.6
C(28)	33.9	33.9	29.6	33.1	29.0	33.7	32.1
C(29)	20.1	20.2	20.4	20.2	20.4	20.5	20.6
C(30)	74.9	74.8	71.6	68.9	69.1	78.8	70.2
C(31)	73.0	73.0	72.9	72.6	73.5	73.2	73.1
C(32)	41.0	41.1	42.1	37.1	38.2	139.7	38.0
C(33)	65.4	65.5	65.1	24.1	125.7	120.5	128.0
C(34)	132.8	135.8	133.0	132.9	135.5	128.4	134.6
C(35)	126.2	129.5	126.3	122.2	130.8	128.3	74.6
C(36)	33.0	73.1	32.9	32.2	123.7	33.2	39.7
Me(37)	19.1	15.9	19.0	19.6	20.3	19.4	23.2
Me(38)	18.1	18.2	19.3	20.0	19.8	19.9	19.6
Me(39)	27.0	27.1	22.5	28.3	22.6	28.2	28.4
Me(40)	25.1	25.4	25.4	24.4	23.8	21.1	24.2
MeO	51.2	51.2	51.2	51.3	51.5	51.2	51.2
Me*C*H_2			55.3		55.1		55.8
MeCH_2			16.5		16.5		15.8

between H–C(21) (δ(H) 3.40) and CH_2(14) (δ(H) 2.76, 2.96) thus assigned to H_α-C(21). The coupling constants of *J*(26,25a) (11.7), *J*(30,29a) (12.2), and *J*(33,32a) (9.5) indicated the axial orientations of H–C(26), H–C(30), and H–C(33). Moreover, the NOE cross-peaks H–C(33)/Me(37), H–C(33)/Me(40), HO–C(33) (δ(H) 4.54)/

H–C(21), HO–C(27) (δ(H) 4.03)/H–C(26), H–C(30)/H_a–C(28), and Me(39)/H_a–C(28) (*Fig.*) secured the configurations of stereogenic centers C(27), C(28), C(30), and C(31) in **1** to be identical to those of desacetylnyalolide, while H–C(33) was β-oriented.



1

Fig. 1. *Key NOE correlations of* **1** *related to rings* B–D

The HR-ESI-MS and NMR data gave the molecular formula of lobophytone V (**2**) as $C_{41}H_{64}O_{10}$, with one O-atom more than **1**. Comparison of the ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*) indicated that **2** presented an additional CH group (δ(H) 4.02 (*d*, J = 7.0); δ(C) 73.1). This H-atom correlated with H–C(2) (δ(H) 3.44 (*d*, J = 7.0)) in COSY, and interacted with C(35) (δ(C) 129.5) and C(34) (δ(C) 135.8) in HMBC. These findings indicated C(36) to be substituted by a OH group. Additional 2D-NMR spectroscopic analysis confirmed the remaining structure of **2** to be identical to that of **1**. The NOE correlations H–C(36)/H–C(14) (δ(H) 2.84 (*d*, J=19.5)) and H–C(14)/H–C(21) (δ(H) 3.31 (*d*, J = 10.5)) determined H_α–C(36), while the relative configurations of other stereogenic centers of **2** were the same as those of **1**, as evidenced by the closely similar NMR data and NOE relationships.

The NMR spectroscopic data of lobophytone W (**3**) were compatible with those of **1**, except for the presence of an EtO group, which was recognized by the ^{1}H-NMR resonances at δ(H) 1.04 (*t*, J=7.0, 3 H), 3.31 (*dq*, J=7.0, 12.0, 1 H), and 3.25 (*dq*, J= 7.0, 12.01, H), and their corresponding ^{13}C-NMR data at δ(C) 16.5 and 55.3, in addition to the molecular weight of **3** being 28 amu higher than that of **1** as determined by HR-ESI-MS data (m/z 746.5174 ($[M+NH_4]^+$)). The EtO group was deduced to be at C(27) on the basis of the HMBC of the MeCH_2O H-atoms to C(27) (δ(C) 73.7). The similar NMR data and NOE relationships indicated the same relative configurations of

3 and **1**. The presence of EtO group in **3** was assumed to be derived during extraction process.

Lobophytone X (**4**) has a molecular formula of $C_{41}H_{64}O_8$, as determined by HR-ESI-MS (*m/z* 685.4689 ($[M+H]^+$)). Its molecular weight is 16 amu less than that of **1**, suggesting the absence of a OH group. Comparison of the NMR data revealed that HO–CH(33) of **1** was replaced by a CH_2(33) (δ(C) 24.1) group in **4**. The relative configurations of **4** were the same as those of **1**, as determined by the similar NOE correlations and NMR data.

The molecular formula of lobophytone Y (**5**) was determined to be $C_{43}H_{66}O_8$ on the basis of HR-ESI-MS data (*m/z* 711.4831 ($[M+H]^+$)), indicating eleven degrees of unsaturation. Its NMR spectroscopic data (*Tables 1* and *2*) were closely related to those of methyl sartortuoate [4], a co-occurring analog isolated from the same specimen. The difference was attributed to the presence of an EtO group, as evident from the ^{1}H-NMR resonances at δ(H) 1.06 (*t*, *J* = 7.0, *Me*CH_2), 3.38 (*dq*, *J* = 7.0, 12.0, 1 H, Me*C*H_2), and 3.24 (*dq*, *J* = 7.0, 12.0, 1 H, MeC*H*$_2$). The corresponding ^{13}C-NMR were δ(C) 16.5 and 55.1, respectively, by HMQC. The position of EtO group as C(27) was determined by the HMBC between CH_2O H-atoms to C(27) (δ(C) 73.3). The relative configurations of **5** were identical to those of methyl sartortuoate, based on the similar NMR data and NOESY relationships. As lobophytone W (**3**), compound **5** is likely to be an artifact formed during the separation process.

The molecular formula of lobophytone Z (**6**) was determined as $C_{41}H_{62}O_8$ by HR-ESI-MS (*m/z* 683.4514 ($[M+H]^+$)). Thus, the molecular weight of **6** is 18 amu less than that of **1**, suggesting a dehydroxylated derivative of **1**. Its NMR spectroscopic data (*Tables 1* and *2*) were closely similar to those of **1**, except for the presence of a couple of olefinic H-atom signals presenting an *AB* spin system at δ(H) 6.32 (*d*, *J* = 16.1) and 5.65 (*d*, *J* = 16.1). The corresponding C-atoms were assigned to δ(C) 120.5 and 139.7, respectively, by HMQC. The HMBCs from one olefinic H-atom (δ(H) 6.32) to the O-bearing quaternary C-atom C(31) (δ(C) 73.2), and the olefinic C-atoms C(34) (δ(C) 128.4) and C(35) (δ(C) 128.3) ascertained this C=C bond to be located between C(32) and C(33). The *J*(32,33) value (16.1 Hz) was indicative of (32*E*)-configuration. The similar NOE and NMR data resulted in the same relative configurations of **6** as those of **1**.

Lobophytone Z_1 (**7**) has the same molecular formula as **3**, as determined by HR-ESI-MS and NMR data. Comparison of ^{1}H- and ^{13}C-NMR data revealed the structure of **7** to be closely related to that of **3**, with the exception of rings *B* and *C*. HMBC Experiment revealed that Me(37) (δ(H) 1.23 (*s*)) correlated to C(35) (δ(C) 74.6, C_q) and C(34) (δ(C) 134.6, C_q), while olefinic H-atom H–C(33) (δ(H) 5.76 (*d*, *J* = 11.3)) correlated to CH_2(32) (δ(H) 2.47–2.30, 1.87–1.72) in COSY, and showed HMBCs with C(35), C(21) (δ(C) 47.8), and C(31) (δ(C) 73.1). Thus, an C=C bond was determined to be located between C(33) and C(34). The HMBC between MeCH_2 H-atoms (δ(H) 3.36, 3.23) and C(35) confirmed the EtO group to be located at C(35). The β-orientation of Me(37) was evident from the NOEs of Me(37)/H_β–C(36) (δ(H) 1.42–1.30) and H_β–C(36)/H–C(2) (δ(H) 3.53). The relative configurations of the remaining stereogenic centers in **7** were the same as those of **3**, based on the similar NOE relationships and NMR data.

In a primary assay, all compounds showed weak cytotoxicities against mouse peritoneal macrophages ($IC_{50} > 10$ μM). However, compound **6** inhibited NO (nitric

oxide) production in mouse peritoneal macrophages induced by lipopolysaccharide (LPS) with an IC_{50} value of 2.6 ± 0.4 μM, while the other compounds showed IC_{50} values in the range of 3–5 μM (*Table 3*). NO is an important signalling molecule that acts in many tissues to regulate a diverse range of physiological and cellular processes. Overproduction of NO is associated with various human diseases including inflammatory and neuronal disorders [16]. The level of NO release may reflect the degree of inflammation and provides an indicator to assess inflammatory processes. This work presents a new structural pattern of biscembranoids which exhibited significant inhibition against NO production.

In addition, the antibiotic test indicated that compound **1** exhibited significant inhibition against *S. aureus* ($IC_{50} = 18.7 \pm 0.6$ μM) and *S. pneumoniae* ($IC_{50} = 19.8 \pm 0.8$ μM), but the other compounds showed weak effects against the two bacterial strains. All compounds were weak inhibitors against the microorganisms *P. aeruginosa*, *E. coli*, *C. albicans*, *A. fumigatus*, *A. flavus*, and *F. oxysporum*.

Table 3. *Inhibition of Compounds* **1**–**9** *against NO Production*[a])

	IC_{50} [μM]
1	4.1 ± 0.2
2	5.0 ± 0.5
3	4.5 ± 0.3
4	4.2 ± 0.2
5	3.8 ± 0.2
6	2.6 ± 0.4
7	3.3 ± 0.1
8	3.7 ± 0.3
9	5.2 ± 0.4

[a]) Testing in mouse peritoneal macrophages induced by lipopolysaccharide (LPS).

Experimental Part

General. Silica gel (SiO_2; 160–200 mesh and 200–300 mesh) for column chromatography (CC) and GF_{254} silica gel for TLC was provided by *Qingdao Marine Chemistry Co. Ltd*. HPLC: *Alltech* instrument (*426*-HPLC pump, *Alltech UV/VIS-200* detector) equipped with *Kromasil* semiprep. (10 μm, *ODS*, 10 mm × 250 mm) and *YMC-Pack* C_8 (5 μm, 10 mm × 250 mm) columns. Optical rotations: *Perkin-Elmer 243B* polarimeter. IR Spectra: *Thermo Nicolet Nexus 470* FT-IR spectrometer. ^{1}H-, ^{13}C-, and 2D-NMR spectra: *Avance-500* FT 500 MHz NMR spectrometer (*Bruker*) with TMS as an internal standard; chemical shifts (δ) in ppm, coupling constants (J) Hz. HR-ESI-MS: *Bruker APEX IV* instrument.

Animal Material. Soft coral *Lobophytum pauciflorum* 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 were frozen immediately. The specimen was identified by *L. v. O.* This coral (HSF-6) was deposited with the State Key Laboratory of Natural and Biomimetic Drugs, Peking University.

Extraction and Isolation. The frozen soft coral *L. pauciflorum* (2.3 kg, wet weight) was homogenized and then extracted with EtOH (6.0 l) at r.t. (25°). The conc. extract was desalted by dissolving in MeOH (500 ml) to yield a residue (92.7 g) which was partitioned between H_2O (350 ml) and AcOEt (200 ml). The H_2O phase was then extracted by BuOH (200 ml). The concentrated AcOEt fraction (12.1 g) was

subjected to CC (SiO_2, 200–300 mesh, 3 × 20 cm; gradient petroleum ether (PE)/acetone 20:1, 10:1, 3:1, 1:1) to obtain seven fractions, *F1–F7*. *Frs. F1–F4* mainly contained lipids and steroids, as detected by ^{1}H-NMR, while *Frs. F5* and *F6* showed the ^{1}H-NMR features of terpenoids. Thus, *Frs. F5* and *F6* were combined (1.69 g) and subsequently subjected CC (*Sephadex LH-20*; CH_2Cl_2/MeOH 1:2) to afford six portions, *Frs. P1–P6*. *Fr. P2* (180 mg) showed blue-green spots after spraying with anisaldehyde reagent, and was separated on reversed-phase (RP) semiprep. HPLC with MeCN/H_2O (61%) as a mobile phase to obtain **2** (8.6 mg, 22.4 min). *Fr. P3* (90 mg) was treated in the same way as *Fr. P2* on HPLC eluting with MeOH/H_2O (80 %) to obtain **1** (26.3 mg, 17.6 min). *Fr. P5* (190 mg) was subjected to CC (SiO_2 PE/acetone 4:1) to afford **6** (6.8 mg) and **9** (8.3 mg). Following by the same protocol, **4** (9.2 mg) and **7** (3.6 mg) were obtained from *Fr. P6* (42.0 mg), while **5** (2.3 mg; t_R 24.2 min), **3** (14.5 mg, t_R 21.6 min), **8** (5.4 mg, t_R 23.6 min), and **2** (6.5 mg, t_R 18.2 min) were purified from *Fr. P4* (120 mg, 19.0 min) by RP semiprep. HPLC (*ODS*) eluting with MeCN/H_2O (87%).

Lobophytone U (= *Methyl* rel-*(1*E,*5*S,*6*R,*9*R,*10*S,*12*R,*14a*S,*17*S,*21*R,*24*S,*26a*S,*26b*S*)-3,5,6,7,8,9,10,11,12,14,14a,15,16,17,18,19,20,21,22,23,24,25,26,26b-Tetracosahydro-6,10,12-trihydroxy-2,6,10,13,17,21-hexamethyl-15,22,25-trioxo-24-(propan-2-yl)-5,9-epoxybenzo[1,2*-a:*3,4*-a′*]di[14]annulene-26a(4*H*)-carboxylate*; **1**). Amorphous powder. $[\alpha]_D^{25} = +60$ ($c = 1.2$, $CHCl_3$). IR (KBr): 3424, 2957, 2931, 1740, 1439, 1373, 1065, 1029. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 723.4458 ($[M+Na]^+$, $C_{41}H_{64}NaO_9^+$; calc. 723.4442).

Lobophytone V (= *Methyl* rel-*(1*E,*5*S,*6*R,*9*R,*10*S,*12*R,*14*S,*14a*R,*17*S,*21*R,*24*S,*26a*S,*26b*S*)-3,5,6,7,8,9,10,11,12,14,14a,15,16,17,18,19,20,21,22,23,24,25,26,26b-Tetracosahydro-6,10,12,14-tetrahydroxy-2,6,10,13,17,21-hexamethyl-15,22,25-trioxo-24-(propan-2-yl)-5,9-epoxybenzo[1,2*-a:*3,4-a′*]di[14]annulene-26a(4*H*)-carboxylate*; **2**). Amorphous powder. $[\alpha]_D^{25} = +58.9$ ($c = 0.71$, $CHCl_3$). IR (KBr): 3409, 2958, 2931, 1740, 1707, 1459, 1373, 1208, 1061, 1028. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 734.4844 ($[M+NH_4]^+$, $C_{41}H_{68}NO_{10}^+$; calc. 734.4838).

Lobophytone W (= *Methyl* rel-*(1*E,*5*S,*6*R,*9*R,*10*S,*12*R,*14a*S,*17*S,*21*R,*24*S,*26a*S,*26b*S*)-6-Ethoxy-3,5,6,7,8,9,10,11,12,14,14a,15,16,17,18,19,20,21,22,23,24,25,26,26b-tetracosahydro-10,12-dihydroxy-2,6,10,13,17,21-hexamethyl-15,22,25-trioxo-24-(propan-2-yl)-5,9-epoxybenzo[1,2*-a:*3,4-a′*]di[14]annulene-26a(4*H*)-carboxylate*; **3**). Amorphous powder. $[\alpha]_D^{25} = +79.6$ ($c = 0.71$, $CHCl_3$). IR (KBr): 3446, 2959, 2927, 1740, 1708, 1458, 1373, 1206, 1069. ^{1}H- and ^{13}C- NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 746.5174 ($[M+NH_4]^+$, $C_{43}H_{68}NO_9^+$; calc. 746.5202).

Lobophytone X (= *Methyl* rel-*(1*E,*5*S,*6*R,*9*R,*10*S,*14a*S,*17*S,*21*R,*24*S,*26a*S,*26b*S*)-3,5,6,7,8,9,10,11,12,14,14a,15,16,17,18,19,20,21,22,23,24,25,26,26b-Tetracosahydro-6,10-dihydroxy-2,6,10,13,17,21-hexamethyl-15,22,25-trioxo-24-(propan-2-yl)-5,9-epoxybenzo[1,2*-a:*3,4-a′*]di[14]annulene-26a(4*H*)-carboxylate*; **4**). Amorphous powder. $[\alpha]_D^{25} = +89.1$ ($c = 0.25$, $CHCl_3$). IR (KBr): 3487, 2958, 2930, 1711, 1459, 1374, 1199, 1066. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 685.4689 ($[M+H]^+$, $C_{41}H_{65}O_8^+$; calc. 685.4674).

Lobophytone Y (= *Methyl* rel-*(1*E,*5*S,*6*R,*9*R,*10*S,*12*Z,*14a*S,*17*S,*21*R,*24*S,*26a*S,*26b*S*)-6-Ethoxy-3,5,6,7,8,9,10,11,14a,15,16,17,18,19,20,21,22,23,24,25,26,26b-docosahydro-10-hydroxy-2,6,10,13,17,21-hexamethyl-15,22,25-trioxo-24-(propan-2-yl)-5,9-epoxybenzo[1,2*-a:*3,4-a′*]di[14]annulene-26a(4*H*)-carboxylate*; **5**). Amorphous powder. $[\alpha]_D^{25} = +108.8$ ($c = 0.27$, $CHCl_3$). IR (KBr): 3448, 2961, 2929, 1745, 1708, 1459, 1375, 1208, 1065. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 711.4831 ($[M+H]^+$, $C_{43}H_{67}O_8^+$; calc. 711.4830).

Lobophytone Z (= *Methyl* rel-*(1*E,*5*S,*6*R,*9*R,*10*S,*11*E,*14a*S,*17*S,*21*R,*24*S,*26a*S,*26b*S*)-3,5,6,7,8,9,10,14,14a,15,16,17,18,19,20,21,22,23,24,25,26,26b-Docosahydro-6,10-dihydroxy-2,6,10,13,17,21-hexamethyl-15,22,25-trioxo-24-(propan-2-yl)-5,9-epoxybenzo[1,2*-a:*3,4-a′*]di[14]annulene-26a(4*H*)-carboxylate*; **6**). Amorphous powder. $[\alpha]_D^{25} = +158.3$ ($c = 0.32$, $CHCl_3$). IR (KBr): 3450, 2957, 2928, 1740, 1708, 1458, 1377, 1206, 1073. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 683.4514 ($[M+H]^+$, $C_{41}H_{63}O_8^+$; calc. 683.4517).

Lobophytone Z$_1$ (= *Methyl* rel-*(1*E,*5*S,*6*R,*9*R,*10*S,*12*Z,*13*R,*14a*S,*17*S,*21*R,*24*S,*26a*S,*26b*S*)-13-Ethoxy-3,5,6,7,8,9,10,11,13,14,14a,15,16,17,18,19,20,21,22,23,24,25,26,26b-tetracosahydro-6,10-dihydroxy-2,6,10,13,17,21-hexamethyl-15,22,25-trioxo-24-(propan-2-yl)-5,9-epoxybenzo[1,2*-a:*3,4-a′*]di[14]annulene-26a(4*H*)-carboxylate*; **7**). Amorphous powder. $[\alpha]_D^{25} = +43.5$ ($c = 0.5$, $CHCl_3$). IR (KBr): 3521, 2959,

2927, 1743, 1707, 1459, 1375, 1207, 1079. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 729.4955 ($[M+H]^+$, $C_{43}H_{65}O_9^+$; calc. 729.4936).

Assay for Inhibition of Nitric Oxide (NO) *Release.* Dexamethasone (DEX, positive control, 20 mM in 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 thioglycolate, RPMI1640, FBS, MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide), and *Griess* reagents were purchased from *Sigma* (St. Louis, MO, USA). Mouse peritoneal macrophages (PEM*Φ*s) were obtained from C57BL6J male mice, and then plated onto 48-well plates and cultured for 2 h in DMEM containing 5% FBS and 5% CO_2 at 37°. PEM*Φ*s were incubated with test compounds for 1 h at 37° before stimulation with 1 μg/ml of lipopolysaccharide (LPS) for 24 h. For blank control (enchylema) test, LPS with compound (1 μM) and DEX (1 μM) were prepared. Cells (5×10^5 cells) were preincubated at 37° 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 produced formazan crystals were dissolved in DMSO (200 μl). Absorbance was measured at 540 nm.

One-way analysis of variance was applied for all statistical analyses by independent experiments, and data were represented as means ± standard error of the measurement. Individual values were compared by *t*-test and a *P*-value < 0.01 to consider as significant. The NO release values were determined in three independent experiments.

Antibiotic Assay. Antimicrobial and antifungal bioassays were conducted in triplicate according to the method recommended by the National Center for Clinical Laboratory Standards (NCCLS) [17][18]. The bacterial strains *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa* were grown on *Mueller–Hinton* agar. The yeasts *Candida albicans* and *Saccharomyces cerevisiae* were grown on *Sabouraud* dextrose agar, and the fungus *Aspergillus fumigatus* was grown on potato dextrose agar. Targeted microbes (3–4 colonies) were prepared from broth culture (bacteria: 37° for 24 h; fungus: 28° for 48 h), and the final spore suspensions of bacteria (in MHB medium), yeasts (in SDB medium), and *A. fumigatus* (in PDB medium) were 10^6 and 10^5 cells/ml and 10^4 mycelial fragments/ml, resp. Testing compounds (10 mg/ml as stock soln. in DMSO and serial dilutions) were transferred to a 96-well clear plate in triplicate, and the suspension of the testing microorganisms was added to each well (200 μl) (antimicrobial peptide AMP, streptomycin, and fluconazole were used as positive controls). After incubation, the absorbance at 595 nm was measured with a microplate reader (*TECAN*), and the inhibition rate was calculated and plotted *vs.* testing concentrations to afford IC_{50} values.

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