Yalongenes A and B, Two New Cembranoids with Cytoprotective Effects from the Hainan Soft Coral *Sarcophyton trocheliophorum* MARENZELLER

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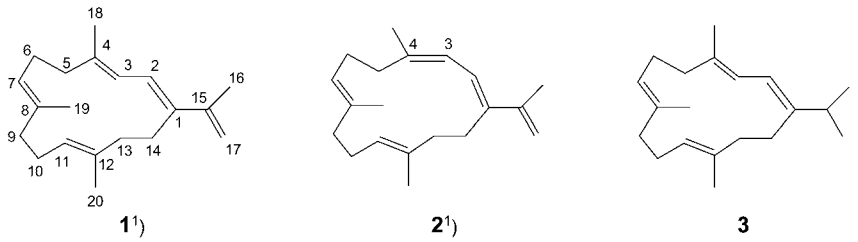
Two new cembranoids, yalongenes A (**1**) and B (**2**), were isolated from the South China Sea soft coral *Sarcophyton trocheliophorum* MARENZELLER. Their structures were elucidated by detailed spectroscopic analysis (IR, MS, and NMR) and by comparison with a related model compound. Yalongenes **1** and **2** were evaluated for their cytoprotective effects on SH-SY5Y cell injury induced by H_2O_2 *in vitro*, and the result showed that only compound **1** had a significant cytoprotective activity at the concentration of 1 μM.

Introduction. – Cembranoids are a family of 14-membered cyclic diterpenoid natural products biosynthesized by a diverse range of organisms from terrestrial and especially from marine habitats [1]. Within the marine environment, all cembrane diterpenes have been reported, with a few exceptions, from both soft corals and gorgonian corals [2]. Biological activities such as anti-inflammatory [3], antifouling [4], Ca-antagonistic [5], and Na^+, K^+-ATPase inhibition [6] properties have been ascribed to some of these cembrane diterpenes, although the most significant results have been described in the antitumor area [7].

The soft coral of the genus *Sarcophyton* (phylum Cnidaria, class Anthozoa, subclass Octocorallia, order Alcyonaceae, family Alcyoniidae) are prolific in the South China Sea. In the course of our ongoing research on the biologically active substances from Chinese marine invertebrates [8–10], we carried out a chemical investigation of the soft coral *Sarcophyton trocheliophorum* MARENZELLER, collected off the Yalong Bay, Hainan Province, China, resulting in the discovery of two new cembranoids, namely yalongenes A (**1**) and B (**2**). This paper deals with the isolation and structural elucidation of these new compounds.

Results and Discussion. – Freshly collected animals of *Sarcophyton trocheliophorum* were immediately put at −20°, and kept frozen prior to extraction. Frozen material was extracted exhaustively with acetone, and the acetone extract was then partitioned between Et_2O and H_2O. The Et_2O-soluble extract was repeatedly subjected to column chromatography (silica gel, *Sephadex LH-20*) followed by reversed-phase HPLC to afford yalongenes A (**1**) and B (**2**) (*Fig. 1*)[1]).

[1]) Arbitrary atom numbering; for systematic names, see *Exper. Part.*



1[1]) **2**[1]) **3**

Fig. 1. *Compounds* **1** *and* **2**, *isolated from* Sarcophyton trocheliophorum, *and cembrene C* (**3**)

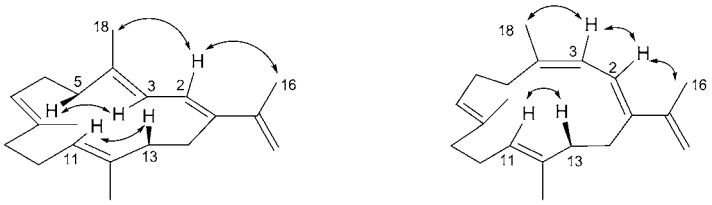
Yalongene A (**1**) was obtained as a colorless oil. The compound was a hydrocarbon with the molecular formula $C_{20}H_{30}$, which was deduced from HR-EI-MS (*m*/*z* 270.2347 (M^+)), implying six degrees of unsaturation. The UV spectrum showed absorption maxima at λ_{max} 234 (log ε 3.82) and 284 nm (log ε 3.72), according to the presence of a conjugated triene chromophore [11]. The ^{13}C-NMR spectrum of **1** (*Table*) displayed signals for 20 C-atoms, which were attributed by DEPT experiment to four Me groups, seven CH_2 groups (one of which was a sp^2 C-atom), four olefinic CH moieties, and five quaternary sp^2 C-atoms. Considering that compound **1** contained a total of 20 C-atoms, including four Me groups and one sp^2 CH_2, and that the secondary metabolites of soft corals of the genus *Sarcophyton* are typically cembrane-type diterpenes, it was reasonable to suggest a cembranoid structure. The presence of five C=C bonds in **1** left

Table. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$) *of* **1** *and* **2**[1]). δ in ppm, *J* in Hz.

	1		**2**	
	δ(H)[a])	δ(C)[b])	δ(H)[a])	δ(C)[b])
C(1)	–	143.5 (*s*)	–	143.6 (*s*)
H–C(2)	6.36 (*d*, *J* = 11.4)	122.9 (*d*)	6.39 (*d*, *J* = 10.8)	124.8 (*d*)
H–C(3)	6.03 (*d*, *J* = 11.4)	122.6 (*d*)	6.04 (*d*, *J* = 10.8)	123.8 (*d*)
C(4)	–	138.3 (*s*)	–	137.9 (*s*)
CH_2(5)	2.12–2.18 (*m*)	39.5 (*t*)	2.75–2.65 (*m*, H_a), 1.86–1.89 (*m*, H_b)	31.2 (*t*)
CH_2(6)	2.16–2.24 (*m*)	25.3 (*t*)	2.32–2.36 (*m*, H_a), 2.14–2.17 (*m*, H_b)	26.3 (*t*)
H–C(7)	4.97 (*m*)	124.3 (*d*)	4.80–4.82 (*m*)	123.3 (*d*)
C(8)	–	134.4 (*s*)	–	134.5 (*s*)
CH_2(9)	2.06–2.16 (*m*)	38.8 (*t*)	2.11–2.16 (*m*, H_a), 1.80–1.84 (*m*, H_b)	39.6 (*t*)
CH_2(10)	2.11–2.18 (*m*)	24.6 (*t*)	2.20–2.26 (*m*, H_a), 1.85–1.92 (*m*, H_b)	25.3 (*t*)
H–C(11)	4.92–5.01 (*m*)	125.1 (*d*)	4.65–4.71 (*m*)	127.1 (*d*)
C(12)	–	135.3 (*s*)	–	132.1 (*s*)
CH_2(13)	2.06–2.15 (*m*)	39.0 (*t*)	2.16–2.21 (*m*, H_a), 2.04–2.12 (*m*, H_b)	38.5 (*t*)
CH_2(14)	2.53 (*m*)	26.5 (*t*)	2.68–2.71 (*m*, H_a), 2.32–2.38 (*m*, H_b)	24.2 (*t*)
C(15)	–	139.1 (*s*)	–	136.1 (*s*)
Me(16)	1.94 (*s*)	21.3 (*q*)	1.92 (*s*)	21.5 (*q*)
CH_2(17)	4.95 (*s*, H_a), 5.05 (*s*, H_b)	111.8 (*t*)	4.92 (*s*, H_a), 4.99 (*s*, H_b)	111.2 (*t*)
Me(18)	1.78 (*s*)	17.2 (*q*)	1.89 (*s*)	23.8 (*q*)
Me(19)	1.52 (*s*)	15.9 (*q*)	1.52 (*s*)	15.7 (*q*)
Me(20)	1.55 (*s*)	17.2 (*q*)	1.54 (*s*)	16.1 (*q*)

[a]) Deduced by ^{1}H,^{1}H-COSY and HMQC experiments. [b]) Deduced by DEPT and HMBC experiments.

one degree of unsaturation, which was attributed to a monocyclic skeleton. The ^{1}H-NMR spectrum showed two *m* at δ(H) 4.97 (*m*, H–C(7)) and 4.92 – 5.01 (*m*, H–C(11)) that were assigned to olefinic H-atoms at two endocyclic trisubstituted C=C moieties. Four further olefinic signals at δ(H) 6.36 (*d*, $J = 11.4$ Hz, H–C(2)), 6.03 (*d*, $J = 11.4$ Hz, H–C(3)), 4.95 (*s*, H_a–C(17)), and 5.05 (*s*, H_b–C(17)) were attributed to the H-atoms of a conjugated triene system with a terminal C=C bond. Four 3-H *s* at δ(H) 1.94 (*s*, Me(16)), 1.78 (*s*, Me(18)), 1.52 (*s*, Me(19)), and 1.55 (*s*, Me(20)) were assignable to four olefinic Me groups. In addition, the intense overlapping signals for 6 CH_2 groups were observed. The NMR data of **1** mentioned above were strongly reminiscent of those of cembrene C (**3**) [12], a cembranoid previously isolated from a soft coral, *Nephthea* sp. Careful comparison of the NMR data of **1** and **3** revealed that the only difference between them resided in the additional terminal olefin C(15)=C(17) (δ(C) 139.1, and 111.8) in **1** instead of the aliphatic Me group at C(15) of **3**, while the rest of the structure of **1** was the same as in **3**. This was in agreement with the two mass units difference between them. Finally, the geometry of four C=C bonds within the 14-membered ring of **1** was established to be the same as those of **3** on the basis of the ^{13}C-NMR chemical shifts of the olefinic Me groups, as well as by extensive analysis of the NOESY data (*Fig. 2*). The configuration of each of the three C=C bonds which bear a Me group, *i.e.*, C(3)=C(4), C(7)=C(8), and C(11)=C(12), was assigned as (*E*) by the ^{13}C-NMR chemical shifts of Me(18), Me(19), and Me(20) resonating at δ(C) 17.2, 15.9 and 17.2 (< 20 ppm), respectively [13]. The NOESY experiment with **1** further confirmed the (*E*) geometry of the three olefinic bonds as judged from the diagnostic cross-peak H–C(2)/Me(18), and by the absence of correlations H–C(7)/Me(19) and H–C(11)/Me(20). In addition, an obvious NOE correlation H–C(2)/Me(16) showed the (*E*) configuration of C(1)=C(2), which was linked to C(3)=C(4), resulting in the configuration of the same s-*trans*-1,1,4,4-tetrasubstituted diene system as in **3**. All ^{1}H- and ^{13}C-NMR resonances of **1** were unambiguously assigned by ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments, as reported in the *Table*. Thus, the structure of yalongene A (**1**) was determined to be (1*E*,3*E*,7*E*,11*E*)-1,7,11-trimethyl-4-(1-methylethenyl)cyclotetradeca-1,3,7,11-tetraene.



1[1]) **2**[1])

Fig. 2. *Selected key NOESY correlations* (H ↔ H) *of compounds* **1** *and* **2**

Yalongene B (**2**) was also isolated as a colorless oil. It had the same molecular formula $C_{20}H_{30}$ as yalongene A (**1**), as established by HR-EI-MS (*m*/*z* 270.2345). Like compound **1**, the UV spectrum of **2** showed also a strong absorption at λ_{max} 282 nm (log ε 4.13) due to a conjugated triene system. The NMR spectra of **2** appeared identical to

those of **1** and revealed that the molecule possessed a cembrane skeleton with an isopropenyl group, two isolated trisubstituted C=C bonds, and two conjugated C=C bonds. A comparison of the NMR data of **2** and **1** revealed that the only difference between them was the C(3)=C(4) geometry ((*Z*) in **2** and (*E*) in **1**). The (*Z*) geometry of C(3)=C(4) in **2** was inferred by the ^{13}C-NMR chemical shifts of Me(18) at δ(C) 23.8 (> 20 ppm) [13] and from a strong NOE correlation H–C(3) (δ(H) 6.04 (*d*, *J* = 10.8 Hz)/Me(18) (δ(H) 1.89 (*s*)). Furthermore, the relative configuration of the other three C=C bonds, *i.e.*, C(1)=C(2), C(7)=C(8), and C(11)=C(12), was deduced to be the same as those of **1**, following the same procedure as described above for **1**. Therefore, the structure of **2** was elucidated as (1*E*,3*Z*,7*E*,11*E*)-1,7,11-trimethyl-4-(1-methylethenyl)cyclotetradeca-1,3,7,11-tetraene.

Compounds **1** and **2** were tested for their cytoprotective effects on SH-SY5Y cells injury induced by hydrogen peroxide *in vitro*, and the results showed that compound **1** had a significant cytoprotective activity (14.53% of increase in cell viability) at the concentration of 1 μM. Other bioassays such as antibacterial and anti-inflammatory activities are currently ongoing.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 and 400–600 mesh; *Qingdao Haiyang Chemical Group Co.*), *Sephadex LH-20* (*Amersham Biosciences*). TLC: precoated SiO_2 plates (*G60*, F_{254}; *Yantai Zifu Chemical Group Co.*). Reversed-phase HPLC: *Agilent-1100* chromatographe; *VWD-G1314A* detector at 210 nm; semi-prep. *ODS-HG-5* (5 μm) column (10 mm (i.d.) × 25 cm). Optical rotations: *Perkin–Elmer-241 MC* polarimeter; in $CHCl_3$. UV Spectra: *Varian-Cary-300-Bio* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Nicolet-Magna-FT-IR-750* spectrometer; $\tilde{\nu}$ in cm^{-1}. NMR-Spectra: *Bruker DRX-400* spectrometer; δ in ppm, with residual $CHCl_3$ (δ(H) 7.26; δ(C) 77.0) as internal standard, *J* in Hz; ^{1}H- and ^{13}C-NMR assignments were supported by ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments. MS: *Finnigan MAT-95* spectrometer; in *m*/*z*.

Biological Material. The specimens of *Sarcophyton trocheliophorum*, identified by *R.-L. Zhou* at the South China Sea Institute of Oceanology, Chinese Academy of Sciences, were collected by scuba at Yalong Bay, Hainan Province, China, in May 2006, and were frozen immediately after collection. A voucher specimen is available for inspection at the Shanghai Institute of Materia Medica, CAS, under registration No. Yal-4.

Extraction and Isolation. The frozen animals (763 g dried weight) were cut into pieces and extracted exhaustively with acetone at r.t. (3 × 1.5 l, 15 min in an ultrasonic bath). The org. extract was concentrated to give a residue which was partitioned between Et_2O and H_2O. The Et_2O soln. was concentrated to give a dark green residue (7.8 g), which was fractionated by CC (SiO_2, light petroleum ether with increasing amounts of acetone): *Fractions 1–8*. *Fr. 2* was further subjected to CC (SiO_2, petroleum ether/Et_2O 99:1) followed by reversed-phase HPLC (semi-prep. *ODS-HG-5*, MeOH/H_2O 97:3, 2.0 ml/min): **1** (7.3 mg) and **2** (8.6 mg).

Yalongene A (= *(1*E*,3*E*,7*E*,11*E*)-1,7,11-Trimethyl-4-(1-methylethenyl)cyclotetradeca-1,3,7,11-tetraene*; **1**): Colorless oil. UV (MeOH): 234 (3.82), 284 (3.72). IR (KBr): 3433, 2926, 2854, 1668, 1634, 1458, 1384, 1095. ^{1}H- and ^{13}C-NMR: *Table.* HR-EI-MS: 270.2347 (M^+, $C_{20}H_{30}^+$; calc. 270.2348).

Yalongene B (= *(1*E*,3*Z*,7*E*,11*E*)-1,7,11-Trimethyl-4-(1-methylethenyl)cyclotetradeca-1,3,7,11-tetraene*; **2**): Colorless oil. UV(MeOH): 282 (4.13). IR (KBr): 3431, 2926, 2854, 1665, 1458, 1103. ^{1}H- and ^{13}C-NMR: *Table.* HR-EI-MS: 270.2345 (M^+, $C_{20}H_{30}^+$; calc. 270.2348).

Neuroprotective Activity Assay. SH-SY5Y Cell survival was evaluated according to the reported protocol with modification [14]. High-passage cells were from the ATCC (American Type Culture Collection) and maintained at 37° in a humidified atmosphere containing 5% CO_2. Cells were seeded into 96-well plates at a density of 1×105 cells/ml in MEM/F12 medium supplemented with 10% (*v*/*v*) fetal bovine serum. Experiments were carried out 24 h after cells were seeded. The 10^{-2} M stock solns. were made of the tested compounds with DMSO and then diluted to corresponding concentrations with cell culture medium. Cell survival was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) reduction [15] to analyze the cytoprotection by the test compounds. In brief, cells were incubated with the test compounds (1 μM) 2 h prior to treatment with 100 μM H_2O_2 for another 24 h without changing the culture medium. Then 10 μl of MTT (5 mg/ml) was added to each well and incubated at 37° for 4 h. The cells were finally lysed with 100 μl of DMSO, and the amount of MTT formazan was measured at 490 nm with a microplate reader.

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