# A New Cembranoid Diterpene and Other Related Metabolites from the South-China-Sea Soft Coral *Lobophytum crassum*

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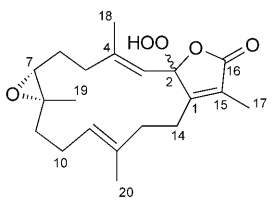
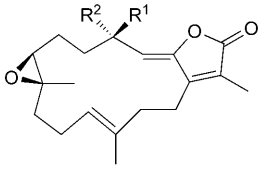
A new hydroperoxy-substituted cembranoid diterpene, 2-hydroperoxysarcophine (=(1a*R**,4*E*,11-*E*,14a*R**)-2,3,6,7,10a,13,14,14a-octahydro-10a-hydroperoxy-1a,5,8,12-tetramethyloxireno[9,10]cyclotetradeca[1,2-*b*]furan-9(1a*H*)-one; **1**), was isolated from the marine soft coral *Lobophytum crassum*. Also isolated were two other cembranoid diterpenes, obtained for the first time from a natural source, *i.e.*, 7$\beta$,8$\beta$-epoxy-4$\alpha$-hydroxycembra-1(15),2,11-trien-16,2-olide (**2**) and 7$\beta$,8$\beta$-epoxy-4$\beta$-hydroxycembra-1(15),2,11-trien-16,2-olide (**3**), along with three further cembranoid derivatives, five sterols, and two open-chain metabolites. Their structures and relative configurations were elucidated on the basis of extensive spectroscopic analyses including 1D- and 2D-NMR, and HR-ESI-MS experiments.

**Introduction.** – A large number of highly functionalized cembranoid diterpenes, sterols, and other related metabolites have been isolated and identified from marine soft corals, especially from the genera *Lobophytum*, *Sarcophyton*, and *Sinularia*, all of which belong to the family Alcyoniidae within the order of Alcyonacea [1][2]. *Lobophytum crassum* is a common soft-coral species widespread in Indo-Pacific reefs [3]. This species has been reported to contain cembranoid diterpenes [4–7], eudesmane-based diterpenoids [7], polyhydroxylated sterols [2][8], and other related compounds [9]. A literature survey revealed that, so far, the reported new cembranoid diterpenes of *L. crassum* origin can be classified into two types: compounds with or without a lactone ring. Interestingly, all new cembranoid diterpenes so far isolated from this species that possess such a lactone ring also have a $\Delta^{15,17}$ unsaturation.

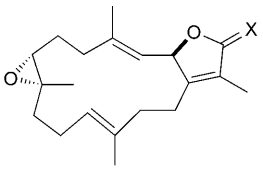
As part of our studies on secondary metabolites from marine organisms from the Chinese Sea, we wish to report in this paper the isolation and structure elucidation of a new cembranoid diterpene, 2-hydroperoxysarcophine (**1**), along with the two cembranoid diterpenes 7$\beta$,8$\beta$-epoxy-4$\alpha$-hydroxycembra-1(15),2,11-trien-16,2-olide (**2**) [10] and 7$\beta$,8$\beta$-epoxy-4$\beta$-hydroxycembra-1(15),2,11-trien-16,2-olide (**3**) [10], which have been isolated for the first time from a natural source. In addition, ten other compounds, including three further cembranoid derivatives (**4**–**6**), five sterols (**7**–**11**), and two open-chain compounds (**12**, **13**) were isolated and identified.



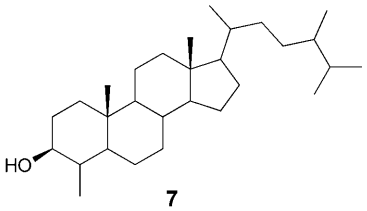

**1**




**2** $R^1$ = Me, $R^2$ = OH
**3** $R^1$ = OH, $R^2$ = Me




**4** X = $H_2$
**5** X = O

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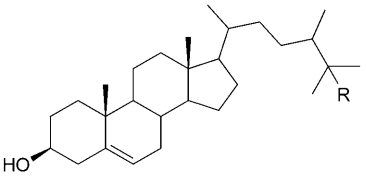
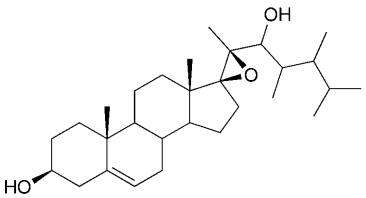



**8** R = H
**11** R = OH

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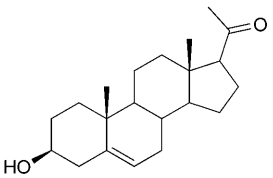
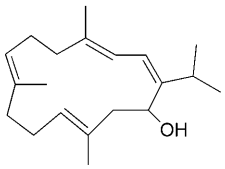
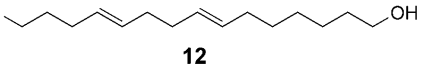


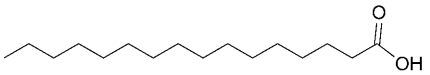

**10**




**6**




**12**




**13**

**Results and Discussion.** – Compound **1**, obtained as colorless crystals, showed the $[M+NH_4]^+$ signal at *m/z* 366.2268 (calc. 366.2280) in the HR-ESI mass spectrum, suggesting the molecular formula $C_{20}H_{28}O_5$, with seven degrees of unsaturation. The structure of **1** was deduced to correspond to 2-hydroperoxysarcophine, as elucidated on the basis of extensive spectroscopic analysis and comparison with the data of sarcophine (**5**), an epoxy cembranoid diterpene isolated from several marine organisms [11][12]. The relative configuration of **1** was determined from a NOESY experiment.

The IR absorptions of **1** at 3353, 1767, 1669, and 1256 $cm^{-1}$ indicated the presence of OH (or OOH), $\gamma$-lactone, olefin, and epoxide functionalities. The $^1$H-NMR spectrum

(*Table*)[1]) clearly indicated the presence of two olefinic H-atoms at $\delta$(H) 5.47 (*s*, 1 H) and 4.99 (*t*, $J=7.5$ Hz, 1 H), and of four Me signals at $\delta$(H) 1.90 (*s*), 1.56 (*s*), 1.17 (*s*), and 1.54 (*s*). The $^{13}$C-NMR (DEPT) spectrum (*Table*) exhibited 20 signals: seven quaternary C-atoms, three CH, six $CH_2$, and four Me groups, suggesting a diterpenoid skeleton for **1**. Four $^{13}$C-NMR signals for the tertiary olefinic C-atoms at $\delta$(C) 151.1 (*s*), 147.9 (*s*), 131.2 (*s*), and 124.7 (*s*), as well as two signals for CH olefinic resonances at $\delta$(C) 127.5 (*d*) and 112.8 (*d*) indicated that the molecule contained three C=C bonds. A further tertiary C-atom signal resonated at $\delta$(H) 170.2 (*s*), which, together with the IR signal at 1767 cm$^{-1}$, suggested a $\gamma$-lactone. The signals at $\delta$(C) 63.0 (*d*), $\delta$(C) 60.2 (*s*), and $\delta$(H) 2.41–2.43 (*m*, 1 H) indicated a trisubstituted epoxide in **1**. These IR and NMR data accounted for three of the five O-atoms. Therefore, the remaining two O-atoms and the oxygenated C-atom signal at $\delta$(C) 85.0 (*s*) implied that **1**, most likely, possessed a hydroperoxy group. A positive reaction in the starch test (KI/AcOH) corroborated, indeed, that **1** was a peroxide [13].

Table. *$^1$H- and $^{13}$C-NMR Data of* **1**. At 400 and 100 MHz, resp., in $CDCl_3$. Assignments[1]) were corroborated by $^1$H,$^1$H-COSY, HMQC, HMBC, and NOESY experiments.

| | $\delta$(H) | $\delta$(C) | | $\delta$(H) | $\delta$(C) |
|---|---|---|---|---|---|
| C(1) | – | 151.1 (*s*) | H–C(11) | 4.99 (*t*, $J=7.5$) | 127.5 (*d*) |
| C(2) | – | 85.0 (*s*) | C(12) | – | 131.2 (*s*) |
| H–C(3) | 5.47 (*s*) | 112.8 (*d*) | $H_a$–C(13) | 2.01–2.05 (*m*) | 34.4 (*t*) |
| C(4) | – | 147.9 (*s*) | $H_b$–C(13) | 2.09–2.15 (*m*) | – |
| $H_a$–C(5) | 1.06–1.13 (*m*) | 37.2 (*t*) | $H_a$–C(14) | 1.31–1.37 (*m*) | 23.2 (*t*) |
| $H_b$–C(5) | 1.83–1.89 (*m*) | – | $H_b$–C(14) | 2.12–2.17 (*m*) | – |
| $H_a$–C(6) | 1.86–1.88 (*m*) | 23.1 (*t*) | H–C(15) | – | 124.7 (*s*) |
| $H_b$–C(6) | 2.00–2.05 (*m*) | – | H–C(16) | – | 170.2 (*s*) |
| $H_\beta$–C(7) | 2.41–2.43 (*m*) | 63.0 (*d*) | Me(17) | 1.90 (*s*) | 9.0 (*q*) |
| C(8) | | 60.2 (*s*) | Me(18) | 1.56 (*s*) | 24.3 (*q*) |
| $H_a$–C(9) | 2.17–2.21 (*m*) | 37.8 (*t*) | Me(19) | 1.17 (*s*) | 17.0 (*q*) |
| $H_b$–C(9) | 2.33–2.37 (*m*) | – | Me(20) | 1.54 (*s*) | 14.8 (*q*) |
| $H_a$–C(10) | 2.56–2.64 (*m*) | 22.4 (*t*) | 2-OOH | 8.78 (*s*) | – |
| $H_b$–C(10) | 2.67–2.71 (*m*) | – | | | |

From the above IR, HR-ESI-MS, and NMR spectroscopic data, compound **1** was identified as a congener of sarcophine (**5**), a cembranoid diterpene previously isolated from the marine soft coral *Sarcophyton glaucum* [11][12]. The only difference was that the H-atom in 2-position of **5** was replaced by a OOH group in **1**.

The olefinic H-atom resonating at $\delta$(H) 5.47 (*s*, H–C(3)) correlated with C(3) at $\delta$(C) 112.8 in the HMQC spectrum of **1**. This H-atom displayed a $^2J$-type HMBC coupling to the oxygenated quaternary C(2) at $\delta$(C) 85.0 (*Figure*). In addition, H–C(3) displayed a $^3J$-type HMBC correlation with the quaternary, olefinic C(1) at $\delta$(C) 151.1. The olefinic resonance at $\delta$(H) 4.99 (*t*), which correlated with C(11) at $\delta$(C) 127.5 in the HMQC spectrum, was assigned to H–C(11). This H-atoms displayed $^3J$-type

[1]) Arbitrary atom numbering.

HMBC correlations with C(9) at $\delta$(C) 37.8, C(13) at 34.4, and C(20) at 14.8, respectively. Further HMBC correlations were observed between H–C(17) and C(1), C(2), C(14), C(15), and C(16); between H–C(18) and C(2), C(3), and C(5); between H–C(19) and C(7), C(8), and C(9); between H–C(20) and C(11), C(12), and C(13); and between H–C(3) and C(1), C(2), C(4), and C(5), respectively (*Figure*).

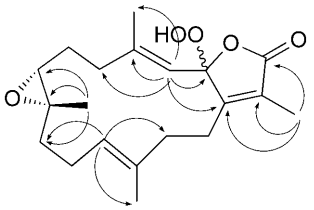



Figure. *Selected HMBC correlations for* **1**

The relative configuration of **1** was established by a NOESY experiment. H–C(7) at $\delta$(H) 2.41–2.43 showed a NOESY correlation with Me(19) at $\delta$(H) 1.17, suggesting the same orientation for both groups. However, the configuration at C(2) remained unknown, since no cross-peak could be detected between the OOH H-atom and any other $^1$H-NMR signal. From the above evidence, compound **1** was identified as 2-hydroperoxysarcophine. Although all experimental procedures, including extraction, isolation, and solvent evaporation, were conducted below 40°, it remains unclear whether **1** is a true natural product or an artifact.

Compounds **2** and **3** were obtained as colorless needles (m.p. 106–108 and 101–102°, resp.). Comparison of their NMR and MS spectroscopic data with those published showed that they correspond to 7$\beta$,8$\beta$-epoxy-4$\alpha$-hydroxycembra-1(15),2,11-trien-16,2-olide and 7$\beta$,8$\beta$-epoxy-4$\beta$-hydroxycembra-1(15),2,11-trien-16,2-olide, respectively, which have previously been obtained by the bioconversion of sarcophine (**5**) [10], but, to the best of our knowledge, not isolated from a natural source.

Several other compounds were isolated and identified from *L. crassum*, including three further cembranoid diterpenes, sarcophytoxide (**4**) [14], sarcorphine (**5**) [11], and 14-hydroxycembra-1,3,7,11-tetraene (**6**) [15]; five sterols, including 4-methyl-ergostan-3-ol (**7**) [16], ergost-5-en-3-ol (**8**) [17], 17$\beta$,20$\beta$-epoxy-23,24-dimethylcholest-5-en-3$\beta$,22-diol (**9**) [18], pregnenolone (**10**) [19], and ergost-5-en-3,25-diol (**11**) [20]; as well as two other metabolites, hexadeca-7,11-dien-1-ol (**12**) [21] and hexadecanoic acid (**13**) [22].

As can be seen from the cembranoid molecular structures reported herein, all compounds contain an $\Delta^{1,15}$ rather than the known $\Delta^{15,17}$ unsaturation identified in previous constituents from *L. crassum*. To the best of our knowledge, this is the first report that *L. crassum* contains cembranoid diterpenes with both an OOH function and a $\Delta^{1,15}$ C=C bonds.

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IOCAS for partial financial support. The program of *Bairen Jihua* from the Chinese Academy of Sciences (awarded to *B.-G. W.*) is also gratefully acknowledged.

## Experimental Part

*General*. Column chromatography (CC) was performed with silica gel (200–300 mesh; *Qingdao Haiyang Co.*, China), and anal. thin-layer chromatography (TLC) was performed with precoated silica gel $GF_{254}$ plates (*Qingdao Haiyang Co.*). UV Spectra: *Varian Cary-300-Bio* spectrophotometer; $\lambda_{max}$ ($\log \varepsilon$) in nm. Optical rotation: *Perkin-Elmer 341* polarimeter. IR Spectra: *Nicolet Magna FT-IR-750* spectrometer; in $cm^{-1}$. $^1H$- and $^{13}C$-NMR Spectra: *Bruker AM-400* apparatus; at 400 ($^1H$) or 100 MHz ($^{13}C$); chemical shifts $\delta$ in ppm rel. to residual $CHCl_3$ [$\delta$(H) 7.26, $\delta$(C) 77.0], coupling constants $J$ in Hz. Assignments were supported by $^1H$,$^1H$-COSY, HMQC, and HMBC experiments. ESI- and HR-ESI-MS: *Q-TOF Micro* LC/MS/MS spectrometer; in $m/z$.

*Animal Material*. The soft coral *Lobophytum crassum* was collected in the sea waters of Hainan island in August 2004 from a depth of 8–10 m, and was identified by Prof. *Z. C. Tang*, Institute of Oceanology, Chinese Academy of Sciences, P. R. China, where a voucher specimen (No. 04B1) was deposited.

*Extraction and Isolation*. The whole specimen (1.5 kg dry weight after extraction) was extracted directly with EtOH at r.t. (3×7 d). The combined extracts were filtered and evaporated at reduced pressure (<40°) to afford a crude residue (105 g), which was partitioned between AcOEt and $H_2O$, and between $H_2O$-sat. BuOH and $H_2O$, to afford an AcOEt and a BuOH-soluble fraction, resp. The AcOEt-soluble fraction (53 g) was purified by CC ($SiO_2$; AcOEt/petroleum-ether (PE) gradient): nine subfractions (*Fr. 1–9*) according to TLC. *Fr. 2* (10 g) was further purified by repeated CC ($SiO_2$; AcOEt/PE 1:10 and AcOEt/$CHCl_3$ 1:30) to afford **6** (550 mg) and **12** (25 mg). *Fr. 3* (4.5 g) was subjected to CC ($SiO_2$; AcOEt/PE 1:5 and AcOEt/$CHCl_3$ 1:15) to afford **1** (61 mg), **2** (10 mg), **3** (9 mg), **4** (300 mg), and **5** (200 mg). *Frs. 4–6* were further separated by CC ($SiO_2$; acetone/PE 1:4 and AcOEt/$CHCl_3$ 1:2) to afford **7** (40 mg), **8** (3.5 g), **9** (460 mg), **13** (2 g), and a mixture of **10** and **11**. The latter was further separated by CC (1. *Sephadex LH-20*, MeOH; 2. *RP-18*, MeOH/$H_2O$ 9:1) to provide **10** (35 mg) and **11** (30 mg).

*2-Hydroperoxysarcophine* (=*(1a*R*,4*E*,11*E*,14a*R*)-2,3,6,7,10a,13,14,14a-Octahydro-10a-hydroperoxy-1a,5,8,12-tetramethyloxireno[9,10]cyclotetradeca[1,2-*b*]furan-9(1a*H*)-one*; **1**). Colorless crystals. M.p. 101–103°. UV (acetone): 212 (1.11). $[\alpha]_D^{20} = -153$ ($c = 1.15$, acetone). IR (KBr): 3353 (OOH), 2929, 1767 ($\gamma$-lactone), 1669 (olefin), 1448, 1385, 1326, 1256 (epoxide), 1064, 1033, 915, 732. $^1H$- and $^{13}C$-NMR: see *Table*. EI-MS: 314 (7, $[M - OOH + H]^+$), 297 (2), 271 (4), 231 (7), 205 (19), 191 (34), 166 (89), 124 (100). HR-ESI-MS: 366.2268 ($[M + NH_4]^+$; $C_{20}H_{32}NO_5^+$; calc. 366.2280).

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