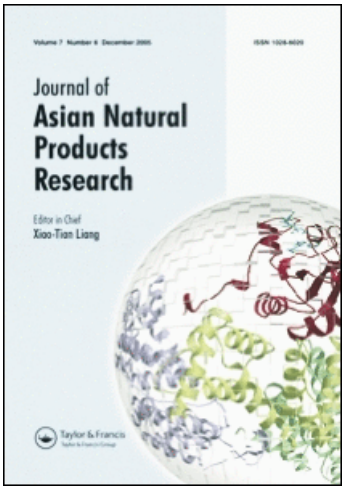

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Two new metabolites from the Hainan soft coral *Sarcophyton crassocaule*

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Two new metabolites, sarcophytonone (**1**) and sarcophytonamine (**2**), have been isolated from the Hainan soft coral *Sarcophyton crassocaule*. Their structures were elucidated on the basis of a detailed analysis of spectroscopic data, and by comparison of their NMR spectral data with those of the related model compounds.

Keywords: soft coral; *Sarcophyton crassocaule*; sarcophytonone; sarcophytonamine

1. Introduction

For recent years, there has been a continuing high number of new and biological secondary metabolites reported from the soft corals [1,2]. Previous works on *Sarcophyton crassocaule* revealed that steroids [3–5], cembrenolides [4,6–10], hippurins [11], and prostaglandins [12] are common metabolites of this soft coral, most of which were reported to exhibit cytotoxic activity.

Recently, in the course of our search for bioactive substances from Chinese marine invertebrates [13], the 95% EtOH extract of the soft coral *S. crassocaule* has been found to have lethal activities towards brine shrimps, *Artemia salina*. The bioassay-guided fractionation of the EtOAc and the *n*-BuOH soluble fractions of the soft coral led to the isolation of a new tetra-substituted quinone sarcophytonone (**1**) and a new quaternary amine sarcophytonamine (**2**), respectively (Figure 1). The present work deals with the isolation and structure elucidation of the new compounds.

2. Results and discussion

Freshly collected specimens of *S. crassocaule* were immediately chilled to $-20^{\circ}C$ and kept frozen until their extraction with 95% EtOH. The EtOH extract was then partitioned between EtOAc and H_2O, and between *n*-BuOH and H_2O. The EtOAc-soluble portion was subjected to repeated column chromatography (silica gel and Sephadex LH-20) to afford sarcophytonone (**1**) (8.0 mg). The *n*-BuOH-soluble portion was applied to repeated column chromatography (silica gel, RP-C18 silica gel, and Sephadex LH-20) to give sarcophytonamine (**2**) (25.6 mg).

Sarcophytonone (**1**) was obtained as an optically active yellow oil with $[\alpha]_D^{25} + 5.82$ ($c = 0.40$, $CHCl_3$). Its ESI-MS displayed a pseudo-molecular ion at *m/z* 373 $[M+Na]^+$. The HR-ESI-MS

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experiment established its molecular formula as $C_{20}H_{30}O_5$ at *m/z* 373.1992 $[M+Na]^+$, indicating six degrees of unsaturation. The UV spectrum of **1** showed absorptions maxima at λ_{max} 255 (log ε 4.0) and 268 (log ε 4.1) nm, similar to those of an α-tocopheryl quinone derivative (**3**), a metabolite previously isolated from the soft coral *Sinularia mayi* [14], indicating the presence of a tetra-substituted quinone system in its molecule. The ^{1}H NMR spectrum of **1** displayed one methoxyl signal at δ_H 3.68 (3H, s, H_3-11) and five methyl signals at δ_H 2.03 (3H, s, H_3-7′), 2.01 (3H, s, H_3-8′), 2.00 (3H, s, H_3-9′), 1.22 (3H, s, H_3-10), and 1.16 (3H, d, $J = 6.9$ Hz, H_3-9). The ^{13}C NMR and DEPT spectroscopic data were in good agreement with the above analysis, and showed 20 carbon signals consisting of one methoxyl, five methyl, five methylene, one methine, and eight quaternary carbons; of which, a group of signals at δ_C 187.7 (s), 187.2 (s), 144.3 (s), 140.6 (s), 140.4(s), and 140.2 (s) confirmed the presence of the tetra-substituted quinone moiety [14]. The methoxycarbonyl group was evident by the NMR signals at δ_H 3.68 (3H, s, H_3-11) and δ_C 177.2 (s, C-1), 51.5 (q, C-11). The final structure of **1** was mainly determined by the extensive study of 2D NMR (^{1}H–^{1}H COSY, HMQC, and HMBC) spectra (Figure 1).

Analysis of ^{1}H–^{1}H COSY (Figure 2) and HMQC spectra, in combination with the ^{13}C NMR spectral data, readily identified two spin–spin systems [**a** (C-7 to C-8) and **b** (C-2 to C-5 and C-9)]. Furthermore, significant HMBC (Figure 2) correlations between H_3-9 (δ_H 1.16), H_2-3 (δ_H 1.39, 1.68) and H_3-11 (δ_H 3.68)/C-1 (δ_C 177.2) suggested that the methoxycarbonyl group was connected to the structure fragment **b** through the quaternary carbon C-1, while HMBC correlations between H_3-10 (δ_H 1.22)/C-5 (δ_C 41.7) and C-7 (δ_C 40.2) supported the linkage of structure fragments **a** and **b** through the quaternary carbon C-6. The HMBC correlations between H_2-8 and C-2′ and C-6′ indicated that C-8 was linked to C-1′. Thus, the structure of **1** was established as shown in Figure 2.

Sarcophytonamine (**2**) was obtained as a white amorphous powder. The molecular formula of **2** was established to be $C_8H_{18}N_2O_5$ from a quasi-molecular ion peak at *m/z* 245.1116 $[M+Na]^+$ by positive HR-ESI-MS. The UV spectrum of **2** showed absorption maximum at λ_{max}

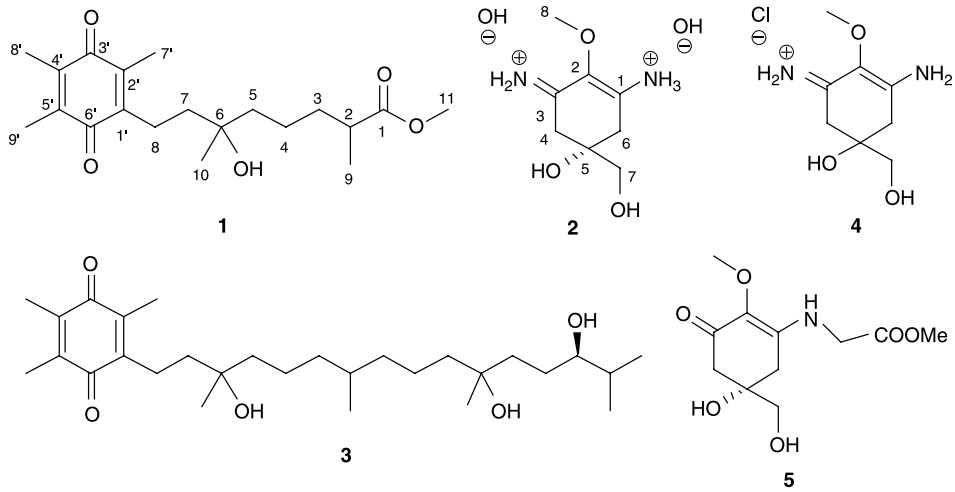

Figure 1. Structures of compounds **1**–**5**.

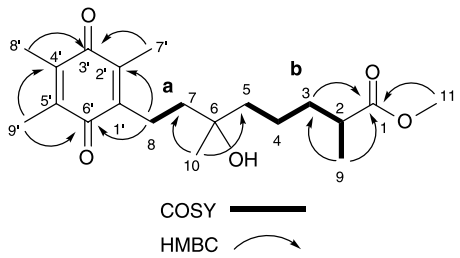

Figure 2. Selected ^{1}H–^{1}H COSY and HMBC correlations for compound **1**.

318 (log ε 4.5) nm, similar to that of an aminocyclohexenimine (**4**), a degradation product of mytilins A and B [15]. In the ^{1}H NMR spectrum (Table 1), **2** displayed the presence of one methoxyl at δ_H 3.52 (3H, s, H_3-8), three methylenes at δ_H 2.58 (1H, d, $J = 17.4$ Hz, H-6a), 2.79 (1H, d, $J = 17.4$ Hz, H-6b), 2.42 (1H, d, $J = 17.0$ Hz, H-4a), 2.77 (1H, d, $J = 17.0$ Hz, H-4b), and 3.26 (2H, d, $J = 4.1$ Hz, H_2-7). According to the ^{13}C NMR and DEPT spectroscopic data, compound **2** has eight carbon signals attributed to one methoxyl at δ_C 59.4, three methylenes at δ_C 68.2, 37.0, and 34.8, and four quaternary carbons at δ_C 162.0, 160.2, 124.9, and 71.6. The above-mentioned structural features of **2** were strongly reminiscent of those of **4** [15]. Careful comparison of their NMR spectral data led to give a quaternary amine as depicted in **2**. Furthermore, the HMBC correlations (Table 1) between H_3-8 (δ_H 3.52), H_2-4 (δ_H 2.42, 2.77), H_2-6 (δ_H 2.58, 2.79), and C-2 (δ_C 124.9) as well as H_2-7 (δ_H 3.26) and C-4 (δ_C 37.0) and C-6 (δ_C 34.8) confirmed the above analysis. The absolute configuration at C-5 was tentatively assigned (*S*) by comparison of the sign of the optical rotation of **2** $\{[\alpha]_D^{25} - 15.3$ ($c = 0.20$, H_2O)$\}$ and the methyl ester of mycosporine-Gly (**5**) $\{[\alpha]_D^{25} - 12$ ($c = 0.40$, H_2O)$\}$ [16].

Sarcophytonone (**1**) was a trimethyl-substituted quinone derivative consisting of a terpene moiety. It may be worthy to point out that these types of quinone derivatives are quite rare in the marine materials. To the best of our knowledge, there is only one analogue isolated before from the soft coral *S. mayi* [14]. Sarcophytonamine (**2**) was an analogue of aminocyclohexenimine. It has been suggested that these kinds of

Table 1. NMR spectral data of compound **2**[a] and ^{13}C NMR spectral data of compound **4**[b] (δ in ppm, *J* in Hz).

	2		**4**[c]	
Position	δ_H, *J* (Hz)	δ_C	HMBC (H—C)	δ_C
1		162.0 (s)[d]		164.5 (s)
2		124.9 (s)		126.4 (s)
3		160.2 (s)[d]		164.5 (s)
4a	2.42 (d, $J = 17.0$)	37.0 (t)[d]	C-2, C-3, C-5, C-6	38.1 (t)
4b	2.77 (d, $J = 17.0$)		C-2, C-3, C-5, C-6	
5		71.6 (s)		72.8 (s)
6a	2.58 (d, $J = 17.4$)	34.8 (t)[d]	C-1, C-2, C-4, C-5	38.1 (t)
6b	2.79 (d, $J = 17.4$)		C-1, C-2, C-4, C-5	
7	3.26 (d, $J = 4.1$)	68.2 (t)	C-4, C-6	69.2 (t)
8	3.52 (s)	59.4 (q)	C-2	59.4 (q)
OH or NH	8.60 (s)		C-2	
	8.52 (s)			
	8.24 (s)		C-2, C-4	

Notes: [a]In DMSO-d_6, ^{1}H NMR (600 MHz); ^{13}C NMR (150 MHz); chemical shifts (in ppm) are referenced to the residual DMSO (δ_H 2.50, δ_C 39.5).
[b] In CD_3OD.
[c] See Ref. [15].
[d] Signals may be interchangeable.

compounds provide protection for the organism from UV radiation damage [17].

At a concentration of 50 μg/ml, compounds **1** and **2** showed lethality to the brine shrimp *A. salina* with lethal rates of 61.2 and 32.3%, respectively. Further studies should be conducted to understand the real biological/ecological role of these metabolites in the life cycle as well as to test their biological activities.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a JASCO P-1020 digital polarimeter. IR spectrum was recorded on a Nicolet Nexus 470 spectrophotometer. UV spectra were recorded on Beckman DU 640 UV spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL Eclips-600 (600 MHz for ^{1}H and 150 MHz for ^{13}C) spectrometer. Chemical shifts (δ) are reported in ppm relative to an internal TMS standard and coupling constant (J) is reported in Hz. ^{1}H and ^{13}C NMR assignments were supported by ^{1}H–^{1}H COSY, HMQC, and HMBC experiments. ESI-MS and HR-ESI-MS were recorded on a Q-TOF Ultima Global GAA076 LC mass spectrometer in *m/z*. Column chromatography: commercial silica gel (200–300 mesh; Qingdao Marine Chemical Group Co., Qingdao, China), octadecylsilyl (ODS) silica gel (45–60 μm; Merck KGaA, Darmstadt, Germany), and Sephadex LH-20 (Amersham Biosciences Inc., Piscataway, NJ, USA).

3.2 Collection of biological materials

Specimens of the soft coral *S. crassocaule* were collected off the Lingshui Bay, Hainan Province, China, in April 2006, and identified by Prof. Hui Huang of South China Sea Institute of Oceanology, Chinese Academy of Sciences. A voucher specimen (SYC-32) is available for inspection at the Herbarium of Ocean University of China.

3.3 Extraction and isolation

The frozen specimen (400 g, dry weight) was cut into pieces and extracted exhaustively with 95% EtOH at room temperature. The organic extract was evaporated to give a residue (15 g), which was partitioned between EtOAc and H_2O, and *n*-BuOH and H_2O, respectively. The EtOAc-soluble portion (7.7 g) was fractionated by silica gel column chromatography (petroleum ether/EtOAc, 0 → 100%) yielding 11 fractions (1–11). Fraction 4 (84.0 mg) was subjected to Sephadex LH-20 column chromatography eluted with $CHCl_3$ to give fractions 4-1, 4-2, and 4-3. Fraction 4-2 (16.6 mg) was further purified by silica gel column chromatography and eluted with petroleum ether/acetone (85:15) to yield sarcophytonone (**1**) (8.0 mg). The *n*-BuOH solution was evaporated to give a dark brown residue (1.3 g). The residue was fractionated by silica gel column chromatography ($CHCl_3$/MeOH, 0 → 100%) to yield five fractions (A–F). Fraction F was purified by column chromatography (Sephadex LH-20, MeOH) followed by reversed-phase silica gel (ODS) column chromatography to afford sarcophytonamine (**2**) (25.6 mg).

Sarcophytonone (**1**). A yellow oil; $[\alpha]_D^{25} + 5.82$ ($c = 0.40$, $CHCl_3$); UV (MeOH): λ_{max} 255 (log ε 4.0), 268 (log ε 4.1) nm; IR ν_{max} (KBr) cm^{-1}: 3747, 3649, 2926, 1733, 1640, 1534, 1448, 672; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 3.68 (3H, s, H_3-11), 2.52 (2H, m, H_2-8), 2.47 (1H, m, H-2), 2.03 (3H, s, H_3-7′), 2.01 (3H, s, H_3-8′), 2.00 (3H, s, H_3-9′), 1.68 (1H, m, Ha-3), 1.44–1.52 (4H, m, H_2-5, H_2-7), 1.39 (1H, m, H_b-3), 1.37 (2H, m, H_2-4), 1.22 (3H, s, H_3-10), 1.16 (3H, d, $J = 6.9$ Hz, H_3-9); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 187.7 (s, C-3′), 187.2 (s, C-6′), 177.2 (s, C-1), 144.3 (s, C-1′), 140.6 (s, C-2′), 140.4 (s, C-4′), 140.2 (s, C-5′), 72.5 (s, C-6), 51.5 (q, C-11), 41.7 (t, C-5), 40.2 (t, C-7), 39.4 (d, C-2), 34.2 (t, C-3), 26.5 (q, C-10), 21.6 (t, C-4), 21.3 (t, C-8), 17.1 (q, C-9), 12.4 (q, C-7′), 12.3

(q, C-8′), 12.0 (q, C-9′); ESI-MS: *m/z* 373 $[M+Na]^+$; HR-ESI-MS: *m/z* 373.1992 $[M+Na]^+$ (calcd for $C_{20}H_{30}O_5Na$, 373.1991).

Sarcophytonamine (**2**). A white amorphous powder; $[\alpha]_D^{25} - 15.3$ ($c = 0.20$, H_2O); UV (MeOH): λ_{max} 318 ($\log \varepsilon$ 4.5) nm; IR ν_{max} (KBr) cm^{-1}: 3430, 3200, 2929, 1275, 1080, 965; ^1H and ^{13}C NMR spectral data: see Table 1; ESI-MS: *m/z* 245 $[M+Na]^+$; HR-ESI-MS: *m/z* 245.1116 $[M+Na]^+$ (calcd for $C_8H_{18}N_2O_5Na$, 245.1113).

Bioassays. The brine shrimp, *A. salina*, lethality assay was performed according to standard protocols [18].

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