Two New Diterpenoids from the Beibu Gulf Gorgonian *Anthogorgia caerulea*

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Two new diterpenoids, anthogonoid A and antsimplexin A (**1**–**2**), as well as a known diterpenoid, klysimplexin G (**3**), were isolated from a Beibu Gulf gorgonian coral, *Anthogorgia caerulea*. The structures of these compounds have been established by detailed spectroscopic analysis and by comparison with spectral data of related known compounds. Compounds **1**–**3** showed significant antifouling activity against the larval settlement of *Balanus amphitrite*.

Key words: Anthogorgia caerulea, Diterpenoid, Antifouling Activity

Introduction

Gorgonian corals have been proven to be rich sources of natural diterpenoid derivatives [1, 2], and some of these diterpenoids possess antifouling activity [3 – 5]. Gorgonian corals of the genus *Anthogorgia* (family Acanthogorgiidae) are prolific in the South China Sea, specially in the Beibu Gulf (Guangxi Province, China). Pertinent literature indicates that the chemical constituents of the gorgonian corals of the genus *Anthogorgia* are mainly diterpenes and polyhydroxylated steroids [6 – 9]. In our study of bioactive compounds from the gorgonian coral *A. caerulea* collected from Beibu Gulf (Guangxi Province, China), two new diterpenoids, anthogonoid A and antsimplexin A (**1**–**2**), as well as a known diterpenoid, klysimplexin G (**3**) [10] (Fig. 1), were obtained. In this paper, we describe the isolation and structure elucidation of the new compounds and the antifouling activity of compounds **1**–**3**.

Results and Discussion

Compound **1** was obtained as a yellow oil. The HR-ESI-MS of **1** revealed a quasi-molecular ion peak at m/z = 445.2204 (calcd. 445.2202 for $C_{23}H_{34}O_7Na$, $[M+Na]^+$) consistent with the molecular formula $C_{23}H_{34}O_7$ and seven degrees of unsaturation. The IR spectrum displayed absorption bands diagnostic of hydroxyl and five-membered lactone and ester groups from absorptions at 3525, 2923, 1772, 1740, 1230, and 1035 cm^{-1}. The ^{13}C NMR data for **1** confirmed the presence of 23 carbon signals (Table 1), characterized by DEPT as five methyls, seven sp^3 methylenes, four sp^3 methines, two sp^3 quaternary carbons, and five sp^2 quaternary carbons. Detailed inspection of the ^{1}H and ^{13}C NMR data (Table 1) of **1** indicated the presence of the key structural feature of a briarane skeleton with a lactone and a methoxy group. A suite of resonances at δ_C = 172.0 (C-19), 156.2 (C-8), 127.4 (C-17), 110.3 (C-7), and 8.6 ppm (C-18) could be assigned to the α-methyl-α,β-unsaturated-γ-lactone moiety in **1**. The resonances of an acetate substituent were displayed at δ_H = 2.06 ppm (3H, s) and δ_C = 23.3, 171.1 ppm. An additional unsaturated functionality was indicated by a ^{13}C NMR resonance at δ_C = 207.3 ppm (C-4), suggesting the presence of a ketonic group. Thus, from the reported data, the skeleton of **1** was suggested to be a briarane diterpenoid with three rings. From the ^{1}H–^{1}H COSY spectrum of **1** (Fig. 2), it was possible to differentiate between the separate spin systems of -CH_2–CH_2-, -CH_2–CH–CH- and

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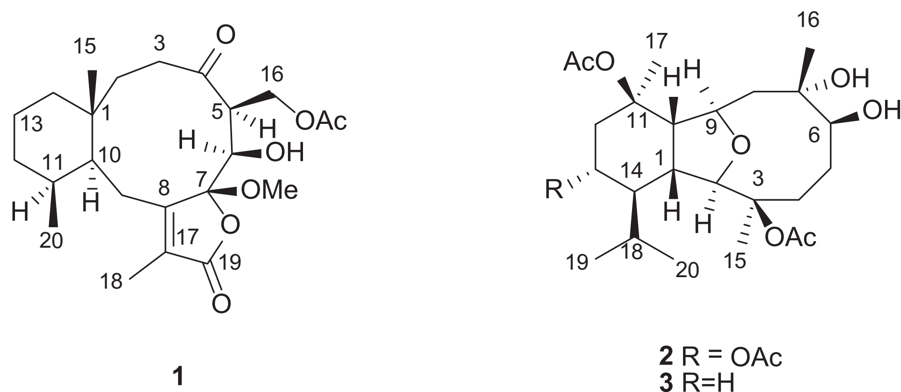

Fig. 1. Structure of compounds **1**–**3**.

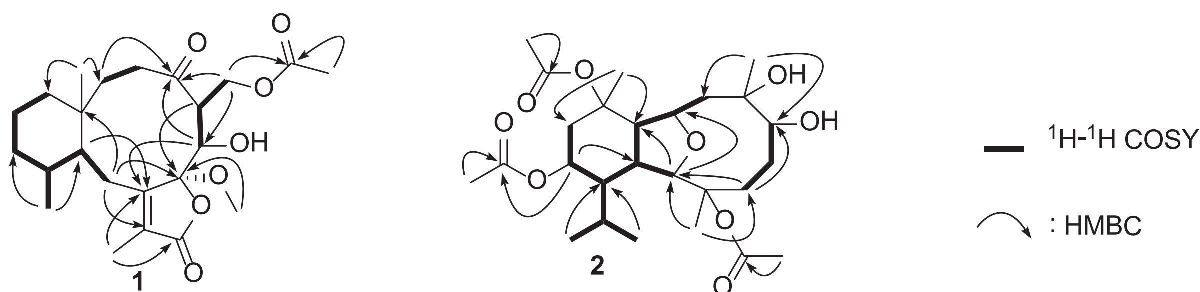

Fig. 2. Key ^{1}H-^{1}H COSY and HMBC correlations of **1** and **2**.

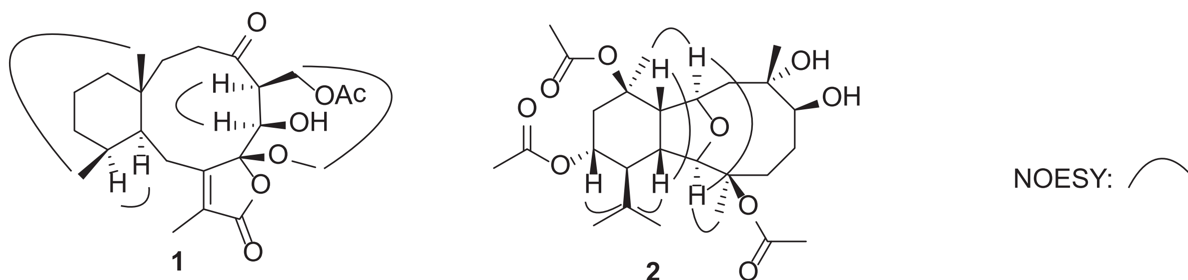

Fig. 3. Key NOESY correlations of **1** and **2**.

-CH_2–CH–CH(CH_3)–CH_2–CH_2–CH_2-. These data, together with the key HMBC correlations between H_2-2 and C-4; H-5 and C-7; H_2-16 and C-4, C-6, 16-OAc; H-6 and C-4, C-8; 7-OMe and C-7; H_3-18 and C-8, C-19; H_2-9 and C-1, C-7, C-17; H-10 and C-8; H_3-20 and C-10, C-12; and H_3-15 and C-2, C-14 (Fig. 2), permitted the elucidation of the carbon skeleton of **1**. In the NOESY experiment (Fig. 3) for **1**, H_3-20 exhibited a correlation with H_3-15, not with H-10, indicating that these two protons (H_3-20 and H_3-15) were situated on the same face and thus assigned as β-protons. In addition, H_2-16 exhibited a correlation with 7-OMe, indicating that these two sets of protons (H_2-16 and 7-OMe) were situated on the same face and thus assigned as β-protons. H-5 displayed a correlation with H-6, implying that the two protons (H-5 and H-6) are situated on the same face and thus to be assigned as α-protons. From the above evidence, the configurations of the chiral carbons of **1** have been assigned as 1*R**, 5*S**, 6*R**, 7*R**, 10*S**, and 11*S**, and thus the structure of **1** determined and designated anthogonoid A.

	1			2	
Position	δ_C	δ_H	Position	δ_C	δ_H
1	29.1 (s)		1	46.0 (d)	2.14 (dd, 11.0, 7.0)
2α	29.4 (t)	1.92 (m)	2	93.4 (d)	3.50 (s)
2β		1.90 (m)			
3α	40.1 (t)	2.59 (m)	3	86.0 (s)	
3β		2.52 (m)			
4α	207.3 (s)		4	36.4 (t)	2.63 (dd, 14.5, 8.4)
4β					1.81 (m)
5α	29.5 (d)	2.04 (m)	5	30.7 (t)	1.61 (m)
5β					1.42 (m)
6	61.2 (d)	4.66 (d, 5.6)	6	80.6 (d)	4.57 (d, 6.0)
7	110.3 (s)		7	77.4 (s)	
8α	156.2 (s)		8	47.4 (t)	2.01 (dd, 11.2, 3.8)
8β					1.87 (dd, 11.2, 6.0)
9α	23.6 (t)	1.68 (dd, 10.2, 4.5)	9	76.4 (d)	4.09 (td, 11.0, 3.8)
9β		1.60 (dd, 10.2, 5.0)			
10	35.6 (d)	1.96 (m)	10	51.0 (d)	3.31 (t, 7.2)
11	29.4 (d)	1.58 (m)	11	83.1 (s)	
12	29.3 (t)	1.57 (m)	12	38.5 (t)	2.25 (dd, 12.1, 4.6)
					1.48 (dd, 12.1, 6.0)
13	22.8 (s)	1.54 (m)	13	69.2 (d)	3.95 (td, 10.5, 3.6)
14	29.5 (t)	1.65 (m)	14	44.0 (d)	1.18 (m)
15	23.3 (q)	1.23 (s)	15	23.4 (q)	1.41 (s)
16	63.4 (t)	3.96 (d, 4.6)	16	22.9 (q)	1.18 (s)
17	127.4 (s)		17	24.6 (q)	1.51 (s)
18	8.6 (q)	1.85 (s)	18	30.2 (d)	1.73 (m)
19	172.0 (s)		19	24.0 (q)	1.04 (d, 7.0)
20	11.0 (q)	1.25 (d, 6.0)	20	16.3 (q)	0.91 (d, 7.0)
7-OMe	50.3 (q)	3.07 (s)			
16-OAc	23.3 (CH_3, q)	2.06 (s)			
	171.1 (s)				
3-OAc				22.4 (CH_3, q)	2.09 (s)
				170.2 (s)	
11-OAc				21.7 (CH_3, q)	2.02 (s)
				169.9 (s)	
13-OAc				22.7 (CH_3, q)	2.05 (s)
				170.4 (s)	

Table 1. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data of **1** (in $CDCl_3$) and **2** (in $CDCl_3$). Chemical shifts δ in ppm, multiplicities and J values (in Hz) in parentheses.

Compound **2** was obtained as a yellow oil with a molecular formula of $C_{26}H_{42}O_9$, implying five degrees of unsaturation, as established by the HR-ESI-MS ($m/z = 499.2847$; calcd. 499.2902 for $C_{26}H_{43}O_9$, $[M+H]^+$). The IR spectrum of **2** revealed the presence of hydroxy and carbonyl functionalities from absorptions at 3453 and 1731 cm^{-1}. The ^{13}C NMR spectrum of **2** exhibited 26 carbon signals (Table 1), which were assigned by the assistance of a DEPT experiment to eight methyls, four sp^3 methylenes, eight sp^3 methines, three sp^3 quaternary carbons, and three sp^2 quaternary carbons. The ^{1}H and ^{13}C NMR spectra also revealed that **2** is a eunicellin-based compound (Table 1). Three ester carbonyls (δ_C = 170.4, 170.2 and 169.9 ppm) were assigned in the ^{13}C NMR spectrum and were HMBC correlated with acetate methyls [δ_H = 2.05 (3H, s), 2.09 (3H, s) and 2.02 ppm (3H, s), respectively]. Therefore, the remaining three degrees of unsaturation identified compound **2** as a tricyclic compound. In the ^{1}H NMR of **2** (Table 1), two doublets at δ_H = 1.04 and 0.91 ppm (each 3H, d, J = 7.0 Hz) were observed for two methyls of one isopropyl group. Furthermore, the three singlets of the tertiary methyls bonded to oxygenated carbons at δ_H = 1.18, 1.41 and 1.51 ppm (each, 3H, s) are due to the resonances of H_3-16, H_3-15 and H_3-17, respectively. Signals resonating at δ_H = 2.14 (1H, dd, J = 11.0, 7.0 Hz), 3.31 (1H, t, J = 7.2 Hz), 3.50 (1H, s), and 4.09 ppm (1H, td, J = 11.0, 3.8 Hz), and at δ_C = 46.0 (C-1), 51.0 (C-10), 93.4 (C-2), and 76.4 ppm (C-9), indicated the presence of a tetrahydrofuran structural unit [11]. The gross structure of the metabolite **2** was elucidated by an analysis of ^{1}H–^{1}H COSY and HMBC correlations (Fig. 2). From the ^{1}H–^{1}H COSY spectrum of **2**, it was possi-

Table 2. Antilarval settlement activities of **1**–**3** against *B. amphitrite*.

Compound	EC_{50} ($\mu g\,mL^{-1}$)	LC_{50} ($\mu g\,mL^{-1}$)
1	0.56	> 200
2	5.28	> 200
3	2.83	> 200

ble to identify three structural units, which were further assembled by HMBC correlations (Fig. 2). Key HMBC correlations from H-2 to C-9 and C-10; H_2-4 to C-2 and C-6; H_3-15 to C-2 and C-4; H_3-16 to C-6 and C-8; H_3-17 to C-10 and C-12; H-13 to C-1 and 13-OAc; and both H_3-19 and H_3-20 to C-14, permitted the connectivity of the carbon skeleton. By means of extensive 2D NMR experiments (COSY, HMQC and HMBC), the structure of **2** was found to be close to that of klysimplexin F which was isolated from the soft coral *Klyxum simplex* [10] except that the *n*-butyryloxy group at C-3 and the hydroxy group at C-13 in klysimplexin F are replaced by two acetoxy groups in **2**. The relative configurations of all chiral centers in **2** were confirmed to be the same as those of klysimplexin F by comparison of the proton shifts, coupling constants, and NOESY correlations (Fig. 3). H_3-16 was found to exhibit an NOESY correlation with H-5β but not with H-6, revealing the β-orientation of the hydroxy group at C-6 and an α-orientation of the hydroxy group at C-7. Thus, the structure of diterpenoid **2** has been established.

Compounds **1**–**3** were evaluated for antilavral (*B. amphitrite*) settlement activity. The results (Table 2) have shown that the ED_{50} values of compounds **1**–**3** are 0.56, 5.28 and 2.83 $\mu g\,mL^{-1}$, respectively, and the LD_{50} values of compounds **1**–**3** are larger than 200 $\mu g\,mL^{-1}$, in comparison with the control dimethyl sulfoxide (DMSO).

Experimental Section

General

UV spectra were recorded in MeOH on a Perkin-Elmer Lambda 35 UV/Vis spectrophotometer. The IR spectra were measured in KBr on a WQF-410 FT-IR spectrophotometer. NMR spectra were recorded on a Bruker AC 600 NMR spectrometer with TMS as internal standard. HR-ESI-MS data were obtained from a Bruker Maxis mass spectrometer. Chromatography: Waters-2695 HPLC system, using a Sunfire^{TM} C_{18} column (250 × 10 mm i. d., 10 μm) coupled to a Waters 2998 phothodiode array detector. Optical rotation data were measured on a Perkin-Elmer Model 341 polarimeter. The silica gel GF_{254} used for TLC were supplied by the Qingdao Marine Chemical Factory, Qingdao, P. R. China. Spots were detected on TLC under UV light or by heating after spraying with 5 % H_2SO_4 in EtOH (*v*/*v*).

Animal material

The *A. caerulea* was collected from Beibu Gulf, South China Sea, in August 2010. The specimen was identified by Dr. Xiubao Li. A voucher specimen (No. 2010-GXAS-2) has been deposited at Guangxi Key Laboratory of Marine Environmental Science, Guangxi Academy of Sciences, P. R. China.

Extraction and isolation

The *A. caerulea* (5 kg, wet, wt) was extracted with ethanol (95 %). The EtOAc extract was concentrated *in vacuo* to afford 24.82 g of a residue, which was subjected to column chromatography (CC) on silica gel, using $CHCl_3$-Me_2CO (10 : 0, 50 : 1, 25 : 1, 3 : 1; *v*/*v*) and $CHCl_3$-MeOH (9 : 1, 8 : 2, 0 : 10; *v*/*v*) as eluents, giving eleven fractions (A–J). Fraction F was subjected to column chromatography, using $CHCl_3$-MeOH (1 : 1; *v*/*v*) as eluent, then it was separated by HPLC, using mixtures of MeOH and H_2O (MeOH-H_2O = 40 : 60, *v*/*v*) to yield **1** (5.3 mg), **2** (3.5 mg) and **3** (2.0 mg).

Anthogonoid A (**1**): Yellow oil. – UV (MeOH): $\lambda_{max}(\log\varepsilon_{max}) = 204$ (2.75) and 282 (3.45) nm. - $[\alpha]_D^{20} = -26.2^\circ$ ($c = 0.13$, MeOH). – IR (KBr): $\nu_{max} = 3525$, 2923, 1772, 1740, 1230 and 1035 cm^{-1}. – ^1H and ^{13}C NMR data: see Table 1. – HRMS ((+)-ESI): $m/z(\%) = 445.2204$ (100) (calcd. 445.2202 for $C_{23}H_{34}O_7Na$, $[M+Na]^+$).

Antimplexin A (**2**): Yellow oil ($CHCl_3$). – UV (MeOH): λ_{max} $(\log\varepsilon_{max}) = 210(3.65)$ nm. – $[\alpha]_D^{20} = -48.2^\circ$ ($c = 0.12$, MeOH). – IR (KBr): $\nu_{max} = 3453$ and 1731 cm^{-1}. – ^1H and ^{13}C NMR data: see Table 1. – HRMS ((+)-ESI): $m/z(\%) = 499.2847$ (100) (calcd. 499.2902 for $C_{26}H_{43}O_9$, $[M+H]^+$).

Larval settlement bioassay

The larval settlement bioassay was carried out by the reported method [12, 13], using sterile 24-well polystyrene plates (∅ = 48 mm; Falcon # 1006, Becton Dickinson, USA). Compounds **1**–**3** in DMSO were dissolved to concentrations ranging from 0.1 to 200 $\mu g\,mL^{-1}$ in autoclaved 0.22 μm-filtered seawater at 30 ppt salinity (FSW). About 20 competent larvae were added to each well in 1 mL of the test solution. The experiment was repeated twice with four replicates each time. Wells containing only FSW with DMSO served as the controls. The plates were incubated at 27 °C for 1 h for *B. amphitrite*. The percentage of larval settlement was determined by counting the settled, live individuals under

a dissecting microscope and expressing the result as a proportion of the total number of larvae in the well. Statistical calculations were performed with the SPSS (version 11) software package. Differences in the larval settlement between the experimental treatments and controls were determined by one-way ANOVA followed by a Dunnett test. EC_{50} (inhibits 50 % of settlement of *B. amphitrite* larvae in comparison with the control) and LC_{50} (the concentration at which 50 % of larvae were dead in comparison with the control) levels of compounds **1**–**3** were calculated by using the probit software program.

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