The Journal of Antibiotics (2010) 63, 699–701

www.nature.com/ja

ORIGINAL ARTICLE

Two novel alkaloids from the South China Sea marine sponge *Dysidea* sp.

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Two new alkaloids, dysideanins A (1) and B (2), along with two known diketopiperazines, cyclo-(Pro-Leu) (3) and cyclo-(Pro-Ile) (4), were isolated from the marine sponge *Dysidea* sp. The structures were established from NMR and MS analysis. Dysideanin B (2) exhibited antibacterial activity. The thiomethylated imidazolinium unit as found in dysideanin A is very rarely encountered in nature.

The Journal of Antibiotics (2010) **63,** 699–701; doi:10.1038/ja.2010.134; published online 10 November 2010

Keywords: alkaloid; antibacterial; diketopiperazine; *Dysidea* sp.; marine sponge

INTRODUCTION

Marine sponge is the oldest and simplest multicellular animal that is widely spread on earth, and has been proved to be a particularly fruitful source of new compounds with novel structures and bioactivities.[1] The genus *Dysidea* (order Dictyoceratida, family Dysideidae) is known as a rich source of diverse classes of secondary metabolites.[2] About 300 compounds, such as terpenoids,[3–5] steroids,[6,7] peptides[8,9] and polychlorinated metabolites,[10–12] were isolated from the genus *Dysidea*. Many of them have significant bioactivities, such as antibacterial,[9,13–15] anti-inflammatory[16] and cytotoxic.[17,18]

In our study of the bioactive compounds from marine sponges, two new (**1**, **2**) and two known (**3**, **4**) alkaloids were isolated from the marine sponge *Dysidea* sp. The structures of two new metabolites were elucidated by interpretation of spectroscopic data. Compound **1** carried a thiomethylated imidazolium nucleus similar to that found in dragmacidonamine A (**5**)[19] and L-ovithiol A disulfide (**6**).[20] Compound **2** was a protonated dimethylamine group constituting the β-carboline alkaloid, similar to the partial structure of denticin C (**7**).[21] In this paper, the isolation, structure elucidation and biological activity of these compounds are described.

RESULTS AND DISCUSSION

Dysideanin A (**1**) was isolated as colorless crystals, and its molecular formula, $C_8H_{14}N_3O_2S^+$, was established by HR-ESI-MS. The ^1H and ^{13}C NMR spectra of the partial structure (thiomethylated imidazolium) were comparable to those of dragmacidonamine A (**5**).[19] The *N*-methyl singlet at δ_H 3.64 was detected by its corresponding carbon resonance at δ_C 32.8. Observed resonances at δ_H 2.41 and δ_C 18.9 were assigned to a thiomethyl group. The *N*-methyl imidazole ring system was verified from the long-range HMBC correlations of the proton at δ_H 7.55 (H-5) with the *N*-methyl carbon at δ_C 32.8 as well as with the quaternary carbons at δ_C 134.7 (C-2) and 128.8 (C-3). The imidazolium proton H-5 (δ_H 7.55) showed a direct heteronuclear single-quantum coherence (HSQC) correlation with the carbon resonating at δ_C 139.1, which was also comparable to that found in **5**. Furthermore, the analysis of NMR data indicated the presence of a carboxylic acid methyl ester (carbonyl carbon, δ_C 172.5, C-7; a methoxy group, δ_H 3.82, δ_C 53.4) and a de-shielded sp^3 aminomethine (δ 5.55, 1H, s, H-6; 64.5, C-6). Analysis of the HMBC data confirmed that the chiral carbon (C-6) was substituted by both NH_2 and methoxycarbonyl moieties. The absolute configuration of C-6 remains to be determined. HMBC correlations from N-Me to C-2, C-3 and C-5, and from H-6 to C-2 and C-3, suggested that dysideanin A was the substituted imidazole ring as shown. Homonuclear (COSY) and heteronuclear (HSQC and HMBC) correlations were used to establish assignments and atom connectivities (Table 1). Thus, the structure of dysideanin A was unambiguously elucidated as **1** (Figure 1).

Dysideanin B (**2**) was obtained as an orange amorphous powder. The molecular formula $C_{14}H_{17}N_3O$ was established by HR-ESI-MS (*m/z* $[M+H]^+$: 243). The ^1H NMR spectra of **2** exhibited three aromatic protons at δ_H 7.47 (1H, d, J=8.5 Hz, H-8), 7.19 (1H, dd, J=2.2, 8.5 Hz, H-7) and 7.58 (1H, brs, H-5) engaged in a 1,2,4-trisubstituted benzene ring and two additional vicinal aromatic protons at δ_H 8.08 (1H, d, J=4.9 Hz, H-3) and 8.55 (1H, d, J=4.9 Hz, H-4), the coupling constants of which were typical of *ortho* protons on a pyrimidine ring. The remaining proton signals

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Received 15 July 2010; revised 15 September 2010; accepted 8 October 2010; published online 10 November 2010

were assigned to a methoxyl singlet at δ_H 4.13 and two N-methyl signals at δ_H 3.50, which were readily detected with the corresponding carbon signals at δ_C 52.8 and 29.7, respectively. Moreover, long-range correlations were observed from the aromatic protons at δ_H 7.58 and 7.47 to the quaternary carbon at δ_C 135.7 and from the aromatic protons at δ 8.55 and 8.08 to the quaternary carbons at δ 121.7 and 138.4, establishing the presence of a 1,6-disubstituted β-carboline moiety. A long-range correlation was found from the proton signal at δ_H 7.58 to the carbon resonating at δ_C 150.3, which positioned the methoxyl group at position C-6 on the β-carboline ring. ^{1}H NMR data showed the presence of a dimethylammonium group. The carbon assignments and multiplicities for compound **2** were confirmed by both HSQC and HMBC experiments. The position of the methoxyl function was confirmed through HMBC correlations and by comparison of its ^{1}H and ^{13}C NMR data with those of both 6-hydroxy-β-carboline alkaloids (gesashidine A[22] and dragmacidonamine A[19]). The appearance of a broad singlet at δ_H 3.50 integrating for six protons revealed the presence of an *N*,*N*-dimethyl quaternary ammonium function. The position of the *N*,*N*-dimethyl quaternary ammonium function was confirmed on observing a HMBC long-range correlation between the methyl protons at δ_H 3.50 and C-1 at δ_C 167.3. Thus, the structure of dysideanin B was unambiguously elucidated.

The ^{1}H and ^{13}C NMR data of compounds **3** and **4** were in agreement with those previously reported for diketopiperazines,[23] namely cyclo-(Pro-Leu) and cyclo-(Pro-Ile).

Table 1 ^{1}H and ^{13}C NMR data, and HMBC correlations of compound 1 (500/125 MHz, in $CDCl_3$)

Position	*^{1}H NMR*	*^{13}C NMR*	*HMBC (H→C)*
2		134.7	
3		128.8	
5	7.55 (1H, s)	139.1	C-2, C-3
6	5.55 (1H, s)	64.5	C-2, C-3, C-7
7		172.5	
OCH_3	3.82 (3H, s)	53.4	C-7
NCH_3	3.64 (3H, s)	32.8	C-2, C-3, C-5
SCH_3	2.41 (3H, s)	18.9	C-2, C-5

The thiomethylated imidazolinium unit found in dysideanin A is very rarely encountered in nature. Dysideanin A (**1**) may be related to a family of thiol-containing amino acids, namely ovothiols A–C,[24] and L-ovithiol A disulfide.[20,25] Dysideanin B (**2**) is structurally related to dragmacidonamines A and B,[19] hyrtiomanzamine,[26] gesashidine A[22] and didemnolines A–D.[27,28]

Owing to the known antibiotic activity of β-carboline alkaloids[28] and diketopiperazines,[29] compounds **1**–**4** were subjected to an antibacterial agar diffusion assay. Dysideanin B (**2**) exhibited antibacterial activity that showed zones of inhibition toward *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Vibrio alginolyticus* (20 μg per 5-mm ϕ disk, 8.0, 7.5, 8.0 and 8.5 mm, respectively), whereas compounds **1**, **3** and **4** were not active.

EXPERIMENTAL PROCEDURE

General experimental procedures

The NMR spectra were recorded on a Bruker AC 500 NMR spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) with tetramethylsilane (TMS) as an internal standard. ESI-MS data were measured on an Agilent 1200 LC-MS spectrometer (Agilent Inc, MA, USA). HR-ESI-MS data were measured on a Bruker Daltonics APEX II 47e spectrometer. The silica gel used for TLC was supplied by the Qingdao Marine Chemical Factory, Qingdao, China. YMC gel (YMC Co., Ltd, Kyoto, Japan, ODS-A, 12 nm, S-50 μm) was used for column chromatography. Spots were detected on TLC under UV light or by heating after spraying with 10% H_2SO_4 in EtOH (v/v).

Animal material

The sponge was collected by hand in August 2005, at Lingshui County of Hainan Island, China. The specimen was identified by Dr Kyung Jin Lee, Wildlife Genetic Resources Center, National Institute of Biological Resources, Environmental Research Complex, Incheon, Korea. A voucher specimen (0507001) has been deposited at the Key Laboratory of Marine Bio-resources Sustainable Utilization, South China Sea Institute of Oceanology, Chinese Academy of Sciences.

Extraction and isolation

The fresh sponge (3 kg wet wt.) was extracted three times with 95% EtOH at room temperature. The extraction was filtered through cotton wool and the solvent removed by rotary evaporation, then partitioned with H_2O and $CHCl_3$. The $CHCl_3$ layer was further partitioned by 70% EtOH and hexane to yield a

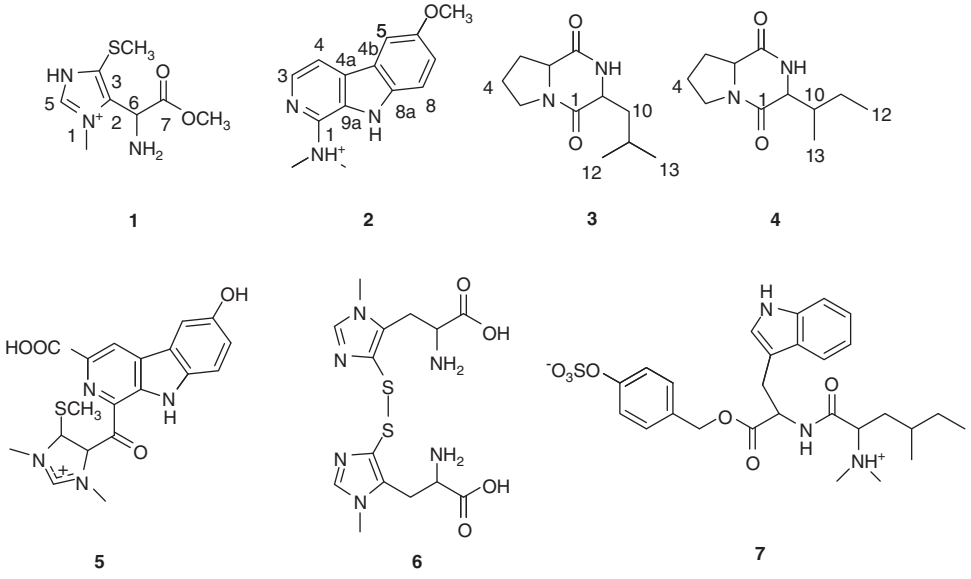

Figure 1 Structures of **1**–**7**.

70% EtOH (13 g) fraction. The 70% EtOH soluble fraction was subjected to reverse-phase column chromatography (YMC gel ODS-A, 12 nm, S-50 μm, 10×20 cm), by eluting with a solvent system of 40→100% MeOH, to afford six fractions. Fraction 1 (40% MeOH portion) was further separated by reverse-phase column chromatography (YMC gel ODS-A, 2.5×80 cm), by eluting with 20→60% MeOH, to afford 11 fractions (A1-A11). Fraction A7 was purified with silica gel column ($CHCl_3$/MeOH, 30:1→15:1) several times to afford **1** (3.0 mg). Fraction A11 was separated by reverse-phase column chromatography (YMC gel ODS-A, 1.0×80 cm, MeOH/H_2O, 2:1) to afford compounds **3** (2.5 mg) and **4** (2.0 mg). Fraction 4 was further separated by a silica gel column (silica gel, 2.5×80 cm), by eluting with $CHCl_3$/MeOH (25:1→1:1), to afford 32 fractions. Fractions 13–16 were combined by monitoring the orange spot and separated by ODS gel column (MeOH/H_2O, 4:1) to provide **2** (4.3 mg).

Antibacterial activity

Compounds **1–4** were tested for antibacterial activity against *B. subtilis*, *S. aureus*, *E. coli* and *V. alginolyticus* using a modified disk diffusion assay. Agar plates were prepared seeded with suspensions of bacteria by adding 20 ml of autoclaved Antibiotic Medium 2 (LB; BD Difco, Franklin Lakes, NJ, USA). Following incubation at 37 °C for 18 h, zones of inhibition resulting from compounds **1–4** were measured.[30]

Dysideanin A (**1**). Colorless crystals. ^{1}H and ^{13}C NMR (500/125 M Hz, $CDCl_3$), see Table 1. ESI-MS *m/z* $[M+H]^+$: 217, $[M+K]^+$: 255; HR-ESI-MS *m/z* 217.0868 $[M+H]^+$ (calcd. for $C_8H_{15}N_3O_2S^+$ 217.0879).

Dysideanin B (**2**). Orange amorphous powder. ^{1}H-NMR (500 M Hz, $CDCl_3$), δ 7.47 (1H, d, J=8.5 Hz, H-8), 7.19 (1H, dd, J=2.2, 8.5 Hz, H-7), 7.58 (1H, brs, H-5), 8.55 (1H, d, J=4.9 Hz, H-4), 8.08 (1H, d, J=4.9 Hz, H-3), 4.13 (3H, s, OCH_3), 3.50 (6H, s, NCH_3); ^{13}C-NMR (125 M Hz, $CDCl_3$), δ 167.3 (C-1), 121.7 (C-4), 150.3 (C-6), 112.6 (C-8), 118.8 (C-8), 106.9 (C-5), 135.7 (C-4a), 136.8 (C-9a), 137.9 (C-8a), 138.4 (C-3), 119.0 (C-7), 53.0 (OCH_3), 50.9 (NCH_3). ESI-MS *m/z* $[M+H]^+$: 243, $[2M+Na]^+$: 507; HR-ESI-MS *m/z* 243.1348 $[M+H]^+$ (calcd. for $C_{14}H_{17}N_3O^+$ 243.1366).

Cyclo-(Pro-Leu) (**3**). Colorless crystals. ^{1}H-NMR (500 M Hz, $CDCl_3$), δ 3.53–3.65 (2H, m, H-3), 1.86–1.94 (1H, m, H-4a), 1.99–2.02 (1H, m, H-4b), 2.13 (1H, m, H-5a), 2.33 (1H, m, H-5b), 4.12 (1H, t, J=8.1 Hz, H-6), 5.89 (1H, brs, N-H), 4.01 (1H, t, J=7.0 Hz), 2.01(1H, m, H-10), 1.69–1.76 (1H, m, H-11), 0.94 (3H, d, J=6.3 Hz, H-12), 1.00 (3H, d, J=6.3 Hz, H-13); ^{13}C-NMR (125 M Hz, $CDCl_3$), δ 170.1 (C-1), 45.5 (C-3), 22.8 (C-4), 28.2 (C-5), 59.0 (C-6), 166.1 (C-7), 53.4 (C-9), 38.7 (C-10), 24.8 (C-11), 22.8 (C-12), 21.2 (C-13).

Cyclo-(Pro-Ile) (**4**). Colorless crystals. ^{1}H-NMR (500 M Hz, $CDCl_3$), δ 3.51–3.61 (2H, m, H-3), 2.15–2.20 (1H, m, H-4a), 1.87–1.95 (1H, m, H-4b), 2.30–2.36 (1H, m, H-5a), 2.10–2.25 (1H, m, H-5b), 4.09 (1H, t, J=7.5 Hz, H-6), 5.90 (1H, brs, N-H), 4.04 (1H, t, J=7.0 Hz), 2.39–2.42 (1H, m, H-10), 1.46–1.52 (1H, m, H-11), 0.92 (3H, t, J=7.4 Hz, H-12), 1.05 (3H, d, J=7.2 Hz, H-13); ^{13}C-NMR (125 M Hz, $CDCl_3$), δ 170.1 (C-1), 45.2 (C-3), 22.4 (C-4), 28.6 (C-5), 58.8 (C-6), 166.1 (C-7), 60.5 (C-9), 35.3 (C-10), 24.1 (C-11), 12.1 (C-12), 16.0 (C-13).

ACKNOWLEDGEMENTS

This study was supported by grants from the National Natural Science Foundation of China (No. 40706046, 30973679 and 20902094), the National Key Basic Research Program of China (973)'s Project (2010CB833800 and 2011CB915503), Knowledge Innovation Program of the Chinese Academy of Science (LYQY200703, SQ200904 and KSCX2-YW-G-073), the Scientific Research Foundation for the Returned Overseas Chinese Scholars, the State Education Ministry and the LMB (091002) Foundation.

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