A new minor diketopiperazine from the sponge-derived fungus
*Simplicillium* sp. YZ-11

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Chemical investigation of the cultures of a sponge-derived fungus *Simplicillium* sp. YZ-11 led to the isolation of a new minor diketopiperazine alkaloid cyclo-(2-hydroxy-Pro-Gly) (1) and a natural lactone (S)-dihydro-5-[(S)-hydroxyphenylmethyl]-2(3\(H\))-furanone (2), together with five known ergostane-type sterols (3–7). Their structures were established based on extensive spectroscopic methods (\(^1\)H and \(^13\)C NMR, \(^1\)H–\(^1\)H COSY, HSQC and HMBC) and optical rotation analysis.

**Keywords:** *Simplicillium* sp; marine sponge; cyclo-(2-hydroxy-Pro-Gly); diketopiperazine; sterols

1. Introduction

Microorganisms associated with marine invertebrates or with algae have attracted considerable attention as a rich and promising sources of novel and bioactive secondary metabolites (Bugni & Ireland, 2004). Among them, the sponge-derived fungi are of special interest because marine sponges are widely distributed in the ocean from the intertidal zone to the deep sea, and are well known to be hosts for large community of microorganisms (Lee et al. 2001). In this study, the chemical constituents of the cultures of a sponge-derived fungus *Simplicillium* sp. YZ-11, a strain that identified from an intertidal sponge *Hymeniacidon perleve*, have been investigated. A new minor diketopiperazine alkaloid cyclo-(2-hydroxy-Pro-Gly) (1) and a natural lactone (S)-dihydro-5-[(S)-hydroxyphenylmethyl]-2(3\(H\))-furanone (2), together with five known sterols (3–7) (Figure 1), were isolated from this strain. Herein, we describe the isolation and structure elucidation of these compounds.

2. Results and discussion

The combined EtOAc and MeOH extract of the cultures of a sponge-derived fungus *Simplicillium* sp. YZ-11 was subjected to column chromatography over silica gel, Sephadex LH-
20, ODS and semi-preparative HPLC to obtain one new minor diketopiperazine alkaloid, named cyclo-(2-hydroxy-Pro-Gly) (1), a natural lactone (2), and five ergostane-type sterols (3–7). The structures of known compounds, (S)-dihydro-5-[(S)-hydroxyphenylmethyl]-2(3H)-furanone (2) (Hargreaves et al. 2002), (22E,24R)-5α,6α-epoxy-ergosta-8(14),22-dien-3β,7α-diol (3) (Yue et al. 2001), (22E,24R)-5α,6α-epoxy-ergosta-8,22-dien-3β,7α-diol (4) (Yue et al. 2001), (22E,24R)-5α,8α-epidioxy-ergosta-6,22-dien-3β-ol (5) (Fang et al. 2013), (22E,24R)-5α,8α-epidioxy-ergosta-6,9(11),22-trien-3β-ol (6) (Fang et al. 2013) and (22E,24R)-6β-methoxy-ergosta-7,22-dien-3β,5α-diol (7) (Kawagishi et al. 1988) were determined by comparing their spectroscopic data with those of literature reported. All these compounds were isolated from the fungi of the genus Simplicillium for the first time.

Compound 1 was isolated as white amorphous solid. Its molecular formula was determined to be C$_7$H$_{10}$N$_2$O$_3$ by HR-EI-MS (m/z 170.0693 [M]$^+$, calculated for 170.0691) and NMR data, which indicated the presence of four degrees of unsaturation. The $^{13}$C NMR and DEPT spectra of 1 (DMSO-$d_6$, 125 MHz) displayed seven carbons, including two carbonyl groups at δ$_C$ 167.8 (s, C-7) and 165.0 (s, C-1), one oxygenated sp$^3$ quaternary carbon at δ$_C$ 86.4 (s, C-6), and four sp$^3$ methylenes at δ$_C$ 45.5 (t, C-9), 44.5 (t, C-3), 35.7 (t, C-5) and 19.6 (t, C-4). The $^1$H NMR spectrum of 1 (DMSO-$d_6$, 500 MHz) showed two one-proton broad signals at δ$_H$ 8.07 and 6.59, which were ascribed to 8-NH and 6-OH, and one characteristic methylene signals at δ$_H$ 4.04 (1H, d, J = 17.0 Hz, H-9a) and 3.57 (1H, d, J = 17.0 and 4.6 Hz, H-9b). In the $^1$H-$^1$H COSY spectrum, the correlations of H-4 with H-3 and H-5 suggested that they were three contiguous methylenes. From the above information, we found that the NMR data of 1 displayed the typical signal patterns for the diketopiperazine derivatives, and were very similar to those of cyclo-(Pro-Gly) (Yu et al. 2014). The only difference between these two compounds was an oxygenated sp$^3$ quaternary carbon in 1 instead of an sp$^3$ methine in cyclo-(Pro-Gly), indicating that a hydroxyl group located at C-6 in 1. This was supported by the HMBC spectrum (Figure S2), which showed correlations between 6-OH with C-5, C-6 and C-7. However, the absolute configuration at C-6 was not determined by Marfey’s method because the hydroxyproline residue decomposed under acidic conditions (Park et al. 2006; Li et al. 2011). Thus, the structure of 1 was elucidated as cyclo-(2-hydroxy-Pro-Gly).

Compound 2 was obtained as colourless oil and was named as (S)-dihydro-5-[(S)-hydroxyphenylmethyl]-2(3H)-furanone. The planar structure of 2 was determined on the basis of 1D and 2D NMR analysis, including HSQC, HMBC and $^1$H-$^1$H COSY (Figure S2), as well as
HR-MS. The absolute configurations of (7S,8S) for isolated 2 were assigned by comparison and analysis of its NMR data and optical rotation with that of literatures reported (Hargreaves et al. 2002; Emmanuvel & Sudalai, 2008; Ube et al. 2010). Previously, it had only been described as a natural product from an Acremonium sp. fungus, and shown weak anti-nematodal activity (Hargreaves et al. 2002).

Cyclo-(2-hydroxy-Pro-Gly) (1) is structurally similar to cyclo-(Pro-Gly), and possesses a 2,5-diketopiperazine unit. In marine environment, diketopiperazines as the smallest cyclic peptides are widely produced by marine microorganisms, especially marine bacteria (Jayatilake et al. 1996; Li et al. 2006; Yu et al., 2014). They are extensively obtained by extraction from natural sources, but may be easily synthesised. For both natural and synthetic diketopiperazines, a wide variety of biological activities was reported, including antitumour, antiviral, antifungal and antibacterial activities (Ivanova et al. 2013). In recent years, the antifouling activities of the diketopiperazines had been studied. Li et al. (2006) isolated five diketopiperazines from a deep-sea bacterium, Streptomyces fungicidicus. The antifouling activity against the larval of the barnacle Balanus amphitrite of those diketopiperazines indicated potential application as novel antifoulants. Yu et al. (2014) reported that the diketopiperazines isolated from the biofouling bacterium Pseudoalteromonas issachenkoni might influence the formation of biofilms and the development of biofouling.

3. Experimental

3.1. General experimental procedures

Optical rotations were determined on a Jasco P-1020 automatic digital polarimeter (Jasco, Tokyo, Japan). UV spectra were recorded on a Shimadzu UV-2401 PC spectrophotometer (Shimadzu, Kyoto, Japan). ECD spectra were recorded on a Chirascan CD spectrometer (Applied Photophysics, Leatherhead, UK). IR spectra were measured on a Jasco FT/IR-4100 spectrometer (Jasco, Tokyo, Japan) with KBr pellets. 1D and 2D NMR spectra were recorded on a Bruker Avance III 500 instruments (Bruker, Fallanden, Switzerland) with TMS as an internal standard. APCI-MS were determined on a LCQ Fleet ion trap mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). HR-EI-MS and HR-ESI-MS were measured on AutoSpec Primier P776 (Waters, Milford, MA, USA) and Agilent G6230 TOF (Agilent Technologies, Santa Clara, CA, USA) mass spectrometers. Column chromatography was performed over silica gel (200–300 mesh; Yantai Xinde Chemical Co., Ltd, Yantai, China), ODS gel (YMC, Kyoto, Japan) and Sephadex LH-20 (Amersham Pharmacia Biotech, Uppsala, Sweden). Semi-Preparative HPLC was performed on Elite Preparative HPLC (Elite Analytical Instruments Co., Ltd, Dalian, China) with a Thermo BDS HYPERSIL column (250 mm × 10.0 mm i.d., 5 μm, Thermo Fisher Scientific, Waltham, MA). TLC was performed on the silica gel plates (Yantai Xinde Chemical Co., Ltd, Yantai, China), and spots were visualised by spraying with 10% H2SO4 in EtOH, followed by heating.

3.2. Fungal materials and culture conditions

The fungal strain YZ-11, was isolated from a marine sponge H. perleve, which was collected from Dalian, Liaoning Province of China in September 2012. This strain was identified as Simplicillium sp. by Takara Biotechnology (Dalian) Co., Ltd., according to its rDNA genes sequence (18S-ITS1-5.8S-ITS2-28S), which showed a 99% similarity to Simplicillium sp. (GenBank ID: 05-F0103). This strain was deposited at the Key Laboratory of Coastal Biology and Biological Resources Utilization, Yantai Institute of Coastal Zone Research, CAS, and the pure strain was stored in 50% glycerol at –80°C. The fungal strain Simplicillium sp. YZ-11 was incubated in 2000 mL × 25 conical flasks containing the liquid medium (800 mL/
flask) composed of peptone (5.0 g/L), yeast powder (2.0 g/L), glucose (20.0 g/L), MgCl\(_2\) (0.5 g/L), KH\(_2\)PO\(_4\) (1.0 g/L) and seawater at 30°C on a rotary shaker (120 rpm) for 30 days.

3.3. Extraction and isolation

The fermented whole broth (20 L) was filtered through cheesecloth to separate it into filtrate and mycelia. The filtrate was extracted three times with EtOAc to yield an EtOAc solution, whereas the mycelia were extracted three times with MeOH to get a MeOH solution. The EtOAc solution and MeOH solution were evaporated under reduced pressure, and combined to afford a crude residue. The crude residue (22.7 g) was subjected to column chromatography on silica gel eluted with a gradient eluent of petroleum ether (PE)-acetone system (15:1 to 1:5, v/v) to give five fractions (A–E). Fraction B (236.1 mg) was subjected to ODS column with gradient eluting (MeOH:H\(_2\)O, 70:30 to 100:0, v/v) to afford three subfractions (B1–B3). Fraction B3 (40.3 mg) was purified on silica gel column (PE:EtOAc, 3:1 and 2:1) to obtain compound 5 (10.5 mg), 6 (3.2 mg) and 7 (3.8 mg). Fraction C (230.4 mg) was also subjected to ODS column (MeOH:H\(_2\)O, 70:30 to 100:0, v/v) and silica gel column (CH\(_2\)Cl\(_2\):acetone, 5:1) to afford 3 (12.8 mg) and 4 (9.5 mg).

Fraction D (3.6 g) was performed on Sephadex LH-20 (CH\(_2\)Cl\(_2\):MeOH, 1:1, v/v) and ODS columns to get two subfractions (D1 and D2). Fraction D1 was purified on silica gel columns with CH\(_2\)Cl\(_2\):MeOH (15:1) and semi-preparative HPLC to yield compound 1 (1.3 mg), and compound 2 (4.4 mg) was obtained from subfraction D2 after purification by silica gel column with PE:acetone (2:1).

3.3.1. cyclo-(2-hydroxy-Pro-Gly) (1)

White amorphous solid; [α]\(_D\)\(^{20.8}\) = −11.9 (c = 0.20, MeOH); IR (KBr) \(\nu_{\text{max}}\) (cm\(^{-1}\)): 3545, 3383, 3205, 1693, 1631, 1454, 1111; \(^1\)H NMR (500 MHz, DMSO-\(d_6\)): \(\delta\) 8.07 (1H, brs, 8-NH), 6.59 (1H, s, 6-OH), 4.04 (1H, d, \(J = \) 17.0 Hz, H-9a), 3.57 (1H, dd, \(J = \) 17.0 and 4.6 Hz, H-9b), 3.05 (1H, m, H-3a), 3.37 (1H, m, H-3b), 2.02 (2H, m, H-5), 1.98 (1H, m, H-4a), 1.83 (1H, m, H-4b); \(^{13}\)C NMR (125 MHz, DMSO-\(d_6\)): \(\delta\) 167.8 (s, C-7), 165.0 (s, C-1), 86.4 (s, C-6), 45.5 (t, C-9), 44.5 (t, C-3), 35.7 (t, C-5), 19.6 (t, C-4); Negative APCI-MS: \(m/z\) 169.04 [M–2H]\(^+\); HR-EI-MS: \(m/z\) 170.0693 [M]+ (calculated for C\(_7\)H\(_{10}\)N\(_2\)O\(_3\)[M]+, 170.0691).

3.3.2. (S)-dihydro-5-[(S)-hydroxyphenylmethyl]-2(3H)-furanone (2)

Colourless oil; [α]\(_D\)\(^{21.1}\) = +74.6 (c = 0.22, MeOH); IR (KBr) \(\nu_{\text{max}}\) (cm\(^{-1}\)): 3425, 1763, 1180, 1041, 702; \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 7.35–7.45 (5H, m, H-2–H-6), 4.73 (1H, d, \(J = \) 6.5 Hz, H-7), 4.68 (1H, dd, \(J = \) 13.5 and 7.0 Hz, H-8), 2.50 (2H, td, \(J = \) 8.6 and 2.1 Hz, H-10), 2.06 (2H, dd, \(J = \) 16.3 and 7.9 Hz, H-9); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): \(\delta\) 176.8 (s, C-11), 138.3 (s, C-1), 128.8 (d × 2, C-3 and C-5), 128.8 (s, C-4), 127.0 (d × 2, C-2 and C-6), 83.4 (d, C-8), 76.6 (d, C-7), 28.5 (t, C-10), 24.0 (t, C-9); Negative APCI-MS: \(m/z\) 190.99 [M – H]\(^-\); HR-ESI-MS (positive): \(m/z\) 215.0681 [M + Na]\(^+\) (calculated for C\(_{11}\)H\(_{12}\)O\(_3\)Na [M + Na]\(^+\), 215.0684).

4. Conclusion

Seven compounds were isolated from the cultures of the sponge-derived fungus Simplicillium sp. YZ-11, including one new minor diketopiperazine alkaloid cyclo-(2-hydroxy-Pro-Gly) (1), one natural lactone (S)-dihydro-5-[(S)-hydroxyphenylmethyl]-2(3H)-furanone (2) and five known ergostane-type sterols (3–7). Their structures were established based on extensive spectroscopic methods and optical rotation analysis. Unfortunately, the absolute configuration of
1 was not determined. To our knowledge, all these compounds were isolated from the genus *Simplicillium* fungi for the first time.

**Supplementary material**

Supplementary material relating to this article is available online, alongside Figures S1–S16.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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**Note**

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