A new diketopiperazine from the gorgonian coral *Menella kanisa*

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Chemical investigation on the gorgonian *Menella kanisa* collected from Beibu Gulf led to the isolation of a new diketopiperazine, named menazepine A (1), as well as three known diketopiperazines, namely cyclo(4-hydroxyprolylleucyl) (2), cyclo(Pro-Leu) (3) and cyclo(4-hydroxyprolylphenylalanyl) (4). The structure of the new diketopiperazine was elucidated on the basis of extensive spectroscopic analysis and by comparing the data with those of related metabolites. Compounds 1–4 were also evaluated for brine shrimp lethality.

**Keywords:** *Menella kanisa*; diketopiperazine; menazepine A; brine shrimp lethality

1. Introduction

Till date, there are a few reports on diketopiperazines from corals (Huang et al. 2010a). In our study of bioactive compounds from the gorgonian coral *Menella kanisa* belonging to the genus *Menella* which has been reported to contain biologically active metabolites (Chai et al. 2010; Kao et al. 2011; Lee et al. 2012), a new diketopiperazine, named menazepine A (1), as well as three known diketopiperazines, namely cyclo(4-hydroxyprolylleucyl) (2) (Shigemori et al. 1998), cyclo(Pro-Leu) (3) (Huang et al. 2010b) and cyclo(4-hydroxyprolylphenylalanyl) (4) (Shigemori et al. 1998), were obtained.

2. Results and discussion

Compound 1, a white needle crystal, was established as C\textsubscript{14}H\textsubscript{19}N\textsubscript{3}O\textsubscript{5} (seven units of unsaturation) based on the HR-ESI-MS ([M + H]\textsuperscript{+} at m/z 310.1401, calcd for C\textsubscript{14}H\textsubscript{20}N\textsubscript{3}O\textsubscript{5}, 310.1403). The \textsuperscript{13}C NMR data for 1 confirmed the presence of 14 carbon signals, characterised by DEPT as a methyl, four sp\textsuperscript{3} methylenes, four sp\textsuperscript{3} methines, a sp\textsuperscript{2} methine, four sp\textsuperscript{2} quaternary carbons. \textsuperscript{1}H NMR chemical shifts of two \textalpha{-}methine protons at δ\textsubscript{H} 4.56 (1H, dd, J = 7.1, 3.0 Hz, H-9) and 4.60 (1H, dd, J = 6.3, 3.1 Hz, H-6), and \textsuperscript{13}C NMR chemical shifts of two carbonyl carbons at δ\textsubscript{C} 169.0 (C-7) and 167.5 (C-1), supported the presence of a peptide fragment. The fact that compound 1 was negative to the ninhydrin test suggested a cyclic or an N-terminus-blocked peptide (Shigemori et al. 1998; Huang et al. 2010b).

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From the $^1$H–$^1$H COSY spectrum of 1 (Figure S2), it was possible to differentiate between the separate spin systems of H$_2$-3/H-4/H$_2$-5/H-6 which indicated the characteristic of a proline residue (Huang et al. 2010b) and H-9/H$_2$-10/H-11/H$_2$-12. These data, together with the key HMBC correlations between H$_2$-3/C-1, C-6; H$_2$-5/C-7; H-9/C-7, C-16; H$_2$-10/C-1; H$_2$-12/C-14 and H$_2$-17/C-14, C-16 (Figure S2), permitted the elucidation of the carbon skeleton of 1. The two hydroxy groups located at C-4 and C-11 were discerned from two carbon signals at $\delta_C$ 69.6 and 67.2, respectively, with the analysis of its molecular formula.

The absolute configuration of compound 1 was determined by optical rotation and NOESY spectrum. In the NOESY spectrum (Figure S2), H-9 exhibited a correlation with H-11, not with H-6, and H-6 did not display a correlation with H-4, which indicated that H-4, H-9 and H-11 were situated on the same face and assigned as $\beta$ protons. The sign for $[\alpha]_D$ for proline-containing diketopiperazines is either negative or positive, depending only on the absolute configuration of Pro (Adamczeski et al. 1995; Huang et al. 2010b). On the basis of $[\alpha]_D^{20}$ (−28) and by comparing the NMR data of the proline residue with those of proline-containing diketopiperazines (Adamczeski et al. 1995; Jayatilake et al. 1996; Huang et al. 2010b), which suggested C-6 has (S)-configuration, Pro in 1 has, therefore, (S)-configuration, and the above-mentioned data approved the absolute configuration of 1 as $4R$, $6S$, $9R$ and $11R$.

Three known diketopiperazines were identified as cyclo(4-hydroxyprolylleucyl) (2), cyclo (Pro-Leu) (3) and cyclo(4-hydroxyprolylphenylalanyl) (4) by comparing with those of earlier reports (Shigemori et al. 1998; Huang et al. 2010b).

The results of brine shrimp lethality of compounds 1–4 are shown in Table S1. The toxicity of compound 3 was significantly stronger than those of the other three compounds (Figure 1).

3. Experimental

3.1. General experimental procedures

$^1$H and $^{13}$C NMR spectra were recorded on a Bruker AC 600 NMR spectrometer with TMS as an internal standard at 600 and 150 MHz, respectively. HR-ESI-MS data were obtained from Bruker Maxis mass spectrometer. Waters-2695 HPLC system, was performed on a Sunfire™ C$_{18}$ column (250 $\times$ 10 mm i.d., 10 $\mu$m) coupled to a Waters 2998 photodiode array detector. Optical rotation data were measured by Perkin-Elmer Model 341 polarimeter. The silica gel GF$_{254}$ used for CC was supplied by the Qingdao Marine Chemical Factory, Qingdao, China. Spots were detected on TLC under UV light or by heating after spraying with 5% H$_2$SO$_4$ in EtOH (v/v).

3.2. Animal material

*M. kanisa* was collected from Xieyang Island of Beibu Gulf, China, in October, 2010. The specimen was identified by Dr Xiubao Li. A voucher specimen (no. 20100401) has been deposited in the Guangxi Key Laboratory of Marine Environmental Science, Guangxi Academy of Sciences, China.
3.3. Extraction and isolation

*M. kanisa* (3 kg, wet wt) was extracted with ethanol (95%). Ethanol was evaporated *in vacuo* to yield a syrupy residue that was suspended in distilled water and fractionated successively with petroleum ether, ethylacetate, and *n*-butanol. The ethylacetate soluble portion (7.21 g) was subjected to column chromatography (CC) on silica gel, using CHCl$_3$:Me$_2$CO (10:0, 9:1, 8:2, 7:3, v:v) and CHCl$_3$:MeOH (10:1, 10:2, 10:3, 0:10, v:v) as eluent, giving 11 fractions (A–K). Fraction E was subjected to column chromatography to yield two subfractions (E1 and E2). Fraction E2 was separated by HPLC, using the mixtures of MeOH and H$_2$O (MeOH: H$_2$O = 5:95, v:v) to yield 2 (5.8 mg) and 3 (5.2 mg). Fraction G was subjected to column chromatography to yield four subfractions (G1–G4). Fraction G4 was separated by HPLC, using the mixtures of MeOH and H$_2$O (MeOH:H$_2$O = 35:65, v:v) to yield 4 (5.1 mg). The *n*-butanol soluble portion (5.01 g) was subjected to column chromatography (CC) on silica gel, using CHCl$_3$:MeOH (CHCl$_3$:MeOH = 10:0, 10:1, 10:2, 10:3.5, 0:10, v:v) as eluent, giving four fractions (L1–L4). Fraction L3 was separated by HPLC, using the mixture of MeOH and H$_2$O (MeOH:H$_2$O = 1:9, v:v) to yield 1 (4.5 mg).

Menazepine A (1): a white needle crystal; m.p.: 203–205°C. [α]$^\text{D}$_{20} = −28 (c 0.23, MeOH). HR-ESI-MS *m/z*: 310.1401 ([M + H]$^+$, calcd for C$_{14}$H$_{20}$N$_3$O$_5$, 310.1403). $^1$H NMR (600 MHz, CD$_3$OD) δ: 7.22 (1H, s, H-16), 4.60 (1H, dd, $J = 6.3, 3.1$ Hz, H-6), 4.56 (1H, dd, $J = 7.1, 3.0$ Hz, H-9), 4.49 (1H, m, H-4), 4.48 (1H, m, H-11), 3.56 (1H, dd, $J = 11.2, 6.3$ Hz, H-12a), 3.52 (1H, dd, $J = 11.2, 3.0$ Hz, H-12b), 2.25 (1H, m, H-5a), 2.16 (1H, m, H-5b), 1.93 (1H, m, H-10b), 1.85 (3H, s, H-17). $^{13}$C NMR (150 MHz, CD$_3$OD) δ: 169.0 (C-7), 167.7 (C-14), 167.5 (C-1), 139.2 (C-16), 110.4 (C-15), 72.5 (C-6), 69.6 (C-4), 67.2 (C-11), 59.9 (C-9), 54.5 (C-3), 43.6 (C-12), 37.6 (C-5), 32.9 (C-10), 12.1 (C-17).

3.4. Brine shrimp lethality bioassay

The brine shrimp lethality bioassay was carried out by the reported method (Lou et al. 2009).

4. Conclusion

In conclusion, our investigation on the chemical constituents of gorgonian *M. kanisa* led to the identification of four diketopeperazines (1–4). Among these compounds, 3 showed significant toxicity towards brine shrimp larvae, while the others displayed moderate toxicity. The results suggest that 3 is a potential toxic natural product.

Supplementary material

Experimental details relating to this article are available online alongside Figures S2–S8 and Table S1.

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