

Alkaloids from the South China Sea Black Coral *Antipathes dichotoma*

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A new carbazole alkaloid, antipathine A (**1**), together with three known zoanthoxanthin alkaloids (**2**–**4**) was isolated from the EtOH/CH₂Cl₂ extracts of the South China Sea black coral *Antipathes dichotoma*. The structure of **1** was determined on the bases of extensive spectroscopic analysis, including 1D and 2D NMR data. Compounds **1** and **2** showed moderate cytotoxicity against human stomach carcinoma SGC-7901 cell line with IC₅₀ of 67.38 and 86.40 μg/ml, respectively, and **1** and **2** also showed weak cytotoxicity toward human liver carcinoma Hep_G2 cell line.

Key words *Antipathes dichotoma*; black coral; carbazole alkaloid; cytotoxicity

Antipathes dichotoma (PALLAS) belongs to zoanthid black corals. It has a series of pharmaceutical functions in Chinese folk, such as relieving fever and softening hard mass. There were only two literatures about the chemical constituents of black corals that reported nine steroids isolated from *Antipathes subpinnata*.^{1,2} In order to obtain new bioactive compounds from the South China Sea black coral *A. dichotoma*, we investigated on the chemical constituents of its EtOH/CH₂Cl₂ extract, and a new carbazole alkaloid, antipathine A (**1**) was obtained together with three known zoanthoxanthin alkaloids zoanthoxanthin **1** (**2**),³ zoanthoxanthin **4** (**3**),³ and paragrachine (**4**).³ In the cytotoxicity assays, we observed that **1** and **2** showed moderate cytotoxicity against human stomach carcinoma SGC-7901 cell line with IC₅₀ of 67.38 and 86.40 μg/ml, respectively, and **1** and **2** also showed weak cytotoxicity toward human liver carcinoma Hep_G2 cell line. This paper deals with the isolation, structural elucidation and cytotoxic activity of **1**.

Compound **1** was obtained as a yellow powder. The molecular formula of C₁₆H₁₃N₃O₂ was deduced from NMR spectra

and HR-ESI-MS. The ultraviolet (UV) spectrum showed sharp absorptions at λ_{max} 220, 240, 284, 301, 336, 348 nm. Its ¹H-NMR spectrum displayed two methyl groups at δ_H 3.57 (3H, s), 3.66 (3H, s), signals of a four-spin proton system at δ_H 7.66 (1H, d, *J*=7.5 Hz), 7.35 (1H, t, *J*=7.5 Hz), 7.60 (1H, t, *J*=7.5 Hz), 8.42 (1H, d, *J*=7.5 Hz), two 1H singlets at δ_H 8.14 (1H, s), 8.66 (1H, s), and a lower-field 1H singlet at δ_H 12.73 (1H, s). The ¹³C-NMR spectrum showed the presence of two methyls (δ_C 28.4, 30.9), six low-field methines (δ_C 104.7, 110.1, 111.9, 119.5, 122.0, 128.4), eight low-field quaternary carbons (δ_C 114.4, 122.8, 129.7, 133.9, 136.8, 143.5, 151.0, 162.6). These data suggested that **1** should have a 1,3-disubstituted-9*H*-carbazole structural unit or 2,3-disubstituted-9*H*-carbazole structural unit.^{4–6}

The suggestion was proved by the heteronuclear multiple bond connectivity (HMBC) spectrum showing correlations of H-2 (δ_H 8.66) with C-1 (δ_C 129.7, s)/C-3 (δ_C 104.7, s), H-4 (δ_H 8.14) with C-1a (δ_C 136.8, s)/C-3 (δ_C 133.9, s)/C-4a (δ_C 114.4, s)/C-5a (δ_C 122.8, s), H-4/H-5 (δ_H 7.66)/H-6 (δ_H 7.35) with C-5a (δ_C 122.8, s), H-6/H-7 (δ_H 7.60) with C-8 (δ_C 111.9, d), and H-7/H-8 (δ_H 8.42) with C-8a (δ_C 143.5, s). Furthermore, HMBC correlations of NH (δ_H 12.73) with C-8/C-8a/C-1a/C-1 indicated that the structure unit of **1** should be 1,3-disubstituted instead of 2,3-disubstituted. Moreover, the HMBC spectrum also showed correlations of H-2 with C-10 (δ_C 162.6, s), Me-15 (δ_H 3.57) with C-10/C-12 (δ_C 151.0, s), and Me-14 (δ_H 3.66) with C-3/C-12, which suggested a group of –CO–NCH₃–CO–NCH₃– attached to C-1/C-3 of the 1,3-disubstituted-9*H*-carbazole structural unit, correspondingly. The 2D nuclear Overhauser effect spectroscopy (NOESY) spectrum of **1** showed correlations of H-4 with Me-14, which further proved the location of –N(13)CH₃– group at C-3. So, the structure of **1** was elucidated as shown, and named antipathine A.

The cytotoxicity of compounds **1**–**3** toward SGC-7901 and Hep_G2 cancer cell lines was evaluated. It was found that **1** and **2** showed moderate cytotoxicity against human stomach carcinoma SGC-7901 cell line with IC₅₀ of 67.38 and 86.40 μg/ml, respectively, and **1** and **2** also showed weak cytotoxicity toward human liver carcinoma Hep_G2 cell line.

Experimental

General Experimental Procedures Optical rotations were measured with a Horiba SEAP-300 spectropolarimeter. UV spectra were measured

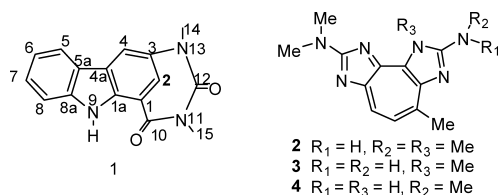


Fig. 1. Structures of Compounds **1**–**4**

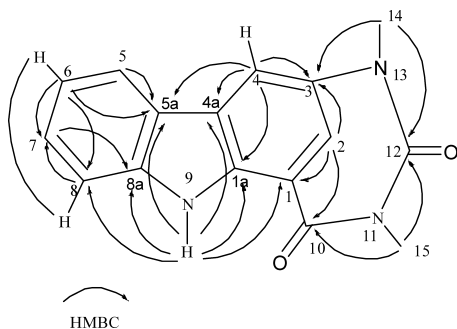


Fig. 2. Key HMBC Correlations of **1**

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with a Shimadzu double-beam 210A spectrophotometer in MeOH solution. ^1H -, ^{13}C -NMR and 2D NMR spectra were recorded on a Bruker AV-500 MHz NMR spectrometer with TMS as internal standard. MS spectral data were obtained on an LCQ^{DECA} XP HPLC/MSⁿ spectrometer for ESI-MS. Si gel (200–300 mesh) for column chromatography and GF₂₅₄ for TLC were obtained from the Qindao Marine Chemical Factory, Qindao, People's Republic of China.

Animal Material The South China Sea black coral *Antipathes dichotoma* (2.5 kg, wet weight) was collected in Sanya, Hainan province, China in October 2005 and identified by Prof. Zou R. L., the South China Sea Institute of Oceanology, Academia Sinica. A voucher specimen (No. 0316) was deposited in the South China Sea Institute of Oceanology, Academia Sinica, Guangzhou, China.

Extraction and Isolation The frozen specimen was extracted with EtOH/CH₂Cl₂ (2:1) three times at room temperature, and the solution was evaporated *in vacuo*. The residue was suspended in H₂O and extracted with EtOAc and *n*-BuOH three times, respectively. The EtOAc and *n*-BuOH layers were concentrated *in vacuo* to afford 27 g and 16 g of residues, respectively. The EtOAc extract was subjected to column chromatography (CC) on silica, using petroleum ether–EtOAc (from 10:0 to 0:10) as eluent. By combining the fractions with TLC (GF₂₅₄) monitoring, eight fractions were obtained. Fraction 6 was chromatographed over Sephadex LH-20 eluting with MeOH, then repeatedly subjected to CC on silica gel, eluted with CHCl₃/Me₂CO (from 8:2 to 7:3) to yield **1** (5 mg). The *n*-BuOH extract was subjected to CC on silica gel, using CHCl₃/MeOH (from 10:0 to 0:10) as eluent to give five fractions. Fraction 1 was chromatographed over Sephadex LH-20 eluting with MeOH, and further purified by reversed-phase HPLC to give **2** (45 mg), **3** (8 mg), and **4** (6 mg).

Antipathine A (**1**): UV (MeOH) λ_{max} 220, 240, 284, 301, 336, 348; ^1H -NMR (C₅D₅N, 500 MHz) δ_{H} : 3.57 (3H, s, Me-15), 3.66 (3H, s, Me-14), 7.35 (1H, t, $J=7.5$ Hz, H-6), 7.60 (1H, t, $J=7.5$ Hz, H-7), 7.66 (1H, d, $J=7.5$ Hz,

H-5), 8.14 (1H, s, H-4), 8.42 (1H, d, $J=7.5$ Hz, H-8), 8.66 (1H, s, H-2), 12.73 (1H, s, NH); ^{13}C -NMR (C₅D₅N, 125 MHz) δ_{C} : 28.4 (Me-15), 30.9 (Me-14), 104.7 (C-4), 110.1 (C-2), 111.9 (C-8), 114.4 (C-4a), 119.5 (C-6), 122.0 (C-5), 122.8 (C-5a), 128.4 (C-7), 129.7 (C-1), 133.9 (C-3), 136.8 (C-1a), 143.5 (C-8a), 151.0 (C-12), 162.6 (C-10); Negative-ion ESI⁻-MS m/z : 278 [M–H]⁻, 263, 238, 211; HR-ESI-MS m/z : 278.1003 [M–H]⁻ (Calcd for C₁₆H₁₃N₃O₂ 278.1007).

Biological Assays Human stomach carcinoma SGC-7901 and liver carcinoma Hep_G2 cell line were purchased from the AMERICAN Type Culture Collection (ATCC, Rockville, MD, U.S.A.). Cytotoxicity assays were measured by MTT methods as described previously.⁷⁾

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