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 the EtOH/CH2Cl2 extracts, and a new carbazole alkaloid, antipathine A (1) was obtained together with three known zoanthoxanthin alkaloids (2—4)3) and paragracine (4).3) In order to obtain new bioactive compounds from the South China Sea black coral, 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 paragracine (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 λmax 220, 240, 284, 301, 336, 348 nm. Its 1H-NMR spectrum displayed two methyl groups at δH 3.57 (3H, s), 3.66 (3H, s), signals of a four-spin proton system at δH 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 δH 8.14 (1H, s), 8.66 (1H, s), and a lower-field 1H singlet at δH 12.73 (1H, s). The 13C-NMR spectrum showed the presence of two methyls (δC 84.3, 80.9), six low-field methines (δC 104.7, 110.1, 111.9, 119.5, 122.0, 128.4), eight low-field quaternary carbons (δC 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-9H-carbazole structural unit or 2,3-disubstituted-9H-carbazole structural unit.4—6) The suggestion was proved by the heteronuclear multiple bond connectivity (HMBC) spectrum showing correlations of H-2 (δH 8.66) with C-1 (δC 129.7, s)/C-3 (δC 104.7, s), H-4 (δH 8.14) with C-1a (δC 136.8, s)/C-3 (δC 133.9, s), C-4a (δC 114.4, s)/C-5a (δC 122.8, s), H-4/H-5 (δH 6.66)/H-6 (δH 7.35) with C-5a (δC 122.8, s), H-6/H-7 (δH 7.60) with C-8 (δH 111.9, d), and H-7/H-8 (δH 8.42) with C-8a (δC 143.5, s).

Furthermore, HMBC correlations of NH (δH 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 (δC 162.6, s), Me-15 (δC 3.57) with C-10/C-12 (δC 151.0, s), and Me-14 (δH 3.66) with C-3/C-12, which suggested a group of CO—NCH2—CO—NCH2— attached to C-1/C-3 of the 1,3-disubstituted-9H-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)CH2— 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](image-url)

![Fig. 2. Key HMBC Correlations of 1](image-url)
with a Shimadzu double-beam 210A spectrophotometer in MeOH solution. 
\(^{1}H, \quad ^{13}C\)-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 LCQDMR XP HPLC/MS \(^{a}\) spectrometer for ESI-
MS. Si gel (200—300 mesh) for column chromatography and GF \(254^\) 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, Acade-
mia Sinica, Guangzhou, China.

**Extraction and Isolation** The frozen specimen was extracted with 
EtOH/CH\(_2\)Cl\(_2\) (2:1) three times at room temperature, and the solution was 
evaporated in vacuo. The residue was suspended in H\(_2\)O and extracted with 
EtOAc and n-BuOH three times, respectively. The EtOAc and n-BuOH lay-
ers were concentrated in vacuo to afford 27 g and 16 g of residues, respec-
tively. 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 
CHCl\(_3\)/MeOH (from 8:2 to 7:3) to yield 1 (5 mg). The n-BuOH extract 
was subjected to CC on silica gel, using CHCl\(_3\)/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) \(\lambda_{max}\) 220, 240, 284, 301, 336, 348; 
\(^{1}H\)-NMR (C\(_5\)D\(_5\)N, 500 MHz) \(\delta_{H}\): 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 (C\(_5\)D\(_5\)N, 125 MHz) \(\delta_{C}\): 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 C\(_{16}\)H\(_{13}\)N\(_3\)O\(_2\) 278.1007).

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

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**References**

5) Ito C., Okahana N., Wu T. S., Wang M. L., Lai J. S., Fu-