Two New Indole Alkaloids from the Marine-Derived Bacterium *Aeromonas* sp. CB101

by **Sheng-Xin Cai**[a]), **De-Hai Li**[a]), **Tian-Jiao Zhu**[a]), **Feng-Ping Wang**[b]), **Xiang Xiao**[b]), **Qian-Qun Gu***[a])

[a]) Key Laboratory of Marine Drugs, Chinese Ministry of Education, Institute of Marine Drugs and Food, Ocean University of China, Qingdao 266003, P. R. China
(phone: +86-532-82032065; fax: +86-532-82033054; e-mail: guqianq@ouc.edu.cn)

[b]) School of Life Science & Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, P. R. China

Two new indole alkaloids, arsindolines A and B (**1** and **2**, resp.), together with six known indole alkaloids, were isolated from a marine-derived bacterium strain CB101, identified as *Aeromonas* sp. Their structures were established by spectroscopic methods, and their antitumor activities were evaluated by SRB and MTT methods.

Introduction. – Bacteria, emerging as a significant chemical resource, exist abundantly in the marine environment. In the past ten years, 659 marine bacterial compounds have been described, and a number of them have yielded most promising therapeutic leads [1]. In our continuous search for new bioactive compounds from marine-derived microorganisms, a bacterium strain CB101, identified as *Aeromonas* sp., was isolated from sea water of the Xiamen sea. The AcOEt extract showed cyctoxicity *in vitro* against the K562 cell line. Studies of the active constituents of this strain led to the isolation of two new indole alkaloids, namely 4-[di(1*H*-indol-3-yl)methyl]quinoline (**1**) and 2,2-di(1*H*-indol-3-yl)ethyl butanoate (**2**), together with six known indole alkaloids, 2-[2,2-di(1*H*-indol-3-yl)ethyl]aniline (**3**) [2], 3,3′,3″-methanetriyltris(1*H*-indole) (**4**) [3], trisindoline (=1*H*,1″*H*-3,3′:3′,3″-terindol-2′(1′*H*)-one; **5**) [4], 1*H*,1″*H*-3,2′:2′,3″-terindol-3′(1′*H*)-one (**6**) [5], 2-(1*H*-indol-3-yl)ethanol (**7**) [6], and 1*H*-indole (**8**) (*Fig. 1*). In this article, we report the isolation, structural identification, and cytotoxicities of these compounds against HL-60 and A-549 cell lines.

Results and Discussion. – The AcOEt extract was concentrated *in vacuo* and then chromatographed by repeated silica gel and *Sephadex LH-20* column chromatography, as well as semipreparative HPLC to yield two new indole alkaloids, **1** and **2**, together with the six known indole alkaloids, **3**–**8**.

Compound **1**, named arsindoline A, was isolated as a colorless amorphous powder. The molecular formula was determined as $C_{26}H_{19}N_3$ by HR-ESI-MS at *m*/*z* 374.1654 ($[M+H]^+$; calc. 374.1657), which indicated 19 degrees of unsaturation. The IR absorption indicated the presence of NH (3405 cm^{-1}) and aromatic rings (1613, 1580, 1507, 1440 cm^{-1}). The ^{1}H-NMR (δ(H) 7.37, 7.19, 7.02, and 6.56) and ^{13}C-NMR (δ(C) 136.7, 126.6, 124.3, 122.2, 119.4, 117.5, and 111.2) data (*Table*) implied the presence of

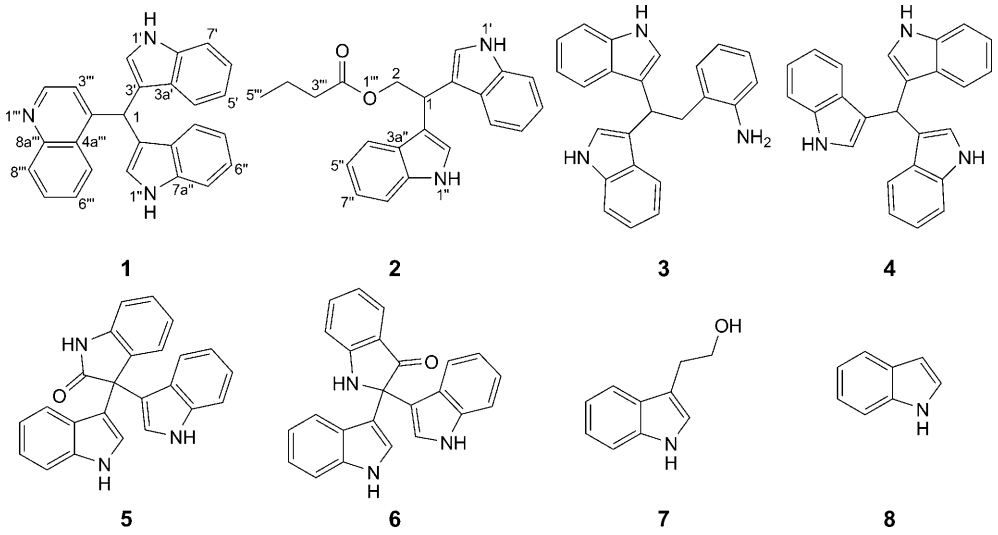

Fig. 1. *Compounds* **1**–**8**, *isolated from* Aeromonas sp. *CB101*

two 3-substituted indole-ring moieties, which was confirmed by the key HMBCs between H–C(1′) and C(3′), C(3a′), and C(7a′); H–C(2′) and C(3′), C(3a′), and C(7a′); H–C(4′) and C(3′), C(6′), and C(7a′); and H–C(7′) and C(5′) and C(3a′) (*Fig. 2*,*a*). In addition, a 4-substituted quinoline unit was deduced by analysis of the ^{1}H-(δ(H) 8.74, 8.15, 7.66, 7.43, and 7.15) and ^{13}C-NMR (δ(C) 150.3, 149.7, 148.3, 129.9, 128.9, 127.3, 126.5, 124.2, and 120.9) data (*Table*). The 4-substituted quinoline structure was consistent with the HMBCs (correlations between H–C(2‴) and C(3‴) and C(4‴); H–C(3‴) and C(2‴) and C(4a‴); H–C(5‴) and C(4‴), C(7‴), and C(8a‴); and H–C(8‴) and C(6‴), C(7‴), and C(8a‴); *Fig. 2*,*b*). The structure assignment of compound **1** was accomplished by connection of the three moieties *via* a CH group using the HMBCs between H–C(1) and C(2′), C(3′), C(3a′), C(2″), C(3″), C(3a″), C(3‴), C(4‴), and C(4a‴) (*Fig. 2*,*c*). These data were sufficient to define the structure of compound **1** as 4-(di-1*H*-indol-3-ylmethyl)quinoline.

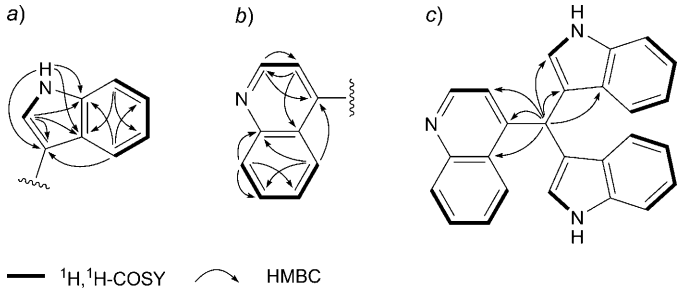

Fig. 2. *The key ^{1}H,^{1}H-COSY and HMBC correlations of compound* **1**

Table. *^{1}H- and ^{13}C-NMR Data of Compound* **1** *and* **2** *in $CDCl_3$*[a])

Position	**1**		**2**	
	δ(C)	δ(H) (J in Hz)	δ(C)	δ(H) (J in Hz)
1	34.5 (*d*)	6.65 (*s*)	33.6 (*d*)	4.95 (*t*, $J=7.1$)
2			67.0 (*t*)	4.74 (*d*, $J=7.1$)
1′/1″		8.05 (br. *s*)		7.98 (br. *s*)
2′/2″	124.3 (*d*)	6.56 (br. *d*, $J=1.9$)	122.1 (*d*)	6.98 (*d*, $J=1.9$)
3′/3″	117.5 (*s*)		116.3 (*s*)	
3a′/3a″	126.6 (*s*)		127.0 (*s*)	
4′/4″	119.4 (*d*)	7.37 (br. *d*, $J=8.2$)	119.5 (*d*)	7.62 (br. *d*, $J=7.8$)
5′/5″	119.4 (*d*)	7.02 (*ddd*, $J=8.2$, 7.3, 1.0)	122.0 (*d*)	7.06 (*ddd*, $J=7.8$, 7.3, 0.9)
6′/6″	122.2 (*d*)	7.19 (*ddd*, $J=8.2$, 7.3, 1.0)	119.3 (*d*)	7.18 (*ddd*, $J=8.3$, 7.3, 0.9)
7′/7″	111.2 (*d*)	7.37 (br. *d*, $J=8.2$)	111.1 (*d*)	7.35 (br. *d*, $J=8.3$)
7a′/7a″	136.7 (*s*)		136.4 (*s*)	
2‴	150.3 (*d*)	8.74 (*d*, $J=4.6$)	173.9 (*s*)	
3‴	120.9 (*d*)	7.15 (*d*, $J=4.6$)	36.2 (*t*)	2.21 (*t*, $J=7.6$)
4‴	149.7 (*s*)		18.3 (*t*)	1.52–1.58 (*m*)
4a‴	127.3 (*s*)			
5‴	124.2 (*d*)	8.15 (*dd*, $J=8.7$, 2.8)	13.5 (*q*)	0.83 (*t*, $J=7.3$)
6‴	126.5 (*d*)	7.43 (*ddd*, $J=8.7$, 6.8, 1.4)		
7‴	128.9 (*d*)	7.66 (*ddd*, $J=8.7$, 6.8, 1.4)		
8‴	129.9 (*d*)	8.15 (*dd*, $J=8.7$, 2.8)		
8a‴	148.3 (*s*)			

[a]) Spectra were recorded at 600 MHz for ^{1}H-NMR and 150 MHz for ^{13}C-NMR using TMS as internal standard.

Compound **2** was obtained as a colorless amorphous powder. The molecular formula was determined as $C_{22}H_{22}N_2O_2$ based on its HR-ESI-MS at m/z 369.1577 $[M+Na]^+$ (calc. 369.1579). The molecular formula indicated 13 degrees of unsaturation. IR absorptions implied the presence of a secondary amino group (3410 cm^{-1}), a conjugated ester group (1712 cm^{-1}), and aromatic rings (1613, 1454 cm^{-1}). Based on the detailed analysis of ^{1}H- and ^{13}C-NMR data, compound **2** showed the same two 3-substituted indolyl systems as compound **1**, but the quinoline unit was replaced by a butanoylmethyl group, which was in agreement with the following data: δ(H) 4.95, 4.74, 2.21, 1.52–1.58, and 0.83; δ(C) 67.0, 173.9, 36.2, 18.3, and 13.5.

The 1D-NMR data of compound **2** were similar to those of streptindole [7], the noticeable difference being an AcO group in streptindole instead of a butanoyl group in compound **2**. Thus, the structure of compound **2** was elucidated as 2,2-di(1*H*-indol-3-yl)ethyl butanoate, with the trivial name arsindoline B (**2**).

The cytotoxicities of all the compounds were first evaluated *in vitro* on the HL-60 and A-549 cell lines by the MTT method [8] and the SRB method [9], respectively. The new compound **2** and the known compounds **3**–**6** showed weak cytotoxicities against the A-549 cell lines with an IC_{50} value of 22.6, 18.1, 13.9, 18.4, and 69.7 μM, respectively.

Considering the structures, all compounds belong to single, bis-, or trisindole derived alkaloids. Compounds **4**–**7** have been previously isolated from different kinds of bacteria [3–6]. Many indole alkaloids have pronounced antiproliferative properties by a G_1 cell cycle arrest and were therefore suggested for the chemoprevention and

treatment of cancer [3]. Beside this, indole alkaloids are also biologically significant in other respects, including antimicrobial, antiviral, insecticidal, antithrombotic, or enzyme inhibitory activity [3][6]. Most of these indole alkaloids from *Aeromonas* sp. CB101 have weak cytotoxicities and might have other bioactivities which need to be investigated in the future.

This work was founded by the *Chinese Ocean Mineral Resource R & D Association* (DY 105-2-04). The antitumor assay was performed at the *Shanghai Institute of Materia Medica*, Chinese Academy of Sciences.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh, 10–40 mm, *Qingdao Marine Chemical Inc.*, P. R. China), *RP-18* (40–63 mm, *YMC Co.*, Japan), *Sephadex LH-20* (*GE Healthcare*, Sweden). TLC: silica gel *GF254* (10–40 mm, *Qingdao Marine Chemical Inc.*, P. R. China). Semiprep. HPLC was performed using an *ODS* column (*YMC-Pack ODS-A*, 10 × 250 mm, 5 μm). IR Spectra: *NICOLET NEXUS470* spectrophotometer; KBr discs. ^{1}H- and ^{13}C-NMR, DEPT, and 2D-NMR spectra: *JEOL Eclipse-600* spectrometer; TMS as internal standard; chemical shifts were recorded as δ values. ESI-MS: *Micromass Q-TOF ULTIMA GLOBAL GAAo76 LC* mass spectrometer.

Fermentation and Extraction. The bacterial strain CB101 was isolated from the sea water of the Xiamen Sea (200 m depth). The bacteria CB101 was cultured in 300 ml marine broth (0.5 g peptone, 0.1 g yeast extract, and 0.1 g $FePO_4$ dissolved in 1 l sea water, pH 7.2–7.6) in 500 ml *Erlenmeyer* flasks. Flasks with a total of 100 l liquid medium were incubated for 3 d on a rotary shaker at 100 rpm at 20°. The broth was centrifuged at 5000 rpm for 30 min to remove the cells and the supernatant was extracted three times with AcOEt. The combined AcOEt extracts were concentrated under reduced pressure to give a crude extract (15.0 g).

Purification. The crude extract (15.0 g) was applied to a SiO_2 (300–400 mesh) column and was separated into six fractions using a step gradient elution of petroleum ether (PE)/$CHCl_3$ and $CHCl_3$/MeOH. *Fr. 1*, eluted with PE/$CHCl_3$ 1:1 was further purified on a SiO_2 column using PE/acetone 20:1, to give compound **8** (5 g). *Fr. 3* (150 mg) was separated on a SiO_2 column using a step gradient elution of PE/acetone and further purified by semiprep. HPLC (70% MeOH, 4 ml/min) to yield compounds **2** (6.7 mg, t_R 15.6 min) and **4** (15 mg, t_R 12.7 min). *Fr. 4* was separated into five subfractions by *Sephadex LH-20* using $CHCl_3$/MeOH 1:1 as the eluent. *Subfr. 4-2* was further purified by extensive semiprep. HPLC (70% MeOH, 4 ml/min) to yield compound **1** (50 mg, t_R 18.6 min). *Subfr. 4-3* was further purified by extensive semiprep. HPLC (70% MeOH, 4 ml/min) to yield compounds **7** (4.1 mg, t_R 4.5 min), **6** (10 mg, t_R 5.7 min), and **3** (10 mg, t_R 9.8 min). *Fr. 5* was separated on a SiO_2 column with $CHCl_3$/MeOH 1:1 and was further purified by semiprep. HPLC (70% MeOH, 4 ml/min) to yield compound **5** (7 mg, t_R 6.4 min).

Arsindoline A (=*4-[Di(1*H*-indol-3-yl)methyl]quinoline*; **1**). Colorless amorphous powder. UV (HPLC, mobile phase): 219, 278. IR (KBr): 3405, 1613, 1580, 1507, 1440, 1334, 1082, 731. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS: 374.1654 ($[M+H]^+$, $C_{26}H_{20}N_3^+$; calc. 374.1657).

Arsindoline B (=*2,2-Di(1*H*-indol-3-yl)ethyl Butanoate*; **2**). Colorless amorphous powder. UV (HPLC, mobile phase): 218, 276. IR (KBr): 3410, 1712, 1613, 1454, 1341, 1188, 1089, 738. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS 369.1577 ($[M+Na]^+$, $C_{22}H_{22}N_2NaO_2^+$; calc. 369.1579).

Biological Assay. In the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay, cell lines were grown in *RPMI-1640* medium supplemented with 10% FBS under a humidified atmosphere of 5% CO_2 and 95% air at 37°. Cell suspensions, 200 μl, at a density of 5×10^4 cell/ml were plated in 96 well microtiter plates and incubated for 24 h. Then, 2 μl of the test solns. (in MeOH) were added to each well and further incubated for 72 h. Then, 20 μl of the MTT soln. (5 mg/ml in *RPMI-1640* medium) was added to each well and incubated for 4 h. The old medium containing MTT (150 μl) was then gently replaced by DMSO and pipetted to dissolve any formazan crystals formed. Absorbance was then determined on a *Spectra Max Plus* plate reader at 540 nm.

In the sulforhodanine B (= SRB) assay, 200 µl of the cell suspensions were plated in 96 cell plates at a density of 2×10^5 cell/ml. Then, 2 µl of the test solns. (in MeOH) was added to each well, and the culture was further incubated for 24 h. The cells were fixed with 12% trichloroacetic acid, and the cell layer was strained with 0.4% SRB. The absorbance of SRB soln. was measured at 515 nm. Dose-response curves were generated, and the IC_{50} values, *i.e.*, the concentration of compound required to inhibit cell proliferation by 50%, were calculated from the linear portion of log dose-response curves.

REFERENCES

[1] W. Fenical, *Chem. Rev.* **1993**, *93*, 1673; P. G. Williams, *Trends Biotechnol.* **2009**, *27*, 45.
[2] B. Pal, V. S. Giri, P. Jaisankar, *Catal. Commun.* **2005**, *6*, 711.
[3] R. Veluri, I. Oka, I. Wagner-Döbler, H. Laatsch, *J. Nat. Prod.* **2003**, *66*, 1520.
[4] M. Kobayashi, S. Aoki, K. Gato, K. Matsunami, M. Kurosu, I. Kitagawa, *Chem. Pharm. Bull.* **1994**, *42*, 2449.
[5] R. Bell, S. Carmeli, N. Sar, *J. Nat. Prod.* **1994**, *57*, 1587.
[6] L. Zheng, X. Yan, J. Xu, H. Chen, W. Lin, *Appl. Biochem. Microbiol.* **2005**, *41*, 29.
[7] G. Bartoli, M. Bosco, G. Foglia, A. Giuliani, E. Marcantoni, L. Sambri, *Synthesis* **2004**, 895.
[8] T. J. Mosmann, *Immunol. Methods* **1983**, *65*, 55.
[9] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenny, M. R. Boyd, *J. Natl. Cancer Inst.* **1990**, *82*, 1107.

Received October 12, 2009