Streptomycindole, an Indole Alkaloid from a Marine *Streptomyces* sp. DA22 Associated with South China Sea Sponge *Craniella australiensis*

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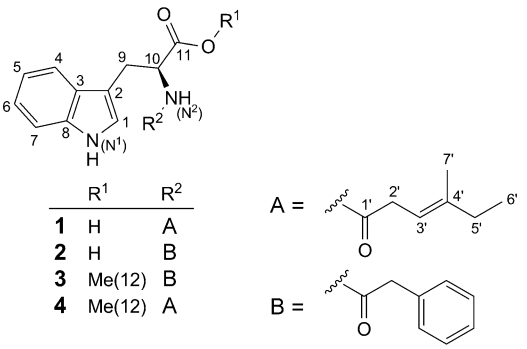
A new indole alkaloid, streptomycindole (**1**), and a known related compound, *N*-phenylacetyl-L-tryptophan (**2**), have been isolated from *Streptomyces* sp. DA22, associated with South China Sea sponge *Craniella australiensis*. Their structures were established on the basis of a combination of mass spectrometry, ^{1}H- and ^{13}C-NMR spectroscopic analyses, and chemical methods.

Introduction. – Actinomycetes are known to be precious sources of natural products of great pharmaceutical potential [1–7]. Marine sponges harbor various microbial symbioses [8], which are presumably the true producers of some natural products isolated from sponges [9]. Recently, sponge-associated microbes, especially actinomycetes, attract more attention [10][11]. However, to the best of our knowledge, the reported metabolites from sponge-associated actinomycetes are relatively rare compared to those isolated from sponge-associated fungi [12][13]. In particular, there is no report about metabolites of South China Sea sponge-associated actinomycetes up to now. In our previous study [14], the strain *Streptomyces* sp. DA22, which showed significant inhibitory activity against *P. variotii*, *E. coli*, *P. fluorescens*, *C. albicans*, *S. aureus*, and *B. subtilis*, was isolated from the sponge *Craniella australiensis* collected in the South China Sea. To track the compounds responsible for the antibacterial activity, a chemical study on the title actinomycete was carried out. This investigation led to the isolation of a novel indole alkaloid, named streptomycindole (**1**), together with a related known compound **2** (*N*-phenylacetyl-L-tryptophan; *Fig.*). Here, we describe the isolation and structure elucidation of the new compound **1**.

Results and Discussion. – The producing strain was cultured and the fermentation was carried out as follows. A small spoon of spores growing on *Gause I* slant was inoculated into a 250-ml *Erlenmeyer* flask containing 100 ml of M1 [14] culture medium and cultured at 28° for 2 d on a rotary shaker at 150 rpm. Then, 1 ml of the resultant seed culture was inoculated into 500 solid plates each containing 100 g of the above culture medium and incubated for 5 d at 28°.



	R^1	R^2
1	H	A
2	H	B
3	Me(12)	B
4	Me(12)	A

Figure. *Structures of compounds* **1**–**4**

Fifty kg of the whole culture medium was exhaustively extracted with AcOEt to give a crude AcOEt extract (8.0 g), which was subjected to silica-gel column chromatography (CC) with $CHCl_3$/MeOH 50:1 to 1:1 as eluent, followed by *Sephadex LH-20* CC with $CHCl_3$/MeOH 1:1 to yield streptomycindole (**1**; 15 mg) and *N*-phenylacetyl-L-tryptophan (**2**; 12 mg).

Though the structure of **2** was already reported previously as a synthetic by-product [15], unfortunately, no NMR data of **2** were given. To avoid any error, the structure of **2** was characterized by detailed NMR spectroscopic analysis, as well as by chemical methods. Thus, compound **2** was treated with CH_2N_2 to afford the expected methyl ester derivative **3**. Careful analysis of ^{1}H,^{1}H-COSY and HMQC spectra of **3** led to identification of two key moieties, tryptophan ester and phenylacetyl segment. Furthermore, the HMBC cross-peak from H–C(10) (δ(H) 4.90) to C(1′) (δ(C) 172.2) confirmed the position of amide function at C(10) according to the assigned structure, namely *N*-phenylacetyl-L-tryptophan methyl ester, for **3**. The complete NMR data of **2** and **3** were compiled in the *Table*. To the best of our knowledge, this is the first report of isolation of compound **2** from a natural source.

Compound **1**, a pale yellow amorphous powder, $[\alpha]_D^{24} = -5$ ($c = 0.25$, $CHCl_3$), provided ^{1}H- and ^{13}C-NMR data (*Table*) very similar to those of co-occurring **2**. HR-EI-MS of **1** led to the molecular formula $C_{18}H_{22}N_2O_3$ (*m*/*z*, 314.1639; calc. 314.1630), indicating nine degrees of unsaturation. The presence of the same *N*-acyltryptophan moiety as in **2** could be easily recognized. In fact, the only difference between **2** and **1** is the substituent (R^2) at N(2). Substraction of the above elaborated tryptophan moiety from the molecular formula of **1** indicated that the rest moiety of the molecule, R^2, is $C_7H_{11}O$ consisting of two CH_2, two Me, one ketone CO, and a trisubstituted olefin groups. Analysis of the ^{1}H,^{1}H-COSY, HMQC, and HMBC spectra led to identification of the spin system including signals as δ(H) 2.80 (*d*, CH_2(2′)), 5.13 (*dd*, H–C(3′)), 1.92 (*q*, CH_2(5′)), and 0.90 (*t*, Me(6′)). The (*E*)-configuration of the C(3′)=C(4′) bond was unambiguously determined by the observation of the diagnostic ROESY correlation between H–C(3′) and CH_2(5′), and no correlation between H–C(3′) and Me(7′). By analogy to **2**, the location of R^2 at N(2) was confirmed by HMBC between H–C(10) (δ(H) 4.38) and C(1′) (δ(C) 170.3).

Table. *^{1}H- and ^{13}C-NMR Data of* **1**, **2**, *and* **3**. Recorded at 400 MHz in (D_6)DMSO (**1**) or $CDCl_3$ (**3**); recorded at 300 MHz in CD_3OD (**2**).

	1		**2**	**3**	
	δ(H)	δ(C)	δ(H)	δ(H)	δ(C)
H–C(1)	7.10 (*s*)	123.2 (*d*)	6.82 (*s*)	7.25 (*s*)	122.5 (*t*)
C(2)	–	110.2 (*s*)	–	–	109.8 (*s*)
C(3)	–	127.4 (*s*)	–	–	127.5 (*s*)
H–C(4)	7.48 (*d*, J = 7.8)	118.2 (*d*)	7.44 (*d*, J = 7.8)	7.44 (*d*, J = 7.8)	118.5 (*d*)
H–C(5)	6.98 (*dd*, J = 7.8, 8.1)	118.2 (*d*)	7.11 (*dd*, J = 7.2, 8.1)	7.11 (*dd*, J = 7.2, 8.1)	122.2 (*d*)
H–C(6)	7.04 (*dd*, J = 7.8, 8.1)	120.8 (*d*)	7.18 (*dd*, J = 7.2, 8.1)	7.18 (*dd*, J = 7.2, 8.1)	119.7 (*d*)
H–C(7)	7.30 (*d*, J = 8.1)	111.2 (*d*)	7.33 (*d*, J = 8.1)	7.33 (*d*, J = 8.1)	111.1 (*d*)
C(8)	–	136.0 (*s*)	–	–	136.0 (*s*)
CH_2(9)	2.98 (*dd*, J = 8.1, 14.7), 3.18 (*dd*, J = 4.8, 14.7)	27.1 (*t*)	3.16–3.20 (*m*)	3.25 (*d*, J = 5.4)	27.3 (*t*)
H–C(10)	4.38 (*dd*, J = 4.8, 8.1)	53.5 (*d*)	3.63–3.67 (*m*)	4.90 (*dt*, J = 5.4, 7.8)	52.8 (*d*)
C(11)	–	170.3 (*s*)	–	–	170.5 (*s*)
Me(12)		–		3.66 (*s*)	52.3 (*q*)
C(1′)	–	170.3 (*s*)	–	–	172.2 (*q*)
CH_2(2′)	2.80 (*d*, J = 6.9)	34.8 (*t*)	3.42 (*s*)	3.53 (*s*)	43.6 (*t*)
H–C(3′)	5.13 (*dd*, J = 6.5, 6.7)	116.4 (*d*)	–	–	134.5 (*s*)
C(4′)	–	138.8 (*s*)	7.12–7.16 (*m*)	7.12–7.16 (*m*)	129.4 (*d*)
CH_2(5′)	1.92 (*q*, J = 7.5)	31.2 (*t*)	7.24–7.28 (*m*)	7.24–7.28 (*m*)	128.9 (*d*)
Me(6′)	0.90 (*t*, J = 7.5)	12.4 (*q*)	7.24–7.28 (*m*)	7.24–7.28 (*m*)	127.2 (*d*)
Me(7′)	1.50 (*s*)	16.0 (*q*)	7.24–7.28 (*m*)	7.24–7.28 (*m*)	128.9 (*d*)
H–C(8′)	–	–	7.12–7.16 (*m*)	7.12–7.16 (*m*)	129.4 (*d*)
H–N(1)	–	–	–	7.97 (br. *s*)	–
H–N(2)	–	–	–	5.92 (br. *d*, J = 7.8)	–

To confirm the assigned structure, a methylation was performed. Compound **1** was treated with CH_2N_2, and the expected methyl ester derivative **4** was obtained. Due to the methylation, H–C(10) (adjacent to the COO group) was apparently downfield shifted (+0.53 ppm), supporting the structure **1** for streptomycindole.

Compounds **1**–**3** were tested for cytotoxicity against several tumor cell lines such as HL-60 leukemic cell line, HCT-116 colon carcinoma cell line, HO-8910 ovarian epithelial carcinoma cell line, and HepG2 human hepatocarcinoma cell lines. Unfortunately, they were all found to be inactive.

Further studies should be conducted to understand the real role of these metabolites in the life cycle of the host sponge and the actinomycete itself, as well as to conduct fermentation of the actinomycete on a large scale and to explore the potential pharmaceutical values of compounds **1** and **2**.

This research work was financially supported by the *Natural Science Foundation of China* (Nos. 30730108, 21021063, and 20911140471). The work was also partially funded by the *STCSM Project* (No. 10540702900), *Hungarian–Chinese Intergovernmental S&T Cooperation Programme* (2009–2011), *EU 7th Framework Programme-IRSES Project* (2010–2014), *SKLDR/SIMM Project* (SIMM1105KF-04 and SIMM1106KF-11), and a grant from *CAS* (KSCX2-YW-R-18).

Experimental Part

General. Column chromatography (CC): commercial silica gel (SiO_2, 200–300 and 400–600 mesh; *Qing Dao Hai Yang Chemical Group Co.*) or *Sephadex LH-20* (*Amersham Biosciences*). TLC: Precoated silica-gel plates (*G60*, *F-254*, *Yan Tai Zi Fu Chemical Group Co.*). Optical rotation: *Perkin-Elmer* polarimeter *341* at the Na D-line, cell length 100 mm. UV Spectra: *756 CRT* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Nicolet-Magna FT-IR 750* spectrometer, $\tilde{\nu}_{max}$ in cm^{-1}. ^1H- and ^{13}C-NMR spectra: *Bruker DRX-400* (400 and 100 MHz for ^1H and ^{13}C, resp.) and *Varian Mercury-300* (300 and 75 MHz for ^1H and ^{13}C, resp.) spectrometers; chemical shift δ in ppm, with residual (D_6)DMSO (δ(H) 2.50, δ(C) 39.5), $CDCl_3$ (δ(H) 7.26, δ(C) 77.0), or CD_3OD (δ(H) 3.30, δ(C) 49.0) as internal standards, coupling constant J in Hz; assignments supported by ^1H,^1H-COSY, HSQC, HMBC, and ROESY experiments. ESI-MS: *Q-TOF Micro* (*Waters*) LC-MS/MS mass spectrometer, in *m*/*z*. EI-MS and HR-EI-MS: *Finnigan-MAT-95* mass spectrometer, in *m*/*z*.

Material. Specimens of sponge *Craniella australiensis*, identified by Prof. *J.-H. Li*, Institute of Oceanology, Chinese Academy of Sciences, were collected off Sanya Bay, Hainan Province, P. R. China, in 2002, at a depth of -20 m, and were frozen immediately after collection. The strain DA22 was isolated from *C. australiensis* on *Gause I* medium (soluble starch, 20 g/l; KNO_3, 1 g/l; NaCl, 0.5 g/l; K_2HPO_4, 0.5 g/l; $MgSO_4$, 0.5 g/l; $FeSO_4$, 0.01 g/l; agar, 18 g/l) prepared with artificial sea water (ASW) containing NaCl (26.518 g), $MgCl_2$ (2.447 g), $MgSO_4$ (3.305 g), $CaCl_2$ (1.141 g), KCl (0.725 g), $NaHCO_3$ (0.202 g), NaBr (0.083 g), and doubly dist. H_2O (1000 ml). The strain was identified as belonging to the genus *Streptomyces* by morphological and molecular (16S rDNA; accession No. DQ180137) phylogenetic methods. A detailed description of the bacterial isolation and growth properties is found elsewhere. To obtain sufficient amount of biological material for chemical study, the strain of *Streptomyces* sp. DA22 was cultured for 5 d on solid plates using M1 [16] medium containing 10 g of starch, 4 g of yeast extract, 2 g of peptone, and 18 g of agar in 1 l of ASW at pH 7.2.

Extraction and Isolation. The culture medium and mycelium were, resp., extracted with AcOEt for four times (4×1 l). After evaporation to dryness, the combined extracts (8.0 g) were chromatographed on a SiO_2 column with a $CHCl_3$/MeOH gradient as eluent, resulting in seven fractions. *Fr. 5* was further purified by CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1; and SiO_2; $CHCl_3$/MeOH 8:2) to yield compound **1** (15 mg). *Fr. 6* was subjected to gel filtration on *Sephadex LH-20* (petroleum ether/$CHCl_3$/MeOH 2:1:1), to afford a mixture that was further purified by CC (SiO_2; $CHCl_3$/MeOH 8:2) to afford compound **2** (12 mg).

Streptomycindole (= N-*[(3*E*)-4-Methylhex-3-enoyl]*-L-*tryptophan*; **1**). Pale yellow powder. $[\alpha]_D^{24} = -5$ ($c = 0.25$, $CHCl_3$). UV (MeOH): 221 (3.42), 276 (2.81). IR (neat): 3288, 2925, 2871, 1722, 1651, 1530–1400. ^1H- and ^{13}C-NMR: see the *Table*. ESI-MS (pos.): 315.2 ($[M+H]^+$). EI-MS: 314 (M^+). HR-EI-MS: 314.1639 (M^+, $C_{18}H_{22}N_2O_3^+$; calc. 314.1630).

Methyl N-*[(3*E*)-4-Methylhex-3-enoyl]*-L-*tryptophanate*; **4**). Freshly prepared CH_2N_2 reagent (0.5 ml) was added to 1 ml of MeOH soln. of **1** (5 mg) at 4° with stirring. This soln. was gradually warmed to r.t. After 2 h, the solvent was removed *in vacuo* to yield 4 mg of **4**. Yellow powder: ^1H-NMR ($CDCl_3$, 300 MHz): 8.11 (br. *s*, H–N(1)); 7.52 (*d*, $J = 8.1$, H–C(4)); 7.35 (*d*, $J = 7.8$, H–C(7)); 7.19 (*dd*, $J = 7.8$, 8.1, H–C(6)); 7.11 (*dd*, $J = 7.8$, 8.1, H–C(5)); 6.95 (*s*, H–C(1)); 6.19 (br. *s*, H–N(2)); 5.17 (*dd*, $J = 7.4$, 7.5, H–C(3′)); 4.91 (br. *t*, $J = 7.2$, H–C(10)); 3.69 (*s*, Me(12)); 3.31 (br. *d*, $J = 7.8$, CH_2(9)); 2.93 (*d*, $J = 7.2$, CH_2(2′)); 1.93 (*q*, $J = 7.5$, CH_2(5′)); 1.49 (*s*, Me(7′)); 0.89 (*t*, $J = 7.5$, Me(6′)). ESI-MS: 329.2 ($[M+H]^+$).

N-*(Phenylacetyl)*-L-*tryptophan* (**2**). Pale yellow powder. ^1H- and ^{13}C-NMR: see the *Table*. ESI-MS: 323.1 ($[M+H]^+$).

Methyl N-*(Phenylacetyl)*-L-*tryptophanate* (**3**). Pale yellow powder. $[\alpha]_D^{20} = -28$ ($c = 1.5$, $CHCl_3$). ^1H- and ^{13}C-NMR: see the *Table*. ESI-MS: 337.1 ($[M+H]^+$).

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Received November 29, 2010