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Sg17-1-4, a Novel Isocoumarin from a Marine Fungus *Alternaria tenuis* Sg17-1

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Abstract One novel isocoumarin, named Sg17-1-4, along with two known isocoumarins AI-77-B and AI-77-F were obtained from a marine fungus *Alternaria tenuis* Sg17-1. Their structures were elucidated based on detailed NMR analysis. The cytotoxicities of these compounds were evaluated *in vitro*.

Keywords marine fungus, isocoumarin, cytotoxicity

Isocoumarins such as the amicoumacins, the AI-77 series of compounds and the xenocoumacins have displayed antibacterial, antitumor and potent antiulcer activities [1～3]. They have been isolated from bacteria. The AI-77 series of compounds were all derived from culture broths of the genus *Bacillus*. In the course of screening bioactive natural compounds from marine microorganisms, three compounds, AI-77-B (**1**), AI-77-F (**2**) and Sg17-1-4 (**3**) were isolated from the culture broth of a marine fungus *Alternaria tenuis* Sg17-1. Compound **3** is a novel isocoumarin, which possesses an unusual 7-number ring in its side chain. This is the first time compounds of this type have been isolated from a fungus. The cytotoxicities of these compounds have been tested using A375 S2 and HeLa cells *in vitro*. Here, we report the isolation, structural elucidation and bioactivities of these compounds.

The fungal strain was isolated from a marine alga collected in Zhoushan Island in 2003 and identified as *Alternaria tenuis* by Prof. Li Tian. A voucher specimen (No. CAAN034015) is deposited in Key laboratory of Marine Biology of State Oceanography Administration, China. The strain was incubated in forty 500-ml flasks each containing 100 ml of medium for 12 days at 25°C on a rotary shaker (150 rpm). The culture medium contained corn steep liquor 200 ml, peptone 2.0 g, yeast powder 1.0 g, dextrose 10 g, NaCl 17 g, $MgCl_2 \cdot 6H_2O$ 1.3 g, KCl 0.1 g and $FePO_4$ 0.01 g in distilled water of 1000 ml. The harvested broth (40 liters) was centrifugated to separate the mycelial mass from the aqueous layer. The aqueous layer was extracted with 1-butanol exhaustively. The organic layer was evaporated under reduced pressure to obtain a crude residue. The residue (20 g) was chromatographed on a silica gel column using a gradient of methanol in chloroform. Fractions eluted with methanol - chloroform 10 : 100 were combined to be and further purified over on a Sephadex LH-20 column (Pharmadex, $CHCl_3$/MeOH 1 : 1). The final purification was carried on reversed-phase silica gel (Chromatorex C_{18}, MeOH/H_2O 1 : 1) to give **1** (10 mg), **2** (12 mg) and **3** (7 mg) respectively.

Compounds **1** and **2** were identified as AI-77-B and AI-77-F, respectively, by comparison with the literature [4] and on the basis of various spectroscopic analyses.

Compound **3**, an amorphous white powder (MeOH), gave a positive color reaction with ninhydrin and $FeCl_3$ reagent and a negative color reaction with bromocresol green reagent. The mp was 147～149°C and $[\alpha]_D^{20}$ −110° (*c* 0.1, MeOH). Its molecular weight was found to be 494 by

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Table 1 ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of sg17-1-4[a,b]

^{13}C shift	^{1}H shift	Assignment	^{13}C shift	^{1}H shift	Assignment
173.3		12′	48.7	2.91 (q, 6.4)	15′
171.8		7′	48.1	4.22 (m)	5′
169.3		1	48.0	3.19 (m)	10′
160.9		8	39.0	1.33 (m), 1.66 (m)	4′
140.9		10	29.1	2.82 (m), 3.10 (m)	4
136.3	7.48 (t, 7.9)	6	28.2	2.21 (m), 2.52 (m)	11′
118.8	6.83 (d, 7.9)	5	24.6	0.93 (s)	18′
115.2	6.84 (d, 7.9)	7	24.2	1.66 (m)	3′
108.4		9	23.4	0.88 (d, 6.3)	2′
94.6		14′	21.6	0.86 (d, 6.3)	1′
81.2	4.70 (ddd, 12.7, 3.4, 2.9)	3	15.3	0.93 (d, 6.4)	17′
70.1	3.81 (d, 8.9)	8′		7.97 (s)	6′
68.5	4.26 (dd, 8.9, 3.1)	9′			

[a] Recorded in DMSO-d_6, coupling constant (Hz).
[b] Assignments are based on HMQC, HMBC, HHCOSY, NOESY data.

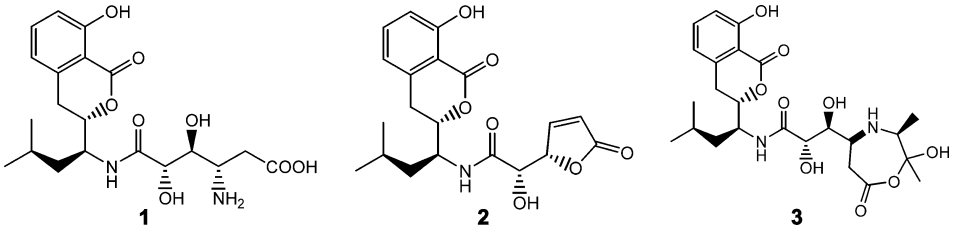

Fig. 1 Structures of Al-77-B (**1**), Al-77-F (**2**) and sg17-1-4 (**3**).

ESI-MS that showed a protonated molecular ion at *m*/*z* 495.1 $(M+H)^+$. The molecular formula was established as $C_{24}H_{34}O_9N_2$ by HR-SIMS [*m*/*z* 495.2334 $(M+H)^+$], which suggested that **3** had 9 units of unsaturation. The UV (MeOH) spectrum displayed bands at 314 nm, 246 nm and 209 nm, which was similar to those of **1** and **2**. The IR spectrum, ν_{max} (KBr) cm^{-1}: 3392, 2930, 1790, 1672, 1570 suggested the presence of a phenolic hydroxyl, an amide group and a saturated ester carbonyl. All these findings above suggested that **3** contained a chromophore similar to 3,4-dihydro-8-hydroxyisocoumarin in its structure. The NMR spectra of **3** (Table 1) were similar to those of compound **1**. Analysis of the 1D and 2D (HMQC, HMBC, HHCOSY and NOESY) NMR spectra of **3** (Fig. 2), suggested a partial structure A as seen in compound **1** (Fig. 1). In addition to the signals for structure A, protons from two methyl at δ 0.93 (3H, s, H-18′) and 0.91 (3H, d, J=6.4 Hz, H-17′) and one methine proton at δ 2.91 (1H, q, J=6.4 Hz, H-15′) can be observed in the ^{1}H NMR spectrum of **3**. One quaternary carbon at δ 94.6 (C-14′), one tertiary carbon at δ 48.7 (C-15′) and two primary carbons at δ 24.6 (C-18′), 15.3 (C-17′) can be observed in the ^{13}C NMR spectrum of **3**. In the HMBC spectrum the proton signal at δ 0.93 (3H, s, H-18′) and 0.91 (3H, d, J=6.4 Hz, H-17′) showed C–H long-range correlations with C-15′ (δ 48.7) and C-14′ (δ 94.6), respectively. By analysis of the 2D NMR and chemical shift values of these data, another partial structure B can be obtained. By further analysis of the HMBC data of **3**, the correlation between the proton at δ 3.19 (H-10′) in structure A and the carbon at δ 48.7 (C-15′) in structure B can be observed. Therefore, considering the degrees of unsaturation and 2D NMR of **3**, we conclude that the new isocoumarin Sg17-1-4 has the planar structure C. Compound **3** was obtained from the same source as compounds **1** and **2**, so the absolute configurations at C3, C5′, C8′, C9′, C10′ should be same as those of **1** and **2**. The CD spectrum of **3** ($\Delta\varepsilon_{326}$ −0.35, $\Delta\varepsilon_{306}$ −0.48, $\Delta\varepsilon_{258}$ −4.2, MeOH) was identical with those of **1** and **2**, which also confirmed this conclusion. The NOE between H-10′ and H-15′ was obviously observed, which indicated C-15′ had an *S*-configuration. As a hemiacetal carbon, the configuration at C14′ couldn't be defined.

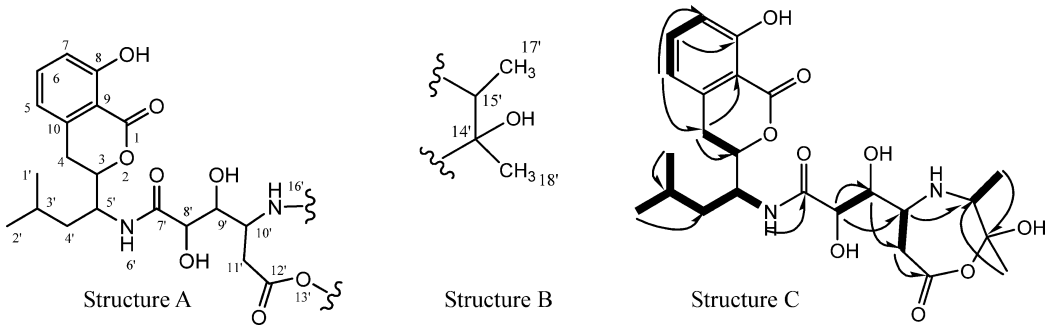

Fig. 2 The selective 2D NMR correlations of **3**. —— ^{1}H-^{1}H COSY ⟶ HMBC.

Finally, the structure of **3** is identified as Fig. 1 shows.

The cytotoxicities *in vitro* against human malignant A375-S2 and human cervicial cancer Hela cells were measured with the MTT assay [5]. Compound **1** exhibited the strongest activity with IC_{50} values of 0.1 and 0.02 mM, respectively. The values of **3** were 0.3 and 0.05 mM. Compound **2** showed only weak activity to Hela cells with an IC_{50} value of 0.4 mM.

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