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Cycloheximide Acid A, a New Cycloheximide Derivative from Marine Derived *Streptomyces* sp. from East China Sea

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Abstract: A new cycloheximide derivative, Cycloheximide acid A (**1**) and two known compounds (–)-anhydrocycloheximide (**2**), and *l*-cycloheximide (**3**) were isolated from the EtOAc extract of the strain of *Streptomyces* sp. Z00045. The structure was determined on the basis of comprehensive 1D and 2D (COSY, HMQC, HMBC) NMR and MS analyses.

Keywords: *cycloheximide derivatives*; *Streptomyces* sp.Z00045; spectroscopic analyses.

1. Introduction

Microbes have always been a rich resource for getting lead molecules with novel scaffold to overcome the limitation of existing drugs. Among the 22 500 biologically active compounds from microbes, 45% are produced by actinomycetes, 38% by fungi and 17% by unicellular bacteria [1]. More and more novel metabolites are being discovering from microbes which act as always a major participant in the global pharmaceutical and neutraceutical industry [2-5]. In the course of screening for new compounds from marine environment, a strain of *Streptomycetes* was isolated from sea water of the East China Sea. Investigation on the chemical constituents of the EtOAc extract yielded a new cycloheximide derivative, cycloheximide acid A (**1**), along with two known compounds, (–)-anhydrocycloheximide (**2**), and *l*-cycloheximide (**3**) [6,7]. In this paper, the compounds isolation and structure elucidation were reported

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2. Materials and Methods

2.1. Microorganism Material

The marine derived *Streptomyces* sp. was isolated from seawater collected from the East China Sea, Wenzhou, Zhejiang Province, China in Oct. 2009.

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2.2 .Fermentation and Isolation

A small spoon of spores growing on Gause's agar slant was inoculated into a 250 mL conical flask containing 40 mL of Gause's synthetic medium and the flask was incubated at 28 ^{o}C for 5 days on a rotary shaker at 160 rpm. Then 20 mL of the resultant seed culture was inoculated into 500 mL >> 1000mL conical flask each containing 300 mL of liquid medium [composed of glucose (1.0%), maltose (1.0%) and yeast extract (0.3%), $CaCl_2$ (0.015%), $MgCl_2$ (0.02%), soybean cake meal (3%) and natural seawater, then adjusting its pH to 7.2], and 20 flasks were cultured at 28°C for 10 days on a rotary shaker at 160 rpm.

The culture broth was extracted with EtOAc exhaustively and the solvent was removed under reduced pressure at <50 °C to yield a brown residue. The EtOAc extract (1.2 g) was subjected to normal phase silica gel (200-300 mesh) column and eluted with a gradient of increasing MeOH (0-100%) in dichloromethane. The fraction eluted by 15% MeOH in dichloromethane was rechromatographed over Sephadex LH-20 using $CHCl_3$-MeOH (3:1) to afford four subfractions. The third subfraction was further separated by reversed-phase preparative HPLC using MeOH-H_2O (70:30) as mobile phase to yield compounds **1** (3.0 mg), **2**, (3.2 mg) and **3** (4.0 mg).

Cycloheximide acid A (**1**), white amorphous powder; $[\alpha]^{20}_{,D}$ + 8.4 (*c* 0.1, MeOH); ESIMS *m/z*: 296 $[M+H]^+$; HRESIMS: *m/z* 296.1498 (calcd for $C_{15}H_{21}NO_5$, 296.1492); for ^{1}H NMR and ^{13}C NMR data, see Table 1.

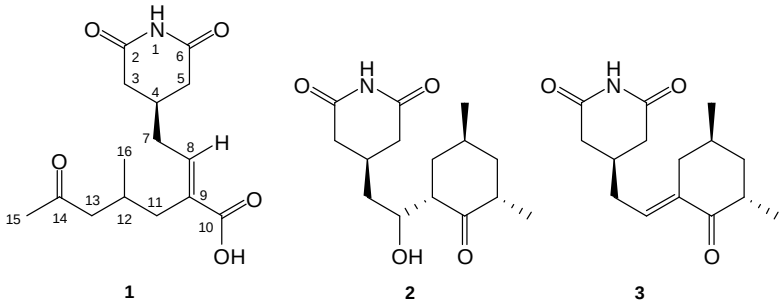

Figure 1. Structures of compounds **1**–**3**

3. Results and Discussion

3.1. Structure elucidation

Compound **1** was obtained as a white amorphous powder. High resolution ESI-MS revealed a pseudo molecular ion peak at m/z 296.1498 for [M+H]+ (calcd. 296.1492) >> pseudo molecular ion peak at m/z 296.1492 for [M+H]+ (calcd. 296.1498) corresponding with the molecular formula $C_{15}H_{21}NO_5$ and six degrees of unsaturation. Analysis of ^{1}H and ^{13}C NMR data for **1**, along with 2D NMR data, indicated the presence of 3-glutarimidyl moiety characteristic of the cycloheximide, including two carbonyl carbons (δ_C 175.1, C-2 and C-6), two methylene groups (δ_C 38.2, C-3 and C-5; δ_H 2.39, 2H, and 2.66, 2H, H_2-3 and H_2-5), and one methine (δ_C 31.7, C-4; δ_H 2.36, H-4). Apart from these moieties, the NMR data for **1** showed one trisubstituted double bond (δ_C 134.7, C-9, and 141,1, C-8; δ_H 6.83, d, J=7.5 Hz, H-8), one carbonyl group (δ_C 211.5, C-14), one carboxyl group (δ_C 170.9, C-10), one methine (δ_C 31.7, C-12; δ_H 2.17, H-12), three methylene [(δ_C 34.6, C-7; δ_H 2.36, H_2-7), (δ_C 34.3, C-11; δ_H 2.29, 2.23, H_2-11) and (δ_C 51.4, C-13; δ_H 2.45, 2.34, H_2-13)], and two methyl groups [(δ_C 30.7, C-15; δ_H 2.12, H_3-15), (δ_C 20.2, C-16; δ_H 0.88, H_3-16)]. Through careful analysis of the ^{1}H-H COSY, the moieties from C-8 to C-7, through C-4 to C-3 and C-5, and from C-11 through C-12 to C-13 and the methyl group at C-12 were established (Figure 1). The HMBC correlations from H-8 to C-9 and C-10 revealed that the carboxyl group (C-10) was attached to the vinylic carbon C-9. The HMBC correlations from H_3-15 to C-14 and C-13 established the connection from C-13 through C-14 to C-15. The HMBC correlations from H_2-3 and H_2-5 to C-2 (δ_C 175.1) revealed the cycloheximide

moiety. The HMBC correlations from H_2-3 and H_2-5 to C-7 and from H-8 to C-4 revealed that the cycloheximide moiety and the side chain were connected between C-4 and C-7. From the ROESY spectra**,** the correlation from H_2-7 to H_3-16 revealed that the double bond of compound **1** was *E* configuration. From the point of biosynthesis, the absolute configuration of C-4 was assigned.

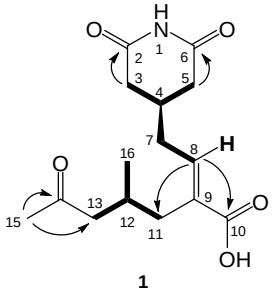

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Figure 2. ^{1}H-^{1}H COSY correlations and the selected HMBC correlations of compound **1** and the structure of compound **1**

The known compounds (–)-anhydrocycloheximide (**2**) and *l*-cycloheximide (**3**) [6, 7] were identified through direct comparison with published data.

Table 1. ^{1}H and ^{13}C NMR data for compound **1** (at 500 MHz in CD_3OD, δ in ppm, *J* in Hz).

No.	H	C	COSY	HMBC
2		175.1 (C)		
3	2.66 (1H, dd, 16.0, 3.5)	38.2 (CH_2)	H-3b, H-4	C-2, C-4, C-5, C-7
	2.39 (1H, m)		H-2b, H-4	C-2, C-4, C-5, C-7
4	2.36 (1H, m)	31.7 (CH)	H-3, H-5	C-3, C-5, C-7
5	2.66 (1H, dd, 16.0, 3.5)	38.2 (CH_2)	H-4, H-5b	C-3, C-4, C-6, C-7
	2.39 (1H, m)		H-4, H-5a	C-3, C-4, C-6, C-7
6		175.1 (C)		
7	2.36 (2H, m)	34.6 (CH_2)	H-8	C-3, C-4, C-5, C-8, C-9
8	6.83 (1H, d, 7.5)	141.1 (CH)	H-7	C-4, C-7, C-9, C-10, C-11
9		134.7 (C)		
10		170.9 (C)		
11	2.29 (1H, m)	34.3 (CH_2)	H-11b, H-12	C-8, C-9, C-10, C-12, C-13, C-16
	2.23 (1H, m)		H-11a, H-12	C-8, C-9, C-10, C-12, C-13, C-16
12	2.17 (1H, m)	31.7 (CH)	H-11, H-13	C-9, C-11, C-13, C-16
13	2.45 (1H, dd, 16.0, 7.0)	51.4 (CH_2)	H-12, H-13b	C-11, C-12, C-14, C-16
	2.34 (1H, m)		H-12, H-13a	C-11, C-12, C-14, C-16
14		211.5 (C)		
15	2.12 (3H, s)	30.7 (CH_3)		C-13, C-14
16	0.88 (3H, d, 7.0)	20.2 (CH_3)	H-12	C-11, C-12, C-13

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