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Cytochalasin H2, a New Cytochalasin, Isolated from the Endophytic Fungus *Xylaria* sp. A23

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Abstract: A new natural product, cytochalasin H2 (**1**), together with cytochalasin H (**2**) were obtained from the agar cultures of the strain *Xylaria* sp. A23, which was isolated from *Annona squamosa*. The chemical structures of them were elucidated by spectroscopic and mass spectrometric analyses, including 1D-, 2D-NMR and HR-FTMS. Compound **1** showed week cytotoxicity against HeLa and 293T cell lines by MTT assay.

Keywords: Cytochalasin H2; cytochalasin H; spectroscopic analyses.

1. Introduction

Annona squamosa L. is native to tropic America, as a folk medicine, it has significant curative effects on dysentery, hysteria, and tumor[1]. A huge number of biological active compounds have been isolated, including alkaloids, annonaceous acetogenins, cyclic peptides, and *ent*-diterpenoids[1-5]. As the endophytes may play important roles in the synthesis or transformation of these bioactive compounds, we embarked on the isolation of endophytic fungi from *Annona squamos*[6] and the search for novel bioactive compounds from them. Here, we report the isolation and structure elucidation of a novel compound cytochalasin H2 (**1**) and a known one cytochalasin H (**2**)[7] from the endophytic fungus *Xylaria* sp. A23 of *Annona squamosa*.

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2. Materials and Methods

2.1 General

Column chromatography (CC): silica gel (200-300 mesh; Qingdao Marine Chemical Factory, Qingdao, P. R. China), and RP-18 (Merck)) were used. Medium Pressure Liquid Chromatography (MPLC): BÜCHI C-605. TLC: precoated silica gel GF254 plates (0.20-0.25 mm, Qingdao Marine Chemical Factory). Optical rotations were obtained on a Perkin-Elmer 341 polarimeter with MeOH as solvent. The IR spectra were measured in KBr on a Nicolet FT-IR 360. HR-FT-MS data were acquired by using Thermo Scientific LTQ Orbitrap mass spectrometer. ^{1}H- and ^{13}C-NMR Spectra: Bruker Avance 600 MHz NMR spectrometer.

2.2. Microorganism Material

The strain A23 was isolated from leaves of *Annona squamosa* L. collected in Xiamen University, Fujian, China. The ITS rDNA sequencing (GenBank accession number: EU009996) was performed to characterize it as *Xylaria* sp., and named A23. The blast query of the NCBI database yielded *Xylaria* strain VegaE4-79 as the closest match to the ITS rDNA of A23 (97%).

2.3. Fermentation and Isolation

The fermentation was performed on PDA (0.5 litre) agar plates for 7 days at 28°C. The culture was diced and extracted with AcOEt/MeOH/AcOH (80 : 15 : 5). The crude extract was partitioned between MeOH and petroleum ether. The MeOH layer was concentrated in vacuum to afford a crude brown syrupy extract (450 mg). It (450 mg) was subjected to MPLC (*RP-18* (30 g); MeOH/H_2O, 1 : 1) to afford *Fr.1* (91.4 mg). This was further subjected to MPLC (*RP-18* (30 g); MeOH/H_2O, 3 : 2, and MeOH) to obtain **2** (12.6 mg) and *Fr.1.b.* (18.7 mg), which was further subjected to CC (SiO_2; petroleum ether/ethyl acetate, 3 : 1) to obtain **1** (5.8 mg).

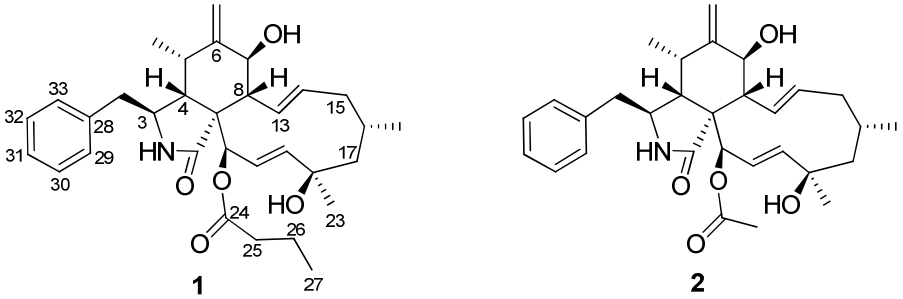

Figure 1. The chemical structures of compounds **1/2**.

3. Results and Discussion

3.1. Structure elucidation

Compound **1** was obtained as white powder. The molecular formula of **1** was determined to be $C_{32}H_{43}NO_5$ by its HR-FTMS (m/z 544.3031 $[M + Na]^+$) and NMR data (Table 1). $[\alpha]_D^{20} = 5.4$ (c = 0.52, MeOH). The IR spectrum showed the absorptions for ester carbonyl groups (1740cm^{-1}), and amide carbonyl groups (1680cm^{-1}). The ^{13}C NMR spectrum of **1** displayed 32 carbon signals for four

methyls, six methylenes, sixteen methines, and six quaternary carbon atoms, including two carbonyl groups, one at δ174.2 C(1), and the other at δ172.2 C(24).

Table 1. ^{1}H and ^{13}C NMR data for **1** (at 600/150 MHz in Acetone-d_6), δ in ppm, J in Hz).

Position	^{1}H	^{13}C	HMBCs
1		174.2 (*s*)	
2	7.05 (*s*)		48.7 (*d*), 52.0 (*s*)
3	3.30 (*m*)	53.2 (*d*)	32.3 (*d*)
4	2.18 (*dd*, *J* = 2.9, 5.2)	48.7 (*d*)	76.7 (*d*), 150.5 (*s*)
5	2.63 (*m*)	32.3 (*d*)	12.7 (*q*), 48.7 (*d*), 53.2 (*d*), 150.5 (*s*)
6		150.5 (*s*)	
7	3.82 (*d*, *J* = 10.1)	70.9 (*d*)	
8	2.93 (*t*, *J* = 10.1)	47.1 (*d*)	52.0 (*s*), 70.9 (*d*), 76.7 (*d*), 128.5 (*d*), 135.9 (*d*)
9		52.0 (*s*)	
10α	2.95 (*dd*, *J* = 5.9, 13.1)	44.7 (*t*)	48.7 (*d*), 52.0 (*s*), 129.7 (*d*), 137.7 (*s*)
10β	2.74 (*dd*, *J* = 8.0, 13.1)		48.7 (*d*), 52.0 (*s*), 53.2 (*d*), 128.4 (*d*), 137.7 (*s*)
11	0.54 (*d, J* = 6.8)	12.7 (*q*)	32.3 (*d*), 48.7 (*d*), 150.5 (*s*)
12a	4.91 (*s*)	111.5 (*t*)	32.3 (*d*), 70.9 (*d*)
12b	5.15 (*s*)		32.3 (*d*), 70.9 (*d*), 150.5 (*s*)
13	5.73 (*dd*, *J* = 9.6, 15.5)	128.5 (*d*)	43.1 (*t*), 47.1 (*d*)
14	5.27 (*ddd*, *J* = 4.9, 10.8, 15.5)	135.9 (*d*)	43.1 (*t*), 47.1 (*d*), 111.5 (*t*)
15α	1.70 (*dd, J* = 10.8, 23.2)	43.1 (*t*)	28.1 (*d*), 54.0 (*t*), 128.5 (*d*), 135.9 (*d*),
15β	1.96 (*dd, J* = 4.8, 12.5)		25.7 (*q*), 28.1 (*d*), 54.0 (*t*), 128.5 (*d*), 135.9 (*d*)
16	1.84 (*m*)	28.1 (*d*)	43.1 (*t*)
17α	1.81 (*m*)	54.0 (*t*)	43.1 (*t*)
17β	1.52 (*dd*, *J* = 3.4, 13.6)		72.8 (*s*)
18		72.8 (*s*)	
19	5.88 (*dd*, *J* = 2.3, 15.5)	126.0 (*d*)	72.8 (*s*), 76.7 (*d*), 128.5 (*d*)
20	5.53 (*dd, J* = 2.3, 15.5)	138.2 (*d*)	30.5 (*q*), 76.7 (*d*), 126.0 (*d*)
21	5.56 (*d, J* = 2.3)	76.7 (*d*)	30.5 (*q*), 47.1 (*d*), 48.7 (*d*), 52.0 (*s*), 76.7 (*d*), 126.0 (*d*), 138.2 (*d*),
22	1.00 (*t, J* = 6.9)	25.7 (*q*)	28.1 (*d*), 43.1 (*t*), 54.0 (*t*)
23	1.26 (*s*)	30.5 (*q*)	28.1 (*d*), 54.0 (*t*), 72.8 (*s*)
24		172.2 (*s*)	
25	2.57 (*m*)	35.9 (*t*)	13.2 (*q*), 18.6 (*t*)
26	1.82 (*m*)	18.6 (*t*)	43.1 (*t*)
27	1.04 (*t*, *J* = 7.4)	13.2 (*q*)	18.6 (*t*), 35.9 (*t*)
28		137.7 (*s*)	
29	7.24 (*m*)	129.7 (*d*)	44.7 (*t*), 126.5 (*d*), 129.7 (*d*)
30	7.31 (*m*)	128.4 (*d*)	128.4 (*d*), 137.7 (*s*)
31	7.24 (*m*)	126.5 (*d*)	129.7 (*d*)
32	7.31 (*m*)	128.4 (*d*)	128.4 (*d*), 137.7 (*s*),
33	7.24 (*m*)	129.7 (*d*)	44.7 (*t*), 126.5 (*d*), 129.7 (*d*)
7-OH	3.48 (s)		54.0 (*t*), 174.2 (*s*)

The HMBCs from H-C(32) to C(28), and H-C(29) to C(10), along with the ^{1}H,^{1}H-COSYs H-C(29)↔H-C(30)↔H-C(31)↔H-C(32)↔H-C(33), and H-C(3)↔H-C(10), let to the establishment of fragment **a**. Fragment **b** was assigned based on the HMBCs from Me(11) to C(4), C(5) and C(6), CH_2(12) to C(6) and C(7), CH (8) to C(1), C(4) and C(9), and the ^{1}H,^{1}H-COSYs H-C(7)↔H-C(8). Meanwhile, the HMBCs from Me(22) to C(15), C(16) and C(17), Me(23) to C(17) and C(18), CH(14) to C(15), CH(19) to C(18), along with the ^{1}H,^{1}H-COSYs H-C(13)↔H-C(14), H-C(15)↔H-C(16)↔H-C(17), H-C(19)↔H-C(20)↔H-C(21), H-C(25)↔H-C(26)↔H-C(27), let to the establishment of

fragment **c**. Fragments **a** and **b** were connected on the basis of the HMBCs from H-C(3) to C(5), and ^{1}H,^{1}H-COSYs H-C(3)↔H-C(4). The connection of fragments **b** and **c** was identified from the HMBCs from the H-C(21) to C(4), and the ^{1}H,^{1}H-COSYs H-C(8)↔H-C(13). Finally, the lactam bond was confirmed by the HMBC correlations from NH(2) to C(8) and C(9) (Figure 2).

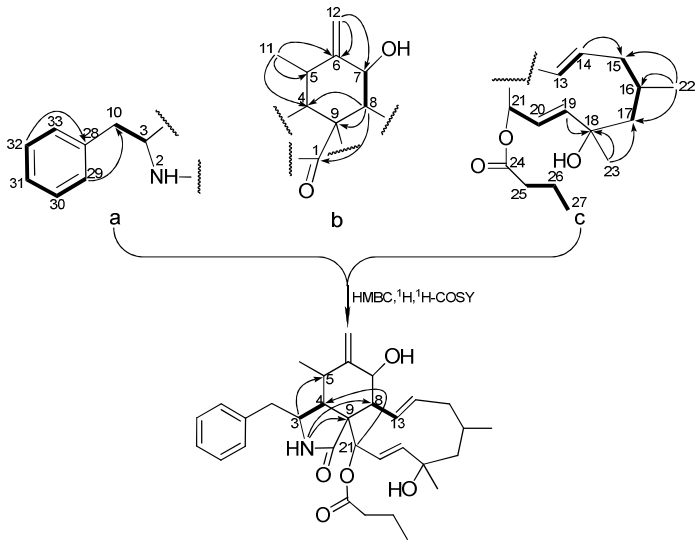

Figure 2. ^{1}H-^{1}H COSY correlations and selected HMBC correlations of **1**.

The relative configuration of **1** was established from the NOESY spectrum. The presence of NOE correlations H-C(3)↔H-C(7)↔H-C(11)↔H-C(21)↔H-C(23) indicated the *α*-orientation of these protons. The NOESY cross-peaks between H-C(4)↔H-C(8)↔H-C(14)↔H-C(16) established the *β*-orientation of H-C(4), H-C(8), H-C(14) and H-C(16) (Figure 3). Therefore, compound **1** was determined to be (3*S*,3a*R*,4*S*,6*S*,6a*R*,7*E*,10*S*,12*R*,13*E*,15*R*,15^{1}*R*)-3-benzyl-6,12-dihydroxy-4,10,12-trimethyl-5-methylene-1-oxo-2,3,3a,4,5,6,6a,9,10,11,12,15-dodecahydro-1*H*-cycloundeca[*d*]isoindol-15-yl butyrate, named cytochalasin H2.

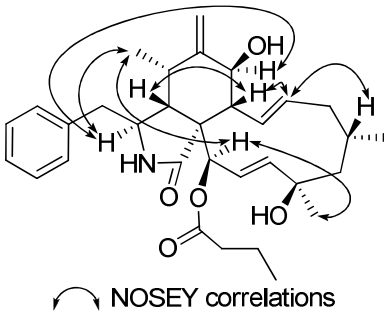

Figure 3. Key NOESY correlations of cytochalasin H2.

3.2. Cytotoxicity and Antimicrobial activity

HeLa cells were cultured in Dulbecco's modified Eagle's media (DMEM) supplemented with 10% fetal bovine serum. The cells were maintained at 37^{o}C in a humidified atmosphere at 95% air and 5% CO_2. Cell viability was measured by MTT assay[8]. The antibacterial activities of **1** were tested against *Bacillus subtilis* (CMCC (B) 63501) using slip method.

Compound **1** exhibited week cytotoxicity against HeLa and 293T cells (1.0 μg/mL, 25.04% and 32.8%, respectively), and induced cell contraction in both cell lines (Figure 4). Compound **1** had no effect on the growth of tested bacteria at 20 μg/disc.

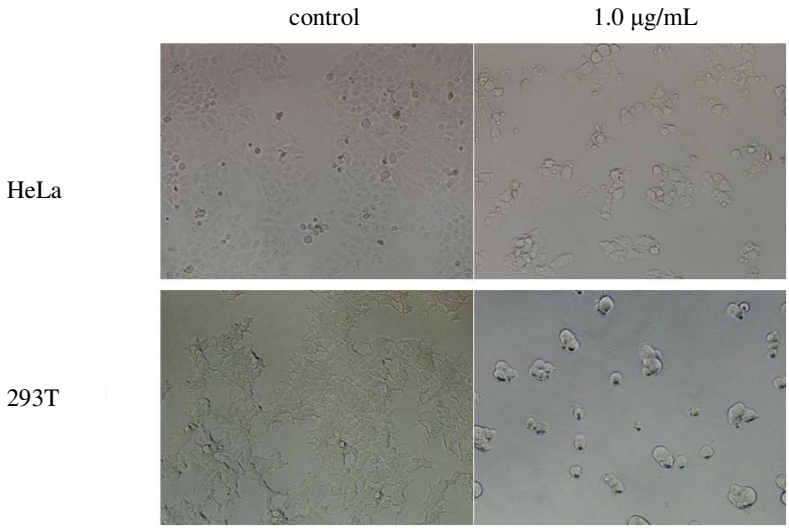

Figure 4. The contraction of HeLa and 293T cells by the treatment of **1** for 72 h.

The cytochalasins are a group of fungal secondary metabolites, related by structure and biological activity. Cytochalasins have the cytotoxic activities include effection of actin filament[9]. Cytochalasin H was first isolated from *Phomopsis* sp. in 1970's[10], which effects the cytoskeletal reorganisation as an inhibitor and shows moderate filament capping activity[11]. The bioactivities of cytochalasin H2 (**1**) need to be further explored.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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