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A New Cyclic Tetrapeptide from Endophytic Fungus *Aspergillus versicolor* E-2

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Abstract: A new cyclic tetrapeptide (**1**) named aspergilpeptide A, together with a known cyclic tetrapeptide penicopeptide A (**2**) and chaetominine (**3**) were obtained from the endophytic fungus *Aspergillus versicolor* E-2 isolated from the medicinal plant *Euphorbia royleana*. The structures of compounds (**1-3**) were elucidated using NMR and MS methods.

Keywords: Cyclic tetrapeptide; endophytic fungus; *Aspergillus versicolor*.

1. Introduction

Microbial natural products have made a significant contribution for constituting half of the pharmaceuticals in the present market [1]. Recent advances in microbial genomics have unequivocally demonstrated that the biosynthetic potential of natural products in endophytic fungus is much higher than previously appreciated [2]. Consequently, we have initiated a program to discover new natural products from endophytic fungus in traditional Chinese medicine (TCM). *Euphorbia royleana* Boiss. is a common thorny succulent species distributed in dry and hot valleys of southwestern mainland China, which usually used as a pesticide in folk, and the mashed fresh stems can be used as a treatment of psoriasis [3]. Previous activity studies in this plant have focused mainly on crude extracts, such as animal poisoning [4-5], anti-inflammatory and anti-arthritic activity [6], antioxidant, antibacterial, cytotoxic activity [7] and immunosuppressive activity [8], and the chemical studies have reported the isolation of some lathyranes and ingol-type diterpenes [9]. But the reports about the endophytic fungus isolated from *E. royleana* and *Euphorbia* or their fermentation and extracts of secondary metabolites was very few [10].

In this work, an endophytic fungus E-2 was derived from *Euphorbia royleana* Boiss. identified as *Aspergillus versicolor* based on internal transcribed spacer gene (ITS) sequence analysis. A new cyclic tetrapeptide (**1**) named aspergilpeptide A, and a known cyclic tetrapeptide, penicopeptide A (**2**) [11] and a known alkaloid, chaetominine (**3**) [12] (Figure 1) was isolated from the culture of E-2. This work describes for the first time the isolation of these compounds from *A. versicolor*. Details of the isolation and identification of compound **1** are presented herein and the known compounds **2-3** were compared spectroscopic data with those reported.

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2. Materials and Methods

2.1. Microorganism Material

The fungal strain *A. versicolor* E-2 was isolated as an endophytic fungus from the roots of fresh *E. royleana*, which was collected at Xuanwei State of Yunnan Province in China, in June 2018. The ITS region of 18 S rDNA sequence data for this fungal strain have been submitted to NCBI with the accession no. MH911364.1. A voucher specimen (No. 201807A) was preserved in Yunnan Minzu University, Kunming.

2.2. Fermentation and Isolation

The seed cultures of *A. versicolor* was prepared in PDA medium at 28 ºC for 7 days. The mass fermentation of this fungus was carried out at 25ºC for 60 days in 100 x 500 mL Erlenmeyer flasks, each containing 50 g of rice, 50 g of perlite and 120 mL of water. The fermented material were soaked in 70% ethanol solution and mashed into small pieces, and then sonicated for 30 min. The combined extracts were evaporated under reduced pressure to afford an aqueous solution, which was further extracted three times with EtOAc (2L x 3) to yield 40 g of the crude extract. The crude extract was subjected to silica gel column chromatography (CC) eluting with a mixed solvent system of CH_2Cl_2/MeOH in a step gradient (from 100/0 to 0/100, v/v) to afford five fractions (A–E). Fr. C (5 g) was further eluted with a silica gel column (dichloromethane: methanol, from 1:0~40:1~20:1~10:1~5:1~0:1, each 0.2 L) to afford six sub-fractions (Fr. C-1~C-6). Fr. C-3 (480 mg) was loaded onto MCI column using a stepwise gradient of MeOH/H_2O (from 30%~45%~60%~75%~95%, each 300 mL) to afford three fractions (Fr. C-3-a~c). Fr. C-3-a was separated over YMC-Pack ODS-A (20×250 mL.

D.S, 5μm, 5mL/min, 254nm) prep. HPLC (80% MeOH/H_2O) and Ultimate XB-C18 (10×250 mm, 5μm, 3mL/min, 203/254/280/300nm) semi-prep. HPLC (72% MeOH/H_2O), yielding **1** (18.1 mg, RT 11.7 min) and **2** (25.0 mg, RT 15.0 min). Fr. D (3 g) was further eluted with a silica gel column (dichloromethane: methanol, from 1:0~70:1~20:1~10:1~5:1~0:1, each 0.1 L) to afford five subfractions (Fr. D-1~C-5). Fr. D-2 (360 mg) was further separated with Venusil XBP C18 (21.2×250 mm, 5 μm, 5mL/min, 254nm) prep. HPLC (69% MeOH/H_2O) and separated over Ultimate XB-C18 (10×250 mm, 5μm, 3mL/min, 203/254/280/300nm) semi-prep. HPLC (80% MeOH/H_2O) to yield **3** (8.2 mg, RT 15.5 min).

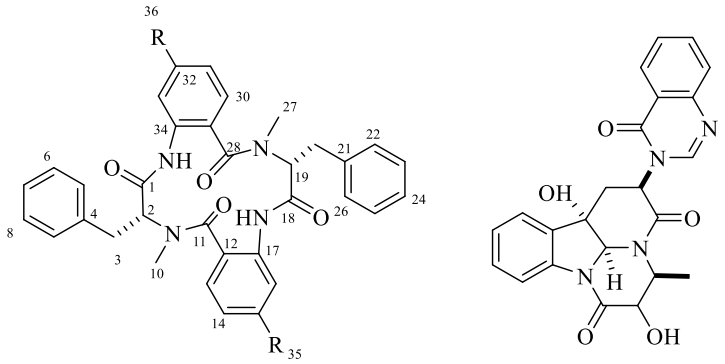

Figure 1. Chemical structures of compounds **1-3**

3. Results and Discussion

Compound **1**, yellow solid, showed a molecular formula of $C_{36}H_{36}N_4O_6$, as deduced from HR-ESI (+) MS ($[M+Na]^+$ at *m/z* 643.2524). Its ^{1}H NMR and ^{13}C NMR (Table 1) spectra showed the presence of the amide N-Me [δ_H 2.91, (3H, s); 3.06, (3H, s) and δ_C 39.8; 29.6] and amino acid protons (δ_H 4.29, dd, *J* = 7.0, 10.4 Hz and δ_H 4.42, t, *J* = 7.60 Hz) inferred that the compound have the properties of cyclopeptides. According to the number of carbons and careful interpretation of the 2D NMR data revealed the presence of two phenylalanine (Phe) and two 2-aminobenzoic acid residues. The above NMR data suggested **1** was a tetracycline peptide and similar to penicopeptide A [11] except **1** have two methoxy signals. This change can be confirmed by the key HMBC correlations from H-35 to C-15, and from H-36 to C-32 (Figure 2). The location of the methoxyl connection also can be confirmed by the cross-peaks of H-13/H-14 and H-30/H-31 in ^{1}H-^{1}H COSY spectrum (Figure 2). Finally, the planar structure of **1** was confirmed by the ^{1}H-^{1}H COSY and HMBC experiments. The absolute configurations of C-2 and C-19 in the Phe units were determined to be R by compare the coupling constant of H-2 and H-19 [δ_H 4.29 (1H, dd, 7.0, 10.4 Hz) and 4.42 (1H, t, 7.6 Hz)] were same with penicopeptide A. Thus, the structure of **1** was defined and named aspergilpeptide A.

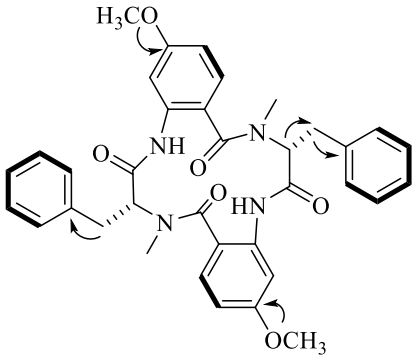

Figure 2. Key HMBC and COSY correlations for compound **1**

*Aspergilpeptide A (**1**):* Yellow solid; $[\alpha]_D^{25}$ -62.0 (*c* 0.26, MeOH); UV (MeOH) λ_{max} (log ε) 211 (2.57), 314 (3.52) nm; ^{1}H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) spectral data see Table 1; (+)HR-ESIMS: *m/z* 643.2524 $[M+Na]^+$ (calcd for $C_{36}H_{36}N_4O_6$, 643.2527).

*Penicopeptide A (**2**):* Yellow solid; ^{1}H-NMR (400 MHz, CD_3OD) δ_H 7.95 (1H, brd, *J* = 7.8 Hz, H-13), 7.80 (1H, brd, *J* = 7.8 Hz, H-30), 7.58 (1H, brd, *J* = 7.9 Hz, H-15), 7.51 (1H, brd, *J* = 7.1 Hz, H-32), 7.34 (1H, t, *J* = 7.5 Hz, H-14), 7.28 (1H, m, H-31), 7.22 (7H, m, H-6,7,8,22,23,25,26), 7.17 (1H, d, *J* = 8.2 Hz, H-16), 7.17 (1H, m, H-24), 7.08 (1H, d, *J* = 8.0 Hz, H-33), 7.02 (1H, d, *J* = 7.2 Hz, H-9), 4.43 (H, t, *J* = 7.6 Hz, H-19), 4.32 (1H, dd, *J* = 10.5, 7.0 Hz, H-2), 3.40 (2H, dd, *J* = 14.5, 7.8 Hz, H-20), 3.25 (2H, dd, *J* =14.5, 7.3 Hz, H-20), 2.79 (2H, dd, *J* = 13.5, 6.9 Hz, H-3), 2.66 (2H, dd, *J* = 13.5, 10.9 Hz, H-3), 3.07 (3H, s, H-27), 2.90 (3H, s, H-10); ^{13}C-NMR (100 MHz, CD_3OD) δ_C 172.3 (C-1), 171.2 (C-18), 170.7 (C-28), 168.3 (C-11), 138.1 (C-21), 138.0 (C-34), 137.2 (C-4), 137.0 (C-17), 134.2 (C-15), 134.0 (C-32), 132.3 (C-13), 132.1 (C-30), 131.8 (C-5), 130.1 (C-9,22), 130.0 (C-26), 129.8 (C-6), 129.6 (C-8), 129.2 (C-25), 128.3 (C-23), 127.8 (C-7), 127.3 (C-29), 126.0 (C-12,24), 125.9 (C-31), 122.3 (C-14), 122.0 (C-33), 121.6 (C-16), 69.8 (C-2), 57.9 (C-19), 39.8 (C-10), 35.2 (C-3), 32.9 (C-20), 29.6 (C-27); ESI-MS *m/z*: 583 $[M+Na]^+$, $C_{34}H_{32}N_4O_4$.

*Chaetominine (**3**)*: Yellow oil; ^{1}H-NMR (400 MHz, CD_3OD) δ_H 8.28 (1H, brs, H-25), 8.18 (1H, brd, *J* = 7.8 Hz, H-19), 7.86 (1H, td, *J* = 7.8,1.0 Hz, H-21), 7.69 (1H, brd, *J* = 7.8 Hz, H-22), 7.58 (1H, brt, *J* = 7.8 Hz, H-20), 7.50 (1H, d, *J* = 7.8 Hz, H-8), 7.49 (1H, brd, *J* = 7.8 Hz, H-5), 7.43 (1H, td, *J* = 7.8, 1.0 Hz, H-7), 7.25 (1H, td, *J* = 7.8, 1.0 Hz, H-6), 5.92 (1H, brs, H-14), 5.60 (1H, s, H-2), 4.61 (1H, q, *J* = 6.8 Hz, H-11), 2.93 (1H, t, *J* = 12.5 Hz, H-13), 2.53 (1H, dd, *J* = 12.5, 2.5 Hz, H-13); ^{13}C-NMR (100 MHz, CD_3OD) δ_C 171.4 (C-10), 167.9 (C-17), 161.2 (C-15), 148.5 (C-25), 148.1 (C-23), 140.2 (C-9), 137.2 (C-4), 136.2 (C-21), 131.0 (C-7), 128.9 (C-22), 128.5 (C-20), 127.7 (C-19), 126.4

(C-6), 125.2 (C-5), 121.8 (C-18), 115.7 (C-8), 82.1 (C-2), 75.8 (C-3), 65.3 (C-11), 55.5 (C-14), 38.1 (C-13), 14.7 (C-12); ESI-MS *m/z* 403 $[M+H]^+$, $C_{22}H_{18}N_4O_4$.

Table 1. ^1H NMR and ^{13}C NMR data for compound **1** (at 400 MHz in CD_3OD, δ in ppm, *J* in Hz)

Position	H	C
1	-	172.0 (C)
2	4.29 (1H, *dd*, *J* = 7.0, 10.4)	69.9 (CH)
3	2.68 (1H, *dd*, *J* = 10.4, 13.5), 2.78 (1H, *dd*, *J* = 7.1, 13.5)	34.2 (CH_2)
4	-	137.3 (C)
5	7.03 (1H, *d*, *J* = 7.0)	130.1 (CH)
6	7.26 (1H, *d*, *J* = 7.4)	130.0 (CH)
7	7.18 (1H, *d*, *J* = 7.2)	129.7 (CH)
8	7.26 (1H, *d*, *J* = 7.4)	130.0 (CH)
9	7.03 (1H, *d*, *J* = 7.0)	130.1 (CH)
10	2.91 (1H, *s*)	39.8 (CH_3)
11	-	170.0 (C)
12	-	129.2 (C)
13	7.45 (1H, *d*, *J* = 2.9)	115.3 (CH)
14	7.17 (1H, *dd*, *J* = 3.0, 8.5)	121.1 (CH)
15	-	158.2 (C)
16	6.98 (1H, *d*, *J* = 7.8)	123.7 (CH)
17	-	130.2 (C)
18	-	171.0 (C)
19	4.42 (1H, *t*, *J* = 7.6)	57.9 (CH)
20	3.23 (1H, *dd*, *J* = 7.2, 14.5), 3.43 (1H, *dd*, *J* = 7.9, 14.5)	32.9 (CH_2)
21	-	138.2 (C)
22	7.23 (1H, *d*, *J* = 7.2)	129.8 (CH)
23	7.22 (1H, *d*, *J* = 8.8)	128.3 (CH)
24	7.16 (1H, *d*, *J* = 7.9)	127.8 (CH)
25	7.22 (1H, *d*, *J* = 8.8)	128.3 (CH)
26	7.23 (1H, *d*, *J* = 7.2)	129.8 (CH)
27	3.06 (1H, *s*)	29.6 (CH_3)
28	-	170.4 (C)
29	-	128.8 (C)
30	7.31 (1H, *d*, *J* = 2.9)	114.7 (CH)
31	7.09 (1H, *dd*, *J* = 3.5, 8.2)	120.9 (CH)
32	-	158.1 (C)
33	7.10 (1H, d, 7.9)	123.3 (CH)
34	-	131.2 (C)
35	3.80 (1H, *s*)	56.2 (OCH_3)
36	3.87 (1H, *s*)	56.1 (OCH_3)

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Supporting Information

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References

[1] A. L. Demain and S. Sanchez (2009). Microbial drug discovery: 80 years of progress, *J. Antibiot.* **62**(**1**), 5-16.
[2] H. S. Yu, L. Zhang, L. Li, C. Zheng, L. Guo, W. C. Li, P. X. Sun and L.P. Qin (2010). Recent developments and future prospects of antimicrobial metabolites produced by endophytes, *Microbiol Res.* **165**(**6**), 437-449.
[3] D. S. Yang, Z. L. Li, J. G. Wei, Y. P. Yang and X. L. Li (2013). Chemical constituents of *Euphorbia royleana*, *Chin. Trad. Herb. Drugs* **44**(**15**), 2039-2043.
[4] P. Singh and A. Singh (2012). Evaluation of latex extract of *Euphorbia royleana* for its piscicidal and muricidal activities, *World J. Agric. Sci.* **8**(**5**), 520-524.
[5] P. ManiRam, K. Abhishek, M. Diwakar, K. S. Sunil and K. S. Ajai (2011). Blood electrolytes of the freshwater catfish *Heteropneustes fossilis* in response to treatment with a botanical pesticide (latex of *Euphorbia royleana*), *Integr. Zool.* **6**(**2**), 150-156.
[6] S. Bani, A. Kaul, B. S. Jaggi, K. A. Suri, O. P. Suri and O. P. Sharma (2000). Anti-inflammatory activity of the hydrosoluble fraction of *euphorbia royleana* late, *Fitoterapia* **71**(**6**), 655-662.
[7] A. Ashraf, R. A. Sarfraz, M. A. Rashid and M. Shahid (2015). Antioxidant, antimicrobial, antitumor, and cytotoxic activities of an important medicinal plant (*euphorbia royleana*) from Pakistan, *J. Food Drug Anal.* **23**(**1**), 109-115.
[8] S. Bani, A. Kaul, B. Khan, S. F. Ahmad, K. A. Suri and N. K. Satti (2005). Immunosuppressive properties of an ethyl acetate fraction from euphorbia royleana, *J. Ethnopharmacol.* **99**(**2**), 185-192.
[9] X. L. Li, Y. Li, S. F. Wang, Y. L. Zhao, K. C. Liu and X. M. Wang (2009). Ingol and ingenol diterpenes from the aerial parts of *Euphorbia royleana* and their antiangiogenic activities, *J. Nat. Prod.* **72**(**6**), 1001-1005.
[10] R. Ashok, R. Natarajan, A. Sathiavelu and S. Mythili (2019). Isolation, screening and optimization of laccase-producing endophytic fungi from *Euphorbia milii*, *Arab. J. Sci. Eng*. **44**, 51-64.
[11] W. Sun, X. Chen, Q. Tong, H. Zhu, Y. He and L. Lei (2016). Novel small molecule 11β-hsd1 inhibitor from the endophytic fungus *penicillium commune*, *Sci. Rep.* **6**, 26418.
[12] R. H. Jiao, S. Xu, J. Y. Liu, H. M. Ge, H. Ding and C. Xu (2006). Chaetominine, a cytotoxic alkaloid produced by endophytic *Chaetomium* sp. IFB-E015, *Org Lett.* **8**(**25**), 5709-5712.