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Chaetominine, a Cytotoxic Alkaloid Produced by Endophytic *Chaetomium* sp. IFB-E015

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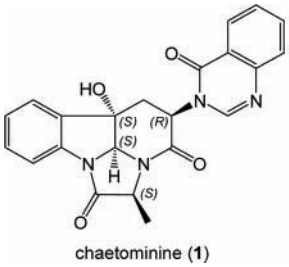
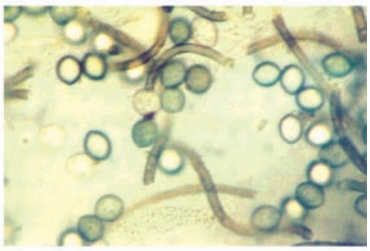
ABSTRACT



chaetominine (**1**)



Chaetominine (1), an alkaloidal metabolite with a new framework, was characterized from the solid-substrate culture of *Chaetomium* sp. IFB-E015, an endophytic fungus on the apparently healthy *Adenophora axilliflora* leaves. Its structure was determined by a combination of its spectral data and single-crystal X-ray diffraction analysis, with its absolute configuration elucidated by Marfey's method. Chaetominine was more cytotoxic than 5-fluorouracil against the human leukemia K562 and colon cancer SW1116 cell lines.

Fungi have been demonstrated to be one of the most productive sources for diverse arrays of secondary metabolites with novel structures and/or significant bioactivities.[1] In our continuous effort to characterize new and/or bioactive natural products from endophytes,[2] marine microorganisms,[3] and medicinal plants,[4] a total of 43 endophytic fungi were isolated from the leaves of *Adenophora axilliflora* (belonging to the family Campanulaceae), whose stem called "Nan-sha-shen" is the traditional Chinese medicine frequently prescribed for cough treatment. Preliminary cytotoxicity assay indicated that one of the fungal endophyte strains, designated as IFB-E015,[5] could produce basic antitumor metabolite(s). In order to predict the type to which the active compound-(s) could belong, the morphology and growth characters were closely inspected to lead to the identification of this fungus

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(1) (a) Herath, K. B.; Jayasuriya, H.; Ondeyka, J. G.; Polishook, J. D.; Bills, G. F.; Dombrowski, A. W.; Cabello, A.; Vicario, P. P.; Zweerink, H.; Guan, Z. Q.; Singh, S. B. *J. Antibiot.* **2005**, *58*, 686−694. (b) Ding, G.; Song, Y. C.; Chen, J. R.; Xu, C.; Ge, H. M.; Wang, X. T.; Tan, R. X. *J. Nat. Prod.* **2006**, *69*, 302−304. (c) Bashyal, B. P.; Wijeratne, E. M. K.; Faeth, S. H.; Gunatilaka, A. A. L. *J. Nat. Prod.* **2005**, *68*, 724−728. (d) Schloerke, O.; Zeeck, A. *Eur. J. Org. Chem.* **2006**, 1043−1049. (e) Kobayashi, M.; Kanasaki, R.; Sato, I.; Abe, F.; Nitta, K.; Ezaki, M.; Sakamoto, K.; Hashimoto, M.; Fujie, A.; Hino, M.; Hori, Y. *Biosci. Biotechnol. Biochem.* **2005**, *69*, 515−521.

(2) (a) Shen, L.; Jiao, R. H.; Ye, Y. H.; Xu, C.; Song, Y. C.; Zhu, H. L.; Tan, R. X. *Chem. Eur. J.* **2006**, *12*, 5596−5602. (b) Liu, J. Y.; Song, Y. C.; Zhang, Z.; Wang, L.; Guo, Z. J.; Tan, R. X. *J. Biotechnol.* **2004**, *114*, 279−287. (c) Zhang, H. W.; Song Y. C.; Tan R. X. *Nat. Prod. Rep.* **2006**, *23*, 753−771.

(3) (a) Yang, L.; Tan, R. X.; Wang, Q.; Huang, W. Y.; Yin, Y. X. *Tetrahedron Lett.* **2002**, *43*, 6545−6548. (b) Liu, C. H.; Liu, J. Y.; Huang, L. L.; Zou, W. X.; Tan, R. X. *Planta Med.* **2003**, *69*, 481−483. (c) Tan, R. X.; Jensen, P. R; Williams, P. G.; Fenical, W. *J. Nat. Prod.* **2004**, *67*, 1374−1382.

(4) (a) Zhou, C. X.; Liu, J. Y.; Ye, W. C.; Liu, C. H.; Tan, R. X. *Tetrahedron* **2003**, *59*, 5743−5747. (b) Chen, J. H.; Cui, G. Y.; Liu, J. Y; Tan, R. X. *Phytochemistry* **2003**, *64*, 903−906. (c) Jiao, R. H.; Ge, H. M.; Shi, D. H.; Tan, R. X. *J. Nat. Prod.* **2006**, *69*, 1089−1091.

(5) (a) All isolated fungal strains were further confirmed to be endophytes according to the methods detailed in ref 2b. (b) A voucher specimen of the title fungus was deposited under the number IFB-E015 at Institute of Functional Biomolecules, Nanjing University.

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as a *Chaetomium* species.[6] This genus seems to be a generous producer of antitumor alkaloid products such as cytochalasins.[1b] However, the ^{1}H NMR spectrum of the extract of the fermentation batches of the fungal strain illustrated the presumable presence of aromatic alkaloid(s) that displayed a D_2O-inexchangeable singlet around δ_H 8.3. The subsequent ^{1}H NMR and bioassay co-guided fractionation of the extract of the endophytic fungus IFB-E015 was therefore performed to afford a cytotoxic alkaloid (**1**) with an unprecedent framework. We herewith wish to report the structure determination and cytotoxic activity of the new compound (**1**), named chaetominine after the scientific name of its producing microbe.

Chaetominine (**1**) was isolated as colorless crystals. The positive-ion mode high-resolution electrospray ionization mass spectrum of **1** gave a pair of precisely weighed quasimolecular ions at *m*/*z* 403.1404 ($[M + H]^+$) and 425.1209 ($[M + Na]^+$), indicative of its molecular formula $C_{22}H_{18}N_4O_4$, which was consistent with its ^{1}H and ^{13}C NMR spectra displaying 18 hydrogen integrals and 22 discrete carbon resonance lines, respectively. Compound **1** was shown to be hexacyclic by subtracting, from the 16 unsaturations of the whole molecule, the 10 obvious unsaturations edited in 3 carbonyls (δ_C 172.5, 165.9 and 160.5) and 14 sp^2-hybridized carbons resonating between δ_C 115 and 148 (Table 1). Furthermore, a scrutiny of the DEPT and HMQC spectra of **1** demonstrated that one of 18 protons in the molecule was involved in a hydroxy or an imine group. The above assumptions were subsequently reinforced by a correlative interpretation of its 2D NMR spectra (^{1}H−^{1}H COSY, NOESY, HMQC, and HMBC) allowing the unambiguous assignment of all ^{1}H and ^{13}C NMR signals (Table 1). From the well-defined coupling sequences and spatial relationships, four structural fragments **A**−**D** could be proposed for **1** (Figure 1, positions numbered as in the whole molecule). Specifically, a 1,2-disubstituted benzene nucleus (**A**) was evidenced from the four intercoupling aromatic proton signals at δ_H 7.49 (br d, J = 7.8 Hz, H-5), 7.25 (td, J = 7.8, 1.0 Hz, H-6), 7.43 (td, J = 7.8, 1.0 Hz, H-7) and 7.50 (br d, J = 7.8 Hz, H-8), and a 2-substituted benzoyl group (**B**) from another four mutually coupled resonances at δ_H 8.18 (br d, J = 7.8 Hz, H-19), 7.58 (br t, J = 7.8 Hz, H-20), 7.86 (td, J = 7.8, 1.0 Hz, H-21) and 7.69 (br d, J = 7.8 Hz, H-22) (Figures 1 and 2). In a similar manner, a 2-substituted propionyl group (**C**) was found to coexist with a 2,4,5,5-tetrasubstituted 3-hydroxypentoyl moiety (**D**), which was indicated by its ^{13}C NMR and NOESY spectra (Figures 1 and 2 and Table 1). The fragments **A**, **C**, and **D** were edited in the partial structure (covering rings **a**−**d**) by the key HMBC correlations of C-4 with HO-3 and H-13, of C-9 with H-2, and of C-15 with H-11, as well as by rationalizing the magnitude of chemical shifts and coupling constants through "appropriately inserting" two nitrogen atoms (Table 1). The leftover isolated aromatic methine (δ_H 8.28, δ_C 147.9), along with fragment **B** and two nitrogen atoms, suggested the presence of a quinazolinone moiety (rings **e** and **f**) that connected to ring **c** through the bonding between C-14 and a lactam nitrogen as evidenced from the magnitude of the 14-methine shift values (δ_H 5.92, δ_C 50.9). It is noteworthy that the broadening of the NMR signals of C(H)-14, -17, -19, and -25 (Figures S3 and S4, Supporting Information) could imply the "N-26 chirality" reversion, presumably initiated by the p-π conjugation between the 17-carbonyl and the electron pair on the lactam nitrogen (Scheme 1). The aforementioned elucidation was confirmed by the electron impact mass spectrum of **1**, which displayed intense typical

Table 1. ^{1}H and ^{13}C NMR Data of Chaetominine in DMSO-d_6

position	δ_C	δ_H	HMBC(H→C)
2	83.0	5.60 (s)	C-3, C-9
3	76.8		
4	137.2		
5	125.4	7.49 (br d, 7.8)	C-3, C-7, C-8
6	126.0	7.25 (td, 7.8, 1.0)	C-3, C-4, C-7, C-8
7	130.4	7.43 (td, 7.8, 1.0)	C-5, C-8, C-9
8	115.0	7.50 (d, 7.8)	C-5, C-6, C-10
9	139.2		
10	172.5		
11	60.1	4.61 (q, 6.8)	C-10, C-12, C-15
12	14.5	1.60 (d, 6.8)	C-10, C-11
13	38.6	α: 2.53 (dd, 12.5, 2.5)	C-3, C-4, C-14, C-15
		β: 2.93 (t, 12.5)	C-2, C-3, C-14, C-15
14	50.9	5.92 (br s)	
15	160.5		
17	165.9		
18	121.6		
19	126.9	8.18 (br d, 7.8)	C-21, C-23
20	127.8	7.58 (br t, 7.8)	C-17, C-18, C-19, C-22, C-23
21	135.2	7.86 (td, 7.8, 1.0)	C-18, C-23
22	127.7	7.69 (br d, 7.8)	C-17, C-18, C-19, C-20, C-21, C-23
23	147.2		
24	147.9	8.28 (br s)	
25	3-OH	6.70 (s)	C-2, C-3, C-4, C-13

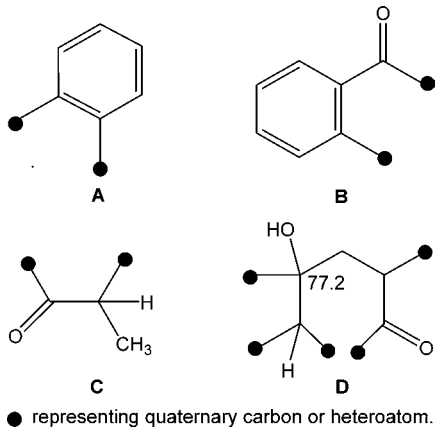


Figure 1. Structural fragments **A**−**D** of **1**.

(6) (a) Wei, J. C. *Manual of Fungal Identification*; Shanghai Science and Technology Press: Shanghai, 1979; pp 195−197. (b) Friedman, A. H. *Neurosurgery* **1998**, *43*, 1469.

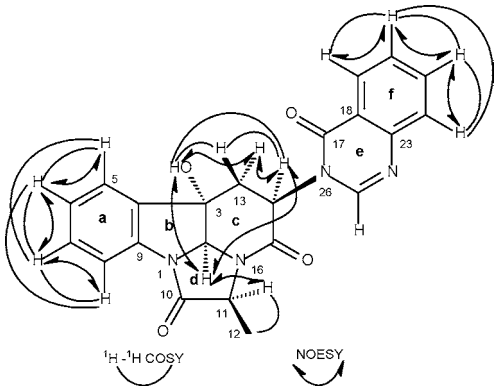
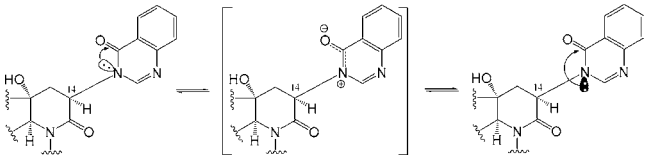


Figure 2. ^{1}H−^{1}H COSY and NOESY correlations for **1**.

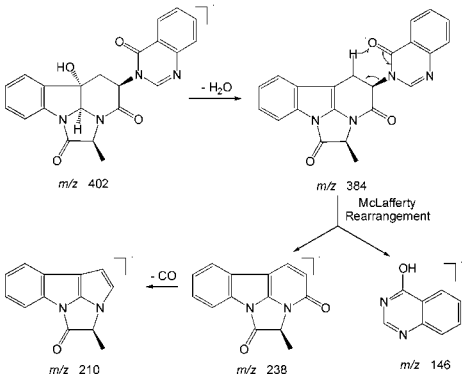
fragment ion peaks at *m*/*z* 384, 238, and 210, presumably being generated through successive eliminations of water,

Scheme 1. Presumable "N-26 Chirality" Reversion of **1** Owing to p-π Conjugation



4-hydroxyquinazoline, and carbon monoxide from the molecular ion at *m*/*z* 402 (Scheme 2).

Scheme 2. EIMS Fragmentation Pattern of **1**



The relative configuration of **1** was given according to the NOESY correlation of H-2 with HO-3, H-11, and H-14. Moreover, this assignment was confirmed by the single-crystal X-ray diffraction analysis of **1**,[7] which revealed that the 3-hydroxy group of chaetominine (**1**) connected to a methanol molecule via a stable intermolecular hydrogen bonding (Figure 3). This solvent co-crystallization explained why the transparent single crystal of "**1**" (actually chaetominine·MeOH) turned to a white powder owing to the volatilization of the crystallized methanol immediately after being taken from the mother liquor. Our successful single-crystal X-ray diffraction analysis was eventually accomplished in a sealed boron-capillary that avoided completely the evaporation of the co-crystallized methanol. The strategy is of referential value for X-ray diffraction experimentation for other solvent-included single crystals.

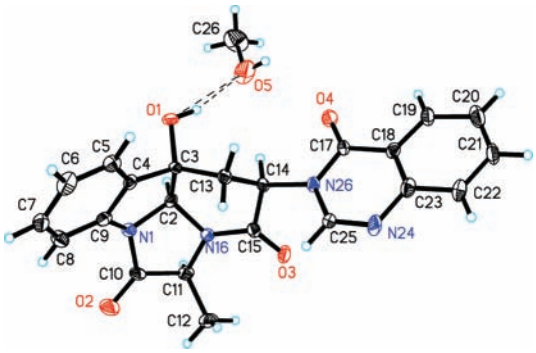


Figure 3. X-ray molecular structure of chaetominine·MeOH with all atoms labeled.

To elucidate its absolute stereochemistry, the CD spectra of **1** was acquired but found to be not informative or decisive enough (Supporting Information). Furthermore, the structure of **1** was acid-sensitive, suggesting that the preparation of its heavy atom carrying derivative such as its hydrobromate for single-crystal diffraction analysis was impractical. To overcome this, we first determined, by Marfey's method,[8] the absolute stereochemistry of the safely (without chiral change) released amino acid such as alanine that could be quantitatively liberated from **1** by HCl hydrolysis. As a result, the retention time of the L-FDAA derivative of the "released alanine" was shown to be identical with that of the authentic L-alanine, highlighting the 11*S*-configuration of **1** (Figure S9, Supporting Information). Relative to the chirality of the carbon, the absolute stereochemistry of other chiral carbons was assigned by the NOESY spectrum and single-crystal X-ray diffraction data. Thus, the absolute configuration of **1** was established as 2*S*, 3*S*, 11*S*, and 14*R*.

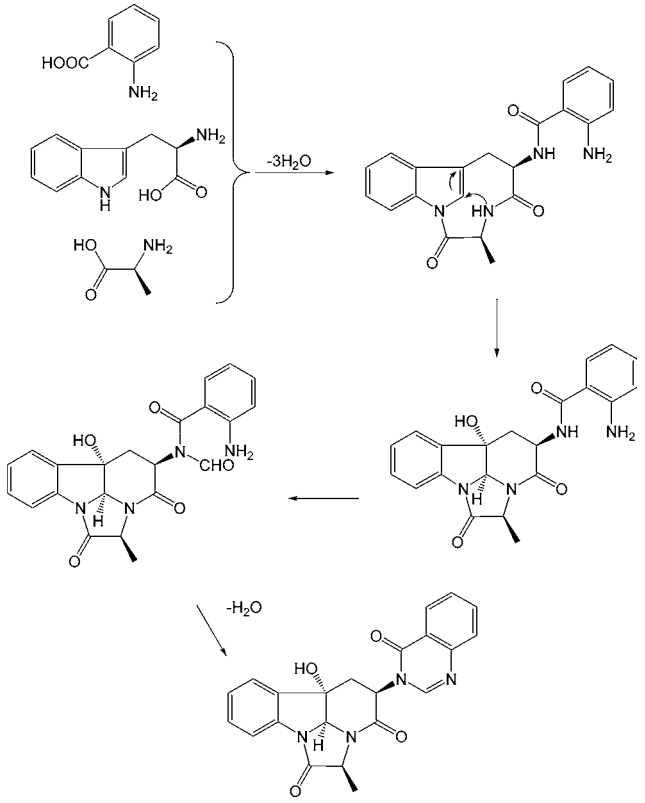
Even in terms of structural fragment(s), chaetominine resembles none of the phytochemicals characterized from *A. axilliflora*, where its producing endophytic fungus used to reside. This observation suggested the striking difference of its biosynthetic pathway from those of the host chemicals. Chaetominine (**1**) was presumably biosynthesized from L-alanine, anthranilic acid, and D-tryptophan.[9] The process should involve tripeptide formation followed sequentially by

(7) Crystal data for chaetominine·MeOH (**1**·MeOH): $C_{22}H_{18}N_4O_4$·CH_3-OH, M_r = 435.1246, monoclinic, space group $P2_12_12_1$, a = 8.4630(17), b = 9.3650(19), c = 26.305(5)Å, U = 2847.1(4) Å^3, Z = 4, D_{calc} = 1.499 g/cm^3. The final R value was 0.1026 (Rw = 0.1707 for 2391 reflections [$I \geq 2\sigma(I)$].

(8) (a) Kochhar, S.; Christen, P. *Anal. Biochem.* **1989**, *178*, 17−21. (b) Lang, G.; Blunt, J. W.; Cummings, N. J.; Cole, A. L. J.; Munro, M. H. G. *J. Nat. Prod.* **2005**, *67*, 1303−1305.

oxidation, formylation (biochemically offered by formylated tetrahydrofolic acid), and construction of quinazolinone through Schiff's reaction. A plausible biosynthetic pathway was thus proposed for **1** as illustrated in Scheme 3.

Scheme 3. Plausible Biosynthetic Pathway for **1**



In vitro cytotoxic assay accomplished by the MTT method[10] showed that chaetominine (**1**) was active against the human leukemia K562 and colon cancer SW1116 cell lines with corresponding IC_{50} values of 21.0 and 28.0 nM, more potent than those (33.0 and 76.0 nM, respectively) of 5-fluorouracil co-tested as a positive reference.

The present work has characterized the tripeptide-derived alkaloidal metabolite chaetominine (**1**) with a new framework from the culture of *Chaetomium* sp. IFB-E015, an endophytic fungus colonizing inside the normal *A. axilliflora* leaves. It was more cytotoxic to the human leukemia K562 and colon cancer SW1116 cell lines than the co-assayed positive reference 5-fluorouracil, which is currently one of most frequently prescribed anticancer drugs. Chaetominine (**1**) seems biogenetically related to the tryptoquivaline-like γ-lactonic metabolites isolated previously from the cultures of *Aspergillus*[11] and *Penicillium* species.[12] Chaetominine was the first tryptoquivaline-related metabolite from the *Chaetomium* species (also an endophyte) with the γ-lactone moiety as in tryptoquivalines rearranged to form a unique δ-lactam ring at the center of the molecule. The tetracyclic system (rings **a**−**d**) of **1** was also possessed by other metabolites such as kapakahines,[13] but the γ-lactam moiety (ring **d**) that they all bear was constructed from phenylalanine or tyrosine and not alanine as in case of chaetominine.

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Supporting Information Available: Experimental details, 1D and 2D NMR spectra, and X-ray crystallographic data of **1** in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) D-Tryptophan is likely formed via the epimerization of L-tryptophan, more abundant in a living system.

(10) (a) Shen, L.; Ye, Y. H.; Wang, X. T.; Zhu, H. L.; Xu, C.; Song, Y. C.; Li, H.; Tan, R. X. *Chem. Eur. J.* **2006**, *12*, 4393−4396. (b) Zhang, C. X.; Huang, K. X. *Toxicol. Appl. Pharm.* **2006**, *210*, 236−245.

(11) (a) Yamazaki, M.; Fujimoto, H.; Okuyama, E. *Chem. Pharm. Bull.* **1978**, *26*, 111−117. (b) Clardy, J.; Springer, J. P.; Buchi, G.; Matsuo, K.; Wightman, R. *J. Am. Chem. Soc.* **1975**, *97*, 663−665.

(12) Ariza, M. R.; Larsen, T. O.; Peterson, B. O.; Duus, J. O.; Barrero, A. F. *J. Agric. Food Chem.* **2002**, *50*, 6361−6365.

(13) (a) Yeung, B. K. S.; Nakao, Y.; Kinnel, R. B.; Carney, J. R.; Yoshida, W. Y.; Scheuer, P. J.; Kelly-Borges, M. *J. Org. Chem.***1996**, *61*, 7168−7173. (b) Nakao, Y.; Kuo, J.; Yoshida, W. Y.; Kelly, M.; Scheuer, P. J. *Org. Lett.* **2003**, *5*, 1387−1390.