Secondary Metabolites of the Endophytic Fungus Chaetomium globosum Isolated From Coptis chinensis

Jia-Cheng Ji¹, Pan-Pan Wei¹, Xiao-Yang Han¹, Zheng-Hui Li¹,², Hong-Lian Ai¹,² and Xin-Xiang Lei¹,²

Abstract
A new steroid, chaetglotone (1), together with 3 known compounds (2-4), were isolated from Chaetomium globosum, which is an endophytic fungus isolated from the root of Coptis chinensis Franch. The new compound was characterized by one-dimensional and two-dimensional nuclear magnetic resonance spectroscopy and high-resolution electrospray ionization mass spectrometer. The relative configuration and absolute configuration of 1 were further determined via the DP4+ and Early Childhood Development protocols, separately.

Keywords
Chaetomium globosum, endophytic fungus, secondary metabolites, chaetglotone, Coptis chinensis

Received: April 10th, 2021; Revised: August 12th, 2021; Accepted: August 13th, 2021.

Introduction
Chaetomium Kunze, the largest genus of Chaetomiaceae, is important for the microbial control of plant diseases. The species within this genus are cosmopolitan and are widespread in soil, air, and plants. The genus Chaetomium has been recognized as an important source of structurally diverse natural products. Approximately 200 metabolites, such as alkaloids, steroids, flavonoids, and terpenoids, have been extracted from Chaetomium spp.¹ Therefore, research on structurally new biologically active products originating from Chaetomium species is continuously increasing.

In recent years, extensive studies on the bioactive chemical constituents of C. globosum have been performed by many research groups. Six new indole alkaloids with antibacterial activity were isolated from marine-fish-derived C. globosum.² The anti-inflammatory effects have been explored of the secondary metabolites from C. globosum, including chaetomugilin I, chaetomugilin J, and 11-epi-chaetomugilin.³ Armnochaeloglacin B, chaetoglobosin V, and chaetoglobosin J, isolated from C. globosum TW1-1, showed weak cytotoxic activity.⁴ Equisetin, with antimicrobial activities, was isolated from the solid fermentation culture of C. globosum.⁵ Chaephilone E, also isolated from C. globosum, showed remarkable growth inhibitory activity against brine shrimp.⁶ Three epipolythiodioxopiperazine alkaloids and a pyridine benzamide isolated from C. globosum 7951 inhibited the growth of MCF-7, MDA-MB-231, H460, and HCT-8 cells.⁷

A new steroid, chaetglotone (1), and three known compounds (2-4) present in C. globosum were found in Coptis chinensis Franch (Figure 1). In this paper, the isolation and structural elucidation of these compounds are described.

Results and Discussion
Chaetglotone (1) was obtained as a yellowish powder. Based on the high-resolution electrospray ionization mass spectrometer (HR-ESI-MS) ion peak at m/z 497.2872 [M+Na]⁺ (calculated for 497.2874), its molecular formula was identified as C₂₈H₄₂O₆, with 8 degrees of unsaturation. The ¹H-NMR, ¹³C-NMR, and HSQC spectral data indicated that compound from C. globosum 7951 inhibited the growth of MCF-7, MDA-MB-231, H460, and HCT-8 cells.

¹School of Pharmaceutical Sciences, South-Central University for Nationalities, Wuhan, People’s Republic of China
²National Demonstration Center for Experimental Ethnopharmacology Education, South-Central University for Nationalities, Wuhan, People’s Republic of China

Corresponding Author:
Hong-Lian Ai, School of Pharmaceutical Sciences, South-Central University for Nationalities, Wuhan 430074, People’s Republic of China.
Email: aihonglian@mail.scuec.edu.cn

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (https://creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access page (https://us.sagepub.com/en-us/nam/open-access-at-sage).
1 had 6 methyl groups (δ_H 0.75 [3H, d, J = 6.9 Hz, H-28], 0.76 [3H, d, J = 6.9 Hz, H-26], 0.93 [3H, d, J = 6.9 Hz, H-27], 1.09 [3H, d, J = 7.0 Hz, H-21], 1.17 [3H, s, H-18], 1.36 [3H, s, H-19]; δ_C 10.4 [C-28], 15.6 [C-26], 22.2 [C-27], 15.0 [C-21], 17.1 [C-18], 22.2 [C-19]), 5 methylene groups (δ_H 1.28 [1H, dd, J = 14.1, 4.3 Hz, H-1], 2.70 [1H, td, J = 13.1, 2.8 Hz, H-1], 1.60 [1H, m, H-2], 1.81 [1H, m, H-2], 2.31 [2H, overlap, H-4], 1.96 [1H, dd, J = 13.3, 6.2 Hz, H-15], 2.41 [1H, dd, J = 13.6, 7.4 Hz, H-15], 2.89 [1H, dd, J = 24.5, 3.9 Hz, H-7], 3.09 [1H, td, J = 24.5, 2.8 Hz, H-7]; δ_C 35.6 [C-1], 32.3 [C-2], 42.4 [C-4], 45.5 [C-15], 28.3 [C-7]), 10 methine groups (δ_H 1.64 [1H, m, H-24], 2.11 [1H, m, H-25], 2.31 [1H, overlap, H-17], 2.60 [1H, m, H-20], 3.30 [1H, overlap, H-23], 3.43 [1H, m, H-3], 3.77 [1H, s, H-12], 4.20 [1H, dd, J = 7.7, 1.2 Hz, H-22], 4.51 [1H, q, J = 6.9 Hz, H-16], 5.45 [1H, t, J = 3.0 Hz, H-6]; δ_C 14.7 [C-24], 27.2 [C-25], 57.9 [C-17], 39.6 [C-20], 74.7 [C-23], 72.2 [C-3], 80.7 [C-12], 85.0 [C-22], 83.7 [C-16], 117.8 [C-6]), 6 quaternary carbons (δ_C 140.3 [C-5], 153.9 [C-8], 155.8 [C-9], 39.1 [C-10], 52.2 [C-13], 83.7 [C-14]), and 1 carbonyl carbon (δ_C 201.3 [C-11]) (Supplemental Table S1). Analysis of the 1H-1H correlation spectroscopy (COSY) spectrum indicated strong correlations between H-1/H-2/H-3/H-4, H-6/H-7, H-15/H-16/H-17/H-20/H-22/H-23/H-24/H-25/H-26, H-20/H-21, H-24/H-28, and H-5/H-27 (Figure 2). The 1H detected heteronuclear multiple bond correlation (HMBC) spectrum revealed correlations between H-19 and C-1/C-5/C-9/C-10, H-6 and C-8, H-7 and C-8, H-15 and C-8, H-12 and C-8, H-15 and C-8, H-12 and C-11, H-18 and C-12/C-13/C-14/C-17, and H-21 and C-20 (Figure 2). The molecular formula revealed that C-16 and C-22 comprise rings with oxygen. Further, the planar structure of compound 1 was confirmed.
The relative configuration of compound 1 was assigned based on the ROESY spectrum and coupling constants (Figure 3). The ROESY spectrum showed correlations between H-19 and H_{19}-1/H_{12}, H-18 and H_{18}-15/H-20, H-16 and H_{16}-15, and H-21 and H-17. In addition, the coupling constants of H-22/H-23 and H-23/H-24 were 1.2 and 8.2 Hz, respectively, indicating that H-22/H-23 were in a gauche conformation and H-23/H-24 were in a staggered conformation. Compound 1 is very similar to the known compound asperforiol except that the position of C-7 in compound 1 is not substituted by carbonyl. The chirality of the chiral carbons is assumed to be 3S, 10S, 12R, 13S, 16S, 17R, 20S, 22R, 23R, and 24R. However, the chirality of the carbon at position 14 is uncertain because it is a quaternary carbon with inadequate coupling information. Therefore, the DP4+ protocol was chosen for chiral analysis. The conformers were optimized at the B3LYP/6-31 + G(d, p) level, and the optimized representative conformations were subjected to NMR GIAO calculations at the B3LYP/6-311 + G(d, p) level. The chirality of C-14 was found to be 3S. Therefore, the relative configuration of compound 1 can be determined as 3S, 10S, 12R*, 13S*, 14S*, 16S*, 17R*, 20S*, 22R*, 23R*, and 24R*.

The absolute configuration of compound 1 was further determined by comparing the experimental and calculated ECD spectra. The calculated ECD data were obtained using time-dependent density functional theory (TDDFT) at the B3LYP/6-311 + G(d, p) level. The predicted ECD spectra of (3S, 10S, 12R, 13S, 14S, 16S, 17R, 20S, 22R, 23R, 24R)-1 and (3R, 10R, 12S, 13R, 14R, 16R, 17S, 20R, 22S, 23S, 24S)-1 were compared with the experimental ECD spectra. The predicted ECD curves of (3S, 10S, 12R, 13S, 14S, 16S, 17R, 20S, 22R, 23R, 24R)-1 were similar to the experimental ECD curve (Supplemental Figure S3). Thus, the absolute configuration of compound 1 was confirmed to be 3S, 10S, 12R, 13S, 14S, 16S, 17R, 20S, 22R, 23R, and 24R. Compound 1 was named chaetglotone.

The known compounds were identified as 13-epi-higginsianin C (2),11 higginsianin B (3),12 and (22S, 24S) -ergoster-5, 7, 22-triene-3β-ol (4).13

Conclusions

In this study, a new compound, chaetglotone (1), and 3 previously reported compounds (2-4) were isolated, purified, and characterized from the endophytic fungus C. globosum isolated from the root of Coptis chinensis Franch.

Experimental Section

General Experimental Procedures

NMR spectra in CD_{3}OD were recorded on a Bruker DXR-600 instrument (600 and 150 MHz), and optical rotation on an Autopol IV automatic polarimeter (RUDOLPH). HR-ESI-MS data were obtained using a UPLC-Q Exactive MS system (Thermo Fisher), and IR spectra with an IRT racer-100 (Shimadzu) with KBr pellets. Column chromatography (CC) was performed using silica gel (300-400 mesh, Qingdao Haiyang Chemical Co., Ltd) or a Sephadex LH-20 (Aladdin). Semipreparative HPLC was carried out on an Agilent 1260 Infinity II system with a diode array detector. A C-18 reversed phase column was used (5 μm, 10×250 mm) (Agela).

Fungal Material

The endophytic fungus was identified as C. globosum. The coverage and maximum similarities were 100%. The GenBank accession number was KU720060.1. The strain was stored in the School of Pharmaceutical Sciences, South Central University for Nationalities.
**Extraction and Isolation**

The fermentation material was solid rice medium (100 g of rice and 100 mL of water in each 500 mL culture flask) and culture was at 28 °C for one month. The culture was extracted with MeOH (1 L × 4) to obtain 423 g of crude extract. This was mixed with hot water in a ratio of 1:1, and then extracted with light petroleum (2 L × 4) and EtOAc (2 L × 4). The ethyl acetate extract weighed 61.16 g. The crude extracts were separated using positive-phase column chromatography. The eluent was composed of light petroleum: EtOAc (15:1-0:1) and Sephadex LH-20 eluting with MeOH, and puriﬁed using positive-phase column chromatography. The eluent was composed of light petroleum: EtOAc (15:1-0:1) and Sephadex LH-20 eluting with MeOH, and puriﬁed using positive-phase column chromatography. Fr. 10 was separated into six fractions (Fr. 10-1-Fr. 10-5) by ODS MPLC. The eluent was composed of MeOH: H2O (Fr. 10-1-Fr. 10-5) by ODS MPLC. The eluent was composed of light petroleum: EtOAc (15:1-0:1) and Sephadex LH-20 eluting with MeOH (15:1 to 0:1). Subsequently, 18 fractions (Fr. 1- Fr. 18) were obtained based on the similarity of the chromatographic behavior. Fr. 8 was recrystallized to yield compound 6 (14.3 mg). Fr. 10 was separated into two fractions (Fr. 10-1-Fr. 10-5) by ODS MPLC. The eluent was composed of MeOH: H2O (1:9-10, stepwise). Fr. 10-3 was separated by Sephadex LH-20 eluting with MeOH and puriﬁed by HPLC (acetone: H2O = 35:65) to obtain compound 1 (5.3 mg, τR = 21.7 min). Fr. 10-4 was fractionated via Sephadex LH-20 eluting with MeOH and puriﬁed by HPLC (acetone: H2O = 64:36) to obtain compound 2 (2.8 mg, τR = 24.5 min) and compound 3 (7.9 mg, τR = 47.8 min).

Chaetoglottone (1): yellowish powder [α]D25 = +52°(c = 0.25, MeOH). UV (MeOH) λmax (logε): 210 (3.49), 255 (3.35) HR-ESI-MS m/z 497.2872 [M+Na]+ (calculated for C28H42O6, 497.2874). 1H and 13C-NMR data see Supplemental Table S1.

**Acknowledgments**

The authors thank the Analytical & Measuring Center, School of Pharmaceutical Sciences, SCUN for their help with NMR measurements.

**Declaration of Conflicting Interests**

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Funding**

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the National Natural Science Foundation of China (grant nos. 31870513, 2019CFA072).

**ORCID iD**

Hong-Lian Ai  
https://orcid.org/0000-0003-2670-2054

**Supplemental Material**

Supplemental material for this article is available online.

**References**

1. Liang HL, Tong ZW, Zhu D. Secondary metabolites from Chaetomium globosum and their bioactivities. Nat Prod Res Dev. 2018;30(4):702–707.
2. Yan W, Zhao SS, Ye YH, et al. Generation of indoles with agrochemical significance through biotransformation by Chaetomium globosum. J Nat Prod. 2019;82(8):2132–2137.
3. Youn UJ, Sripisut T, Park EJ, et al. Determination of the absolute conﬁguration of chaetoviridins and other bioactive azaphilones from the endophytic fungus Chaetomium globosum. Bioorg Med Chem Lett. 2015;25(21):4719–4723.
4. Gao W, Sun W, Li F, et al. Armochaetoglasins A-I: cytochalasan alkaloids from fermentation broth of Chaetomium globosum TW1-1 by feeding L-tyrosine. Phytochemistry. 2018;156:106–115.
5. Yang SX, Zhao WT, Chen HY, et al. Auroneons A and B, two new C15-polyketides from Chaetomium globosum, an endophytic fungus in Salvia miltiorrhiza. Chem Biodiversity. 2019;16(9):e1900364.
6. Song CG, Ding G, Wu G, et al. Identiﬁcation of a unique azaphilone produced by Chaetomium globosum isolated from Polygonatum sibiricum. Chem Biodiversity. 2020;17(3):e1900744.
7. Wang F, Zhao WI, Zhang CH, et al. Cytotoxic metabolites from the endophytic fungus Chaetomium globosum 7951. RSC Adv. 2019;9:16035–16039.
8. Gu BB, Wu W, Jiao FR, et al. Asperﬂotone, an 8(14→15)-abeo-ergostane from the sponge-derived fungus Aspergillus fuscus. J Org Chem. 2010;75(24):7072–7074.
9. Grimblat N, Zanardi MM, Sarotti AM. Beyond DP4: an improved probability for the stereochemical assignment of isomeric compounds using quantum chemical calculations of NMR shifts. J Org Chem. 2015;80(24):12526–12534.
10. Smith SG, Goodman JM. Assigning stereochemistry to single diastereoisomers by GIAO NMR calculation: the DP4 probability. J Org Chem. 2015;80(24):12526–12534.
11. Dallery JF, Le Goff G, Adelin E, et al. Deleting a chromatin remodeling gene increases the diversity of secondary metabolites produced by Colletotrichum higginsianum. J Nat Prod. 2019;82(4):813–822.
12. Cimmino A, Mathieu V, Masi M, et al. Higginsianins A and B, two diterpenoid α-pyrones produced by Colletotrichum higginsianum, with in vitro cytostatic activity. J Nat Prod. 2016;79(1):116–125.
13. Guan LP, Zhou TT, Li DX, et al. Isolation, identiﬁcation and anti-tumor activity of secondary metabolite of Aspergillus Niger 2HL-M-8. Chin J Med Chem. 2016;26(1):49–55.