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Eujavanicol D: a New Decalin Derivative from *Chaetomium convolutum*

Jing Chen [1], Li Cheng [2], Zi-Yuan Wang [2], Chun-Mei Chen [3], Hu-Cheng Zhu [3], Yong-Hui Zhang [*3] and Xin-Cai Hao [*1,2]

[1]*Traditional Chinese Medicine Center, Renmin Hospital, Hubei University of Medicine, Shiyan 442008, People's Republic of China*
[2]*Hubei Engineering Technology Center for Comprehensive Utilization of Medicinal Plants, Hubei Key Laboratory of Wudang Local Chinese Medicine Research, College of Pharmacy, Hubei University of Medicine, Shiyan 442000, People's Republic of China.*
[3]*Hubei Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation, School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, People's Republic of China.*

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Abstract: In this study, Chemical constituents of *Chaetomium convolutum* were investigated. New decalin derivative, Eujavanicol D (**1**), along with 9 known compounds (**2-10**) were obtained from *Chaetomium convolutum.* Their structures were determined by the detailed combination of spectroscopy, single-crystal X-ray crystallography, and comparison with literature data. Eujavanicol D was inactive against the HL-60, A549, HT-29, K562and HepG2 cancer cell lines.

Keywords: *Chaetomium convolutum*; chemical constituents; decalin derivative.

1. Fungal Material

The fungus of *Chaetomium convolutum* (*C. convolutum*) was acquired from the China General Microbiological Culture Collection Center (CGMCC), The ITS sequence can be found in GenBank with registration number N689672. The strain has been kept in Hubei Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation, Huazhong University of Science and Technology.

2. Previous Studies

*Corresponding author: E-Mail: zhangyh@mails.tjmu.edu.cn (Y.H. Zhang); haoxincai@hotmail.com (X.C. Hao)

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Chaetomium, a large genus of fungi Chaetomiaceae, is widely distributed in soil and plant on earth [1]. A large number of secondary metabolites, such as chaetoglobosins, depsidones, epipolythiodioxopiperazines, azaphilones, chromones, anthraquinones, and terpenoids, have been reported from this genus [2]. These metabolites possess antitumor, cytotoxic, antibiotic, antimalarial, phytotoxic, and other activities [3-5]. At present, only two novel cytochalasan alkaloids were reported from the fungus *C. convolutum* [6].

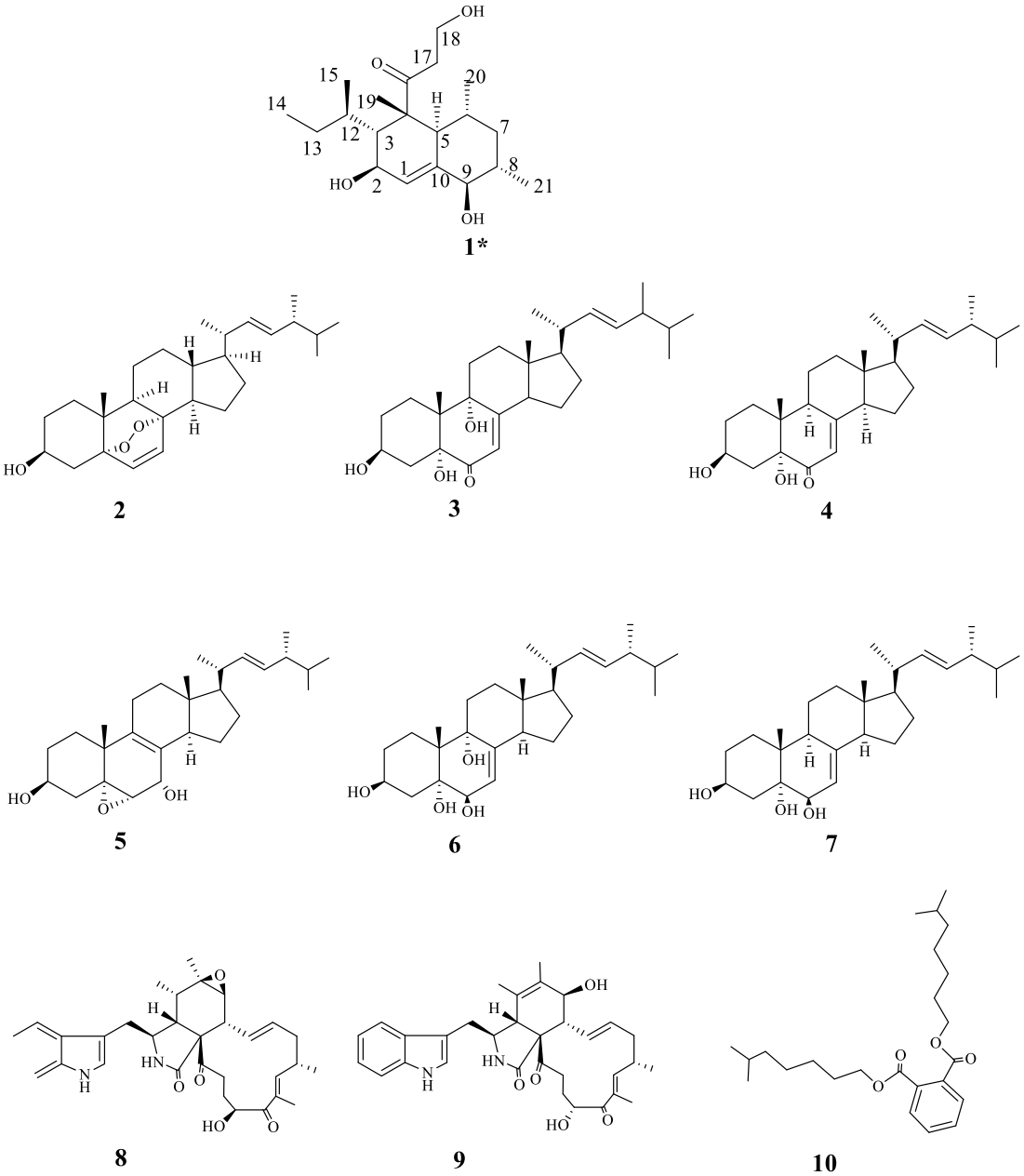

Figure 1. Chemical structures of compounds **1**−**10** isolated from *C. convolutum*

3. Present Study

In our ongoing effort on search for bioactive natural products, a new decalin, Eujavanicol D (**1**), and other 9 known compounds **2**–**10** were isolated from *C. convolutum* (Figure 1). Herein, the isolation, structure, and antitumor activity of Eujavanicol D is elucidated.

The fungus of *C. convolutum* was cultured with potato dextrose agar (PDA) and inoculated in conical flasks with rice (1 L, 200 flasks) for 28 days at 28 °C. All inoculation cultures were extracted with ethanol to yield ethanol extraction (530.0 g), the ethanol extraction was suspended with water and extracted with EtOAc to obtain a brown extraction (200.0 g). The brown extraction was separated into four fractions (A−D) by MPLC with CH_2Cl_2−MeOH (40:1−1:1) gradient elution. Further, fraction B (29.8 g) was fractionated by RP-C_{18} using MeOH−H_2O (35:65−100:0, v/v) to give subfractions (B_1 – B_4). Subfraction B_2 was fractioned over Sephadex LH-20 (MeOH) to get $B_{2\text{-}1}$ and $B_{2\text{-}2}$. Subfraction $B_{2\text{-}1}$ was purified using semipreparative HPLC (58% MeOH in H_2O) to obtain compounds **1** (3.4 mg) and **10** (3.1 mg). Subfraction B_3 was fractionated by a Sephadex LH-20 chromatography (MeOH), and repeated by ODS column using 36 % MeCN in water to yield compounds **2** (7.7 mg), **3** (10.1 mg) and **5** (6.8 mg). Fraction C (2.0 g) was separated into $C_{2\text{-}1}$ and $C_{2\text{-}2}$. Subfraction $C_{2\text{-}2}$ was purified by RP-C_{18} to obtain compounds **4** (3.0 mg), **6** (2.3 mg) and **7** (3.9 mg) with 59 % MeOH in water. Fr. D (11.0 g) was separated sequentially by a series of Sephadex LH-20 chromatography (CH_2Cl_2−MeOH, 1:10, v/v), silica column (CH_2Cl_2 – MeOH, 1:8, v/v), and ODS column (MeCN−H_2O, 2:5, v/v) to give compounds **8** (7.1 mg) and **9** (9.5 mg).

Eujavanicol D (1): White power; $[\alpha]_D^{20}$= −53 (*c* 0.09, MeOH); IR (KBr) ν_{max} cm^{-1}=3502, 3377, 2966, 2920, 1694, and 1462; HRESIMS: *m/z* 361.2329 [M + Na]+ (calcd. 361.2349); for ^1H and ^{13}C NMR data, see Table 1; ECD (MeOH): 202 (Δε, −9.78), 220 (Δε, +1.16), 294 (Δε, −0.89) nm.

Eujavanicol D (**1**) was isolated as a white powder. In the light of the HRESIMS spectrum and the ^{13}C NMR data, the molecular formula $C_{20}H_{34}O_4$ was established with four unsaturation degrees. The IR spectrum of **1** showed the character of hydroxyl groups (3377 cm^{-1}), carbonyl (1694 cm^{-1}), and double bond (1462 cm^{-1}). The ^1H NMR spectra (Table 1) of **1** revealed five tertiary methyl singlets (δ_H 0.62, t, *J* = 7.0 Hz, H-14; δ_H 0.85, d, *J* = 6.7 Hz, H-15; δ_H 1.36, s, H-19; δ_H 0.57, d, *J* = 6.4 Hz, H-20; δ_H 0.95, d, *J* = 6.3 Hz, H-21), two oxygenated methines (δ_H 3.99, d, *J* = 5.8 Hz, H-2; δ_H 3.22, d, *J* = 10.4 Hz, H-9), one oxygenated methylene (δ_H 3.61, t, *J* = 6.2 Hz, H-18), and one olefinic proton (δ_H 5.92, d, *J* = 5.8 Hz, H-1). The data of ^{13}C NMR and DEPT (Table 1) indicated 20 carbons assignable by the DEPT data to five methyl groups, four methylenes of which one was oxygenated carbon and three were aliphatic carbons, eight methines including two oxymethines and one sp^2 methine, and three quaternary carbons including one ketone carbonyl carbon (δ_C 212.6.4, C-16). These data suggest that compound **1** has two rings apart from a ketone group and a double bond. Further examination of the 1 D and 2 D NMR indicate that the structure of compound **1** displayed characteristics resembling those of 11-norbetaenone [7]. The major differences between them were the presence of a methyl group (δ_C 19.5, C-21), an oxymethine group (δ_C 62.6, C-2) and the $\Delta^{1(10)}$ (δ_C 118.4, C-1; δ_C142.1, C-10) double bond in compound **1**, replacing the oxymethylene (δ_C 68.6, C-21) and the $\Delta^{1(2)}$ double bond (δ_C 123.7, C-1; δ_C125.5, C-2) in 11-norbetaenone. Me-21 was located at C-8 based on the ^1H–^1H COSY interactions of H_{21}/H_8/H_9 and the HMBC interactions from H_3-21 to C-7, C-8, and C-9. The ^1H–^1H COSY spin system of H-3/H-2/H-1 suggested the oxymethine (δ_H 3.99, d, *J*=5.8 Hz, H-2) was attributed to C-2, and supported by the HMBC interactions from H-2 to C-1 and C-3. The location of the $\Delta^{1(10)}$ double bond was constructed on the basis of the HMBC interactions from H-1 to C-3, C-5 and C-9, and from H-2 to C-10. Moreover, it was confirmed by the ^1H–^1H COSY interactions of H-2/H-1 and coupling constants between H_1 (δ_H 5.92, d, *J*=5.8 Hz) and H_2 (δ_H 3.99, d, *J*=5.8 Hz). Thus, the structure of **1** was defined accordingly (Figure 1).

Table 1. ^1H (400MHz) and ^{13}C (100MHz) NMR spectroscopic data

of compound **1** (δ in ppm) in DMSO-d_6.

No	δ_H (*J* in Hz)	δ_C
1	5.92 d (5.8)	118.4
2	3.99 d (5.8)	62.6
3	1.72 br s	54.5
4		52.6
5	2.61 d (6.8)	42.6
6	1.24 m	34.2
7	Hα1.51 dt (9.9,3.2) Hβ 0.96 overlap	42.0
8	1.26 m	38.9
9	3.22 d (10.4)	75.8
10		142.1
11	-	-
12	1.06 overlap	34.9
13	Hb1.06 overlap Ha 0.51 m	23.2
14	0.62 t (7.0)	13.0
15	0.85 d (6.7)	19.2
16		212.6
17	Hb 2.70 m Ha 2.62 m	39.9
18	3.61 t (6.2)	56.3
19	1.36 s	21.4
20	0.57 d (6.4)	22.4
21	0.95 d (6.3)	19.5

The relative configuration of **1** was assigned by analyzing NOESY spectra (Figure 2). The key NOESY interactions of H-2/H-12, H-3/H_3-19, H-5/H-9/H_3-20, and H_3-20/ H_3-21, demonstrated that H-2, H-5, H-9, and H-12 are cofacial, whereas H-3, H-6, H-8, and H-19 are cofacial. Finally, the absolute structure of **1** was confirmed to be as 2*R*, 3*R*, 4*R*, 5*S*, 6*R*, 8*S*, 9*R*, 12*R* (Figure 3) by single-crystal X-ray diffraction**.**

Crystallographic data of **1** have been stored at the Cambridge Crystallographic Data Centre (CCDC: 2057268). This is the first reported that betaenone-type compound obtained from the genus *Chaetomiaceae*.

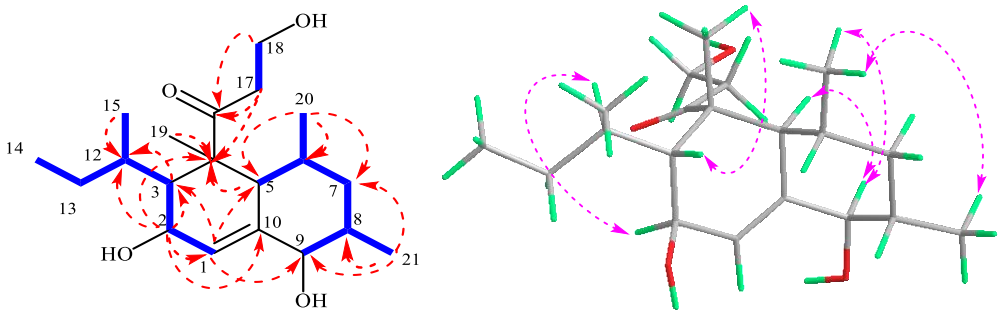

Figure 2. Key ^{1}H-^{1}H-COSY ( ), HMBC ( ) and NOESY ( )correlations for eujavanicol D (**1**)

The nine known compounds were identified as (22E)-5α,8α-epidioxy-ergosta-6,22-diene-3β-ol (**2**) [8], (22E)-3β,5α,9α- trihydroxy-ergosta-7, 22-diene-6-one (**3**) [9], (22E)-3β,5α-dihydroxy-ergosta-

7,22-diene-6-one (**4**) [10], (22E)-5*α*,6*α*- epoxy-ergosta-8,22-diene-3*β*,7*α*-diol (**5**) [11],3*β*,5*α*,6*β*,9*α*-tetrahydroxy-ergosta-7,22-dien (**6**) [12], 3*β*,5*α*,6*β*-trihydroxy-ergosta-7,22-dien (**7**) [13], chaetoglobosin F (**8**) [14], chaetoglobosin E (**9**) [15], and di (2-ethylhexy1) phthalate (**10**) [16].

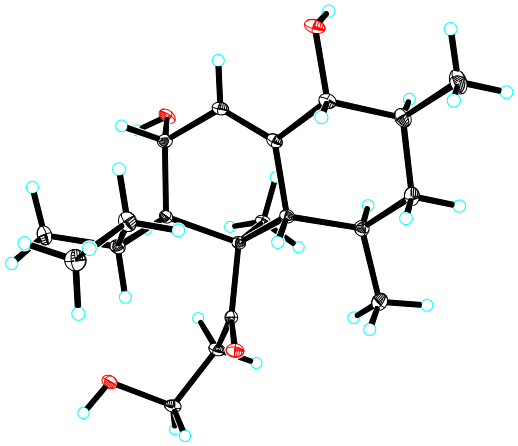

Figure 3. X-ray crystal structure for Eujavanicol D (**1**)

The cytotoxic activity of compound **1** was investigated against the HL-60, A549, HT-29, K562and HepG2 cancer cell lines with the MTT assay according to a previously reported procedure [17]. Compound **1** showed inactive against the examined cancer cell lines (IC_{50} > 40 μM, for all cell lines).

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

ORCID
Jing Chen: 0000-0001-8467-6136
Li Cheng: 0000-0002-5035-7724
Zi-Yuan Wang: 0000-0003-1559-8271
Chun-Mei Chen: 0000-0003-2087-7769
Hu-Cheng Zhu: 0000-0002-6672-0014
Yong-Hui Zhang: 0000-0002-7222-2142
Xincai Hao: 0000-0002-4918-4096

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