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NOTE

A New Isocoumarin from Fermentation Products of Endophytic Fungus of *Aspergillus versicolor*

BING-KUN JI, WEI DONG, YUE-DE WANG, KUN ZHOU, YIN-KE LI, MIN ZHOU, GANG DU, QIU-FEN HU, YAN-QING YE and HAI-YING YANG*

Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry of Education, Yunnan Minzu University, Kunming 650031, P.R. China

*Corresponding author: E-mail: huqiufena@aliyun.com

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A new isocoumarin, versicoumarin D, was isolated from the fermentation products of an endophytic fungus *Aspergillus versicolor*. Its structure was elucidated by spectroscopic methods, including extensive 1D NMR and 2D NMR techniques. Compound **1** was also tested for its cytotoxicity against five human tumor cell lines (NB4, A549, SHSY5Y, PC3 and MCF7) and it showed high cytotoxicity against A549 and MCF7 cell with IC_{50} values of 5.8 and 8.0 μM, respectively.

Keywords: Versicoumarin D, Isocoumarin, *Aspergillus versicolor*, Cytotoxicity.

Aspergillus versicolor is a highly ubiquitous fungus species commonly isolated from soil, plant debris, marine environments and indoor air environments[1,2]. Many metabolites produced by *A. versicolor* exhibit antibacterial, fungicidal, insecticidal and cytotoxic properties[3,4]. Isocoumarins comprise a class of polyphenolic natural products present in a variety of plant species displaying several pharmacological activities, including antibacterial and antifungal[3,5,6], cytotoxicity[7,8], anti-virus[9], antioxidant[10], anti-inflammatory[11], *etc*.

Motivated by a search for new bioactive metabolites from the fermentation products of microbe, an endophytic fungus *Aspergillus versicolor* was isolated from the rhizome of *Paris marmorata* Stearn, collected in Dali, Yunnan, PR China and the chemical constituents of its fermentation products were investigated. A new (**1**) isocoumarin was isolated. This paper deals with the isolation and structural characterization of this compound and its cytotoxicity against five human tumor cell lines (NB4, A549, SHSY5Y, PC3 and MCF7).

Optical rotations were measured in a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. ECD spectra were measured on a JASCO J-810 spectropolarimeter. IR spectra were obtained in KBr disc on a Bio-Rad Wininfmred spectrophotometer. ESI-MS were measured on a VG Auto Spec-3000 MS spectrometer. ^1H, ^{13}C and 2D NMR spectra were recorded on Bruker DRX-400 instruments with TMS as internal standard. Column chromatography was performed on silica gel (200-300 mesh), or on silica gel H (10~40 μm, Qingdao Marine Chemical Inc., China). Second separate was used an Agilent 1100 HPLC equipped with ZORBAX-C_{18} (21.2 mm × 250 mm, 7.0 μm) column and DAD detector.

The culture of *Aspergillus versicolor* was isolated from the rhizome of *Paris marmorata* Stearn, collected from Dali, Yunnan, P.R. China, in 2012. The strain was identified by one of authors (Gang Du) based on the analysis of ITS sequence. It was cultivated at room temperature for 7 days on potato dextrose agar at 28 °C. Agar plugs were inoculated into 250 mL Erlenmeyer asks each containing 100 mL potato dextrose broth and cultured at 28 °C on a rotary shaker at 180 rpm for 5 days. Large scale fermentation was carried out in 100 Fernbach asks (500 mL) each containing 100 g of rice and 120 mL of distilled H_2O. Each flask was inoculated with 5 mL of cultured broth and incubated at 25 °C for 45 days.

Extraction and isolation: The fermentation products were extracted four times with 70 % acetone (4 × 5 L) at room temperature and filtered. The crude extract (128 g) was applied to silica gel (200-300 mesh) column chromatography, eluting with a $CHCl_3$-$(CH_3)_2CO$ gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions A-F. The further separation of fraction B (9:1, 11.4 g) by silica gel column chromatography, eluted with petroleum ether-EtOAc (9:1, 8:2, 7:3, 6:4, 1:1), yielded mixtures B1-B5. Fraction B2 (8:2, 3.52 g) was subjected to preparative HPLC (60 % MeOH, flow rate 12 mL/min) to give **1** (18.2 mg).

Spectroscopic data: Versicoumarin D (**1**), $C_{15}H_{16}O_5$, white amorphous powder, $[\alpha]_D^{24.8}$ 65.8 (c 0.10, CH_3OH). UV (CH_3OH)

λ_{max} nm (log ε): 212 (3.78), 275 (3.50), 295 (3.22). CD (c 0.05, MeOH), nm (Δε) 228 (-6.9), 248 (+12.5), 265 (-12.0); IR (KBr, ν_{max}, cm^{-1}): 3065, 1676, 1644, 1618, 1545, 1486, 1438, 1350, 1278, 1162, 1112, 868, 805. ^1H and ^{13}C NMR data (400 and 100 MHz, in $CDCl_3$) (Table-1). ESI-MS: 299; HR-ESI-MS *m/z*: 299.0890 $[M+Na]^+$ (calcd for $C_{15}H_{16}NaO_5$, 299.0895).

TABLE-1
^1H NMR (400 MHz) AND ^{13}C NMR (100 MHz) DATA
FOR COMPOUND **1** (δ IN ppm AND *J* IN Hz)

No.	δ_C	δ_H (m, *J*, Hz)	No.	δ_C	δ_H (m, *J*, Hz)
1	169.2 s		8a	123.6 s	
3	76.8 d	4.58 m	9	21.7 q	1.48 d (6.3)
4	36.5 t	2.80 dd (16.2,10.8)	1′	191.3 s	
		2.88 dd (16.2,3.9)	2′	47.0 t	2.68, 2.65 s
4a	126.2 s		3′	79.2 s	
5	117.8 s		4′	25.5 q	1.52 q
6	152.5 s		5′	25.5 q	1.52 q
7	143.0 s		7-OHe		10.24 s
8	122.0 d	7.42 s			

The fermented substrate was extracted with 70 % aqueous acetone. The extract was subjected repeatedly to column to column chromatography on Silic gel, Sephadex LH-20, RP-18 and Preparative HPLC to afford compound **1**. The structures of the compounds **1** was shown in Fig. 1 and the ^1H NMR and ^{13}C NMR data of **1** were listed in Table-1.

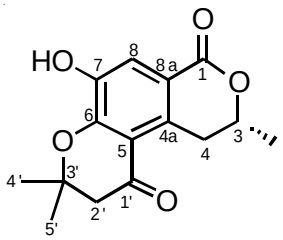

Fig. 1. Structure of compound **1**

Compound **1** was isolated as a white amorphous powder. Its molecular composition was found to be $C_{15}H_{16}O_5$ by ESIHRMS, *m/z* 299.0890 $[M+Na]^+$ (calcd. 299.0895 for $C_{16}H_{18}NaO_5$). The UV spectrum showed absorption maxima at 212, 275, 295 nm and the IR spectrum showed absorption bands at 1676, 1644, 1618, 1545, 1486 cm^{-1}, suggesting the presence of an aromatic ring and unsaturated lactone. The ^1H NMR and ^{13}C NMR spectra (Table-1) exhibited signals assignable to a pentasubstituted aromatic ring (δ_C 126.2 s, 117.8 s, 152.5 s, 143.0 s, 122.0 d, 123.6 s; δ_H 7.42 s), a oxidated methine carbon (δ_C 76.8 d, δ_H 4.58 m), a methylene carbon (δ_C 36.5 t; δ_H 2.80, dd, *J* = 16.2, 10.8 and 2.88 dd, *J* = 16.2, 3.9), a methyl group (δ_C 21.7 q, δ_H 1.48 d, *J* =6.3), a ester carbonyl group (δ_C 169.2 s), an isoamyl ketone group[12] [δ_C 191.3 s, 47.0 t, 79.2 s, 25.5 q (2C); δ_H 2.65 s, 2.68 s (2H), 1.52 q, (6H)] and a phenolic hydroxy group (δ_H 10.24 s). These spectral data indicated **1** should be processed a 3-methyl-3,4-dihydroisocoumarin skeleton[13]. These were also confirmeed by the HMBC correlations (Fig. 2) of H-3 with C-1, C-4, C-4a and C-9, of H-4 with C-3, C-9, C-4a, C-5 and C-8a. Long-range HMBC correlations of H-22 to C-5, C-32 and C-42,52 were observed in **1**. This indicated that the isoamyl ketone moiety was attached to the aromatic ring at positions C-5 and C-6, forming a dimethyl-2,3-dihydropyran-4-one moiety. The phenolic hydroxy group located at C-7 was supported by the HMBC correlation of methoxy proton with C-6, C-7 and C-8. The CD spectrum of **1** showed the Cotton effects at 228, 248 and 265 nm, respectively, which was similar to those of periplanetin B, indicating the presence of R configuration at C-3 in **1**. Therefore, the structure of versicoumarin D (**1**) was established.

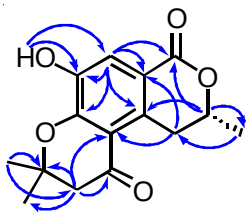

Fig. 2. Key HMBC ( ) correlation of compound **1**

Since some isocoumarins are known to exhibit potential cytotoxicity, the cytotoxicity of compound **1** was tested using a previously reported procedure. The cytotoxic abilities against NB4, A549, SHSY5Y, PC3 and MCF7 tumor cell lines by MTT-assay (with taxol as the positive control. The results revealed that compound **1** showed high cytotoxicity against A549 and MCF7 cell with IC_{50} values of 5.8 and 8.0 μM.

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