SHORT REPORT

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(8*S*, 9*R*)-Dihydroisoflavipucine, a New Isoflavipucine Derivative from the Endophytic Fungi *Botryosphaeria dothidea*

Hui Chen [†1, 2], Wendan Li [†1], Lian Xue [3], Xiuji Liu [2], Yiqing Zhou [*4] and Zhangshuang Deng [*1]

[1] *Key Laboratory of Functional Yeast, China National Light Industry & Hubei Key Laboratory of Natural Products Research and Development, College of Biological and Pharmaceutical Sciences, China Three Gorges University, Yichang 443002, P.R. China*
[2] *Center for Industrial Microbiology and Brewing Technology, Angel Yeast Co., Ltd, Yichang 443003, P.R. China*
[3] *HEC API Yichang Co., Ltd, HEC 3rd Industrial Park, Yidu 443311, P.R. China*
[4] *School of Biotechnology and Food Engineering, Changshu Institute of Technology, Changshu 215500, P.R. China*

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Abstract: A new isoflavipucine derivative, (8*S*, 9*R*)-dihydroisoflavipucine (**1**), is isolated from an ethyl acetate extract of endophytic fungi *Botryosphaeria dothidea* (D4-2) derived from *Taxus mairei*. The structure of **1** is elucidated based on nuclear magnetic resonance, circular dichroism, and mass spectrometry. The antiproliferative activity of the isolates against breast cancer cells MDA-MB-231, MCF7 and 4T1 are evaluated.

Keywords: Dihydroisoflavipucine; isoflavipucine; *Taxus mairei*; endophytic fungi; *Botryosphaeria dothidea*; antiproliferative activity. © 2022 ACG Publications. All rights reserved.

1. Plant Source and Fungal Material

The plant of *T. mairei* collected at Shennongjia National Nature Reserve, Hubei province, P. R. China in April 2013, was identified by Professor Yubing Wang (plant taxonomist) from China Three Gorges University and recorded as No. 2013T0401 at Hubei Key Laboratory of Natural Products Research and Development. The endophytic fungi *B. dothidea* (no. D4-2) was isolated from a fresh sample of the plant *T. mairei*, identified by analyzing rDNA-ITS sequences and deposited at the same laboratory.

[†]Hui Chen and Wendan Li contributed equally to this work.
[*] Corresponding authors: E- Mail: dzs163@163.com (Z. Deng); zhyq2012@gmail.com (Y. Zhou)

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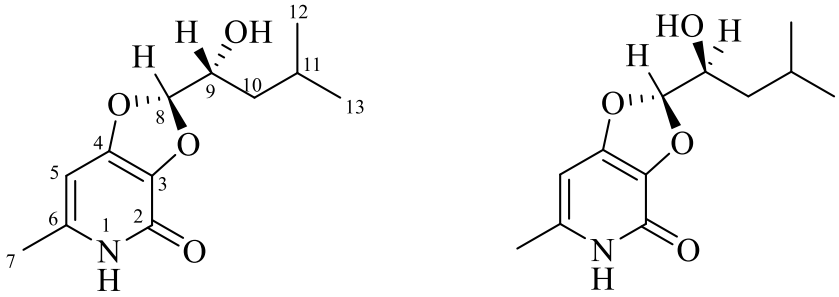
2. Previous Studies

Previous studies on *B. dothidea* as the pathogen of plants were focused on plant hosts, infection process and interaction with hosts [1, 2]. However, few literature has reported the metabolites of *B. dothidea* [3]. Dihydroisoflavipucines are natural products derived from fungus and produced through reduction of isoflavipucines [4]. Although dihydroisoflavipucines possessing two chiral centers at C-8 and C-9 generate four stereoisomers, only two of them, (8*S*, 9*S*)-dihydroisoflavipucine and (8*R*, 9*S*)-dihydroisoflavipucine, have been isolated and identified from natural sources [5-8].

3. Present Study

In the course of our continuing search for new active compounds from endophytic fungi of *T. maire* [9, 10], two dihydroisoflavipucines **1** and **2** (Figure 1) were isolated from *B. dothidea* (D4-2). In this study, we elucidate the structure of the new dihydroisoflavipucine **1**. The isolation of (8*S*, 9*R*)-**1** in our work and that of (8*S*, 9*S*)-**1** and (8*R*, 9*S*)-**1** in previous literatures [6, 7] supported that biosynthetic pathway of dihydroisoflavipucine is not stereospecific [4, 8].



1 **2**

Figure 1. Structures of (8*S*, 9*R*)-dihydroisoflavipucine (**1**) and (8*S*, 9*S*)-dihydroisoflavipucine (**2**)

Fungal strains of *B. dothidea* were grown on rice solid medium for 30 days at room temperature. The solid culture was exhaustively extracted with ethyl acetate in an ultrasonic bath at room temperature for 1 h. The combined ethyl acetate solution was concentrated under reduced pressure to give an extract (2.346 g), which was further defatted with methanol/n-hexane (v/v, 50:50) to obtain methanol-soluble extract (1.357 g). For further separation, the methanolic extract was subjected to column chromatography on silica gel (200–300 mesh) eluting with a $CHCl_3$-CH_3OH gradient (v/v, from 100:0 to 100:100) to afford five fractions. Further purification of fraction 4 by column chromatography on Sephadex LH-20 eluting with $CHCl_3$-CH_3OH (v/v, 1:1) and then by semi-preparative HPLC (YMC-Pack ODS-A C18 column; 10 mm × 250 mm, 5 μm; 60% CH_3CN-H_2O) to afford compounds **1** (5.0 mg) and **2** (3.0 mg).

*(8S, 9R)-Dihydroisoflavipucine (**1**):*Pale colorless solid; $[\alpha]_D^{25}$ = -4.0° (c 0.025, MeOH); ECD (c 4.18 × 10^{-3} M, MeOH) λ_{max} ($\Delta\varepsilon$) 185 (20.83), 201 (0.12), 215 (2.03) nm; ^{1}H and ^{13}C NMR data, see Table 1; (+)-HRESIMS $[M+H]^+$ *m/z* 240.12262 (calcd. for $C_{12}H_{18}NO_4$, 240.12358).

*(8S, 9S)-Dihydroisoflavipucine (**2**)*: Pale colorless solid; $[\alpha]_D^{25}$ = +225.0° (c 0.02, MeOH); ECD (c 4.18 × 10^{-3} M, MeOH) λ_{max} ($\Delta\varepsilon$) 185 (38.80), 200 (-0.92), 221 (1.21) nm; ^{1}H and ^{13}C NMR data, see Table 1; (+)-HRESIMS $[M+H]^+$ *m/z* 240.12256 (calcd. for $C_{12}H_{18}NO_4$, 240.12358).

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) spectroscopic data for **1** and **2** (in MeOD, δ in ppm)

Position	1		2	
	δ_H (*J* in Hz)	δ_C , type	δ_H (*J* in Hz)	δ_C , type
2		155.2, C		155.1, C
3		132.8, C		133.0, C
4		158.1, C		158.0, C
5	6.12,brs	95.1, CH	6.13, brs	95.3, CH
6		143.6, C		143.6, C
7	2.27, s	19.0, CH_3	2.27, s	19.0, CH_3
8	6.06, d (2.8)	115.9, CH	6.06, d (3.2)	116.0, CH
9	3.88, dt (3.2, 10.4)	70.7, CH	3.89, dt (3.2, 10.8)	70.6, CH
10	a: 1.57, ddd (4.0, 9.8, 14.4) b: 1.35, ddd (3.2, 9.6, 14.0)	40.6, CH_2	a: 1.55, ddd (4.0, 9.8, 14.5) b: 1.35, ddd (3.2, 9.6, 14.0)	40.7, CH_2
11	1.89, m	25.4, CH	1.89, m	25.4, CH
12	0.94, d (6.8)	22.0, CH_3	0.95, d (6.8)	21.9, CH_3
13	0.99, d (6.8)	24.2, CH_3	0.99, d (6.8)	24.2, CH_3

Although compounds **1** and **2** exhibited two independent peaks with the retention time of 34.5 and 35.5 min respectively in semi-preparative HPLC system, their MS and NMR spectrums are almost identical. Thus, compounds **1** and **2** were deduced to be stereoisomers.

Compound **1** was obtained as pale colorless solid with the molecular formula $C_{12}H_{17}NO_4$ as determined from the hydrogen adduct ion $[M+H]^+$ peak at *m/z* 240.12262 (calcd. for $C_{12}H_{18}NO_4$, 240.12358) in the HRMS spectrum. The structure was deduced by detailed analysis of the 1D and 2D NMR. The ^{1}H NMR spectrum of **1** displayed the presence of 3 sp^2 quaternary carbons [δ_C 132.8 (C-3), 158.1 (C-4) and 143.6 (C-6)], 1 sp^2 methine group [δ_H 6.12 (1H, brs, H-5) / δ_C 95.1 (C-5)] and 1 carbonyl carbon [δ_C 155.2 (C-2)]. In addition, signals corresponding to 2 sp^3 oxygenated methine groups [δ_H 6.06 (1H, d, *J* = 2.8 Hz, H-8) / δ_C 115.9 (C-8) and δ_H 3.88 (1H, dt, *J* = 3.2, 10.4 Hz, H-9) / δ_C 70.7 (C-9)], 1 sp^3 methine group [δ_H 1.89 (1H, m, H-11) / δ_C 25.4 (C-11)], 1 sp^3 methylene group [δ_H 1.57 (1H, ddd, *J* = 4.0, 9.8, 14.4 Hz, H-10a) and 1.35 (1H, ddd, *J* = 3.2, 9.6, 14.0 Hz, H-10b) / δ_C 40.6 (C-10)], and 3 sp^3 methyl groups [δ_H 2.27 (3H, s, H-7) / δ_C 19.0 (C-7), δ_H 0.94 (3H, d, *J* = 6.8 Hz, H-12) / δ_C 22.0 (C-12) and δ_H 0.99 (3H, d, *J* = 6.8 Hz, H-13) / δ_C 24.2 (C-13)] were observed. In the ^{1}H-^{1}H COSY spectrum (Figure 2), the cross peaks of H-8/H-9, H-9/H-10, H-10/H-11, H-11/H-12 and H-11/H-13 suggested the presence of 2-methylpentane structure, $CHCHCH_2CH(CH_3)_2$, in **1**. Moreover, the correlation of H-5/H-7 in ^{1}H-^{1}H COSY spectrum and the higher field carbonyl signal [δ_C 155.2 (C-2)] in ^{13}C spectrum, combined with the HMBC correlations of H-7/C-5, H-7/C-6, H-7/C-4 (weak), H-7/C-3 (weak), H-5/C-7, H-5/C-3, H-5/C-6, H-5/C-4, revealed the other structural skeleton of 3,4-disubstitued-6-methylpyridin-2(1*H*)-one in **1** (Figure 2). In view of the only remaining degree of unsaturation and the HMBC correlations of H-8/C-3, H-8/C-4, it was determined that the three oxygenated carbons (C-3, C-4 and C-8) are connected through two oxygen bridges to build linkage unit of dioxolane. The hydroxyl group was connected to C-9 based on the HMBC correlations of H-10/C-8, H-10/C-12 and H-10/C-13, combined with the lower field carbonyl signal [δ_C 70.7 (C-9)]. Therefore, the planar structure of **1** was deduced as dihydroisoflavipucine.

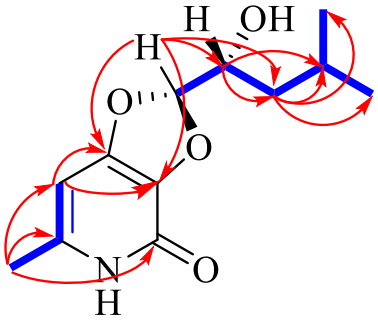


Figure 2. Key HMBC (H ⤻ C) and ^{1}H-^{1}H COSY (▬) correlations in **1**

The absolute configuration of **1** was elucidated by comparing the experimental and the quantum-chemically calculated electronic circular dichroism (ECD) spectrum of (8*S*, 9*R*)-dihydroisoflavipucine [6]. The CD spectrum of **1** showed positive Cotton effects at 185, 201 and 215 nm, indicating the absolute configurations at C-8 and C-9 were assigned as 8*S*, 9*R* in **1**. However, the CD spectrum of **2** showed positive Cotton effects at 185 and 221 nm and negative one at 200 nm, which is in accordance with the CD spectral pattern of (8*S*, 9*S*)-dihydroisoflavipucine elucidated in previous literature [7]. Thus, the absolute configuration of **2** was 8*S*, 9*S*.

Finally, two dihydroisoflavipucines **1** and **2** were evaluated for *in vitro* antiproliferative potential against breast cancer cells MDA-MB-231, MCF7 and 4T1 according to reported procedures [11,12]. **1** and **2** showed no inhibitory activity against the three cell lines (IC_{50} > 0.02 μg/μL) and other pharmacological studies such as anti-inflammatory and antibacterial effects are still needed to be explored.

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Supporting Information

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ORCID
Hui Chen: 0000-0001-9634-9861
Wendan Li: 0000-0002-0819-7763
Lian Xue: 0000-0002-7507-3744
Xiuji Liu: 0000-0001-5373-0646
Yiqing Zhou: 0000-0002-6391-3259
Zhangshuang Deng: 0000-0001-5929-235X

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