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Phenylspirodrimane Derivatives From Cultures of the Fungus *Stachybotrys chartarum* YIM DT 10079

Zhang-Gui Ding[1]*, Jian-Hai Ding[2]*, Jiang-Yuan Zhao[1]*, Ming-Gang Li[1], Dong-Bao Hu[3], Xian-Jun Jiang[4], Shao-Jie Gu[4], Fei Wang[4], and Meng-Liang Wen[1]

Abstract

A new phenylspirodrimane derivative, stachartin F (**1**), and 2 known secondary metabolites stachybonoid E (**2**) and stachybonoid F (**3**) were isolated from cultures of the tin mine tailings-associated fungus *Stachybotrys chartarum* YIM DT 10079. Their structures were determined with the help of extensive spectroscopic analyses and absolute configuration of compound **1** was rationalized by quantum chemical calculations of the electronic circular dichroism spectra.

Keywords

Stachybotrys chartarum, fungus, secondary metabolite, phenylspirodrimane derivative

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The phenylspirodrimanes, structurally characterized by the fusion of a drimane-based sesquiterpene with a phenyl moiety, are known as characteristic secondary metabolites of the fungus *Stachybotrys chartarum*. These compounds have exhibited diverse pharmacological activities, such as antihyperlipidemic activities,[1] anti-HIV activity,[2] osteoclast differentiation inhibitor,[3] and antimalarial activity.[4] As part of our ongoing search for novel molecules from extremophiles,[5,6] *S. chartarum* YIM DT 10079 was isolated from a soil sample collected from the Datun tin mine tailings area, Yunnan, China. An EtOAc extract of cultures of *S. chartarum* YIM DT 10079 was subjected to investigation, which resulted in the isolation of a new phenylspirodrimane derivative, stachartin F (**1**), and the known stachybonoid E (**2**) and stachybonoid F (**3**)[7] (Figure 1). Herein, we report the isolation and structure elucidation of these compounds, and stachartin F (**1**) was tested for its cytotoxicity against 5 human cancer cell lines.

Compound **1** was isolated as a white powder. Its molecular formula was established as $C_{28}H_{39}NO_6$ by high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) at m/z 508.2671 $[M+Na]^+$ (calcd 508.2670), indicating 10 degrees of unsaturation. Infrared Spectroscopy (IR) absorption bands at 3429, 1725, and 1658 cm^{-1} implied the presence of hydroxy and carbonyl functionalities. Comparison of the ^1H and ^{13}C Nuclear magnetic resonance spectroscopy (NMR) data (Table 1) with those of chartarlactam H[1] showed the presence of the same phenylspirodrimane skeleton, except the side chain moiety on the nitrogen was replaced by a methyl butyrate group in **1**, which was further confirmed by the Heteronuclear Multiple Bond Correlation (HMBC) correlations (Figure 2) from H_3-5" (δ_H 3.54) and H_2-2" (δ_H 1.79) to C-4" (δ_C 173.1) and from H_2-1" (δ_H 3.35) to C-7' (δ_C 49.3) and C-8' (δ_C 166.1), and ^1H-^1H correlation spectroscopy (COSY) correlations of H-2"

[1]Key Laboratory for Microbial Resources, Ministry of Education, Yunnan Institute of Microbiology, Yunnan University, Kunming, P.R. China
[2]Engineering and Technology Research Center of Liupanshan Resources, College of Chemistry and Chemical Engineering, Ningxia Normal University, Guyuan, P. R. China
[3]School of Chemical Biology and Environment, Yuxi Normal University, P.R. China
[4]BioBioPha Co., Ltd., Kunming, P.R. China

*The authors Zhang-Gui Ding, Jian-Hai Ding and Jiang-Yuan Zhao contributed equally to the work.

Corresponding Authors:
Jian-Hai Ding, Engineering and Technology Research Center of Liupanshan Resources, College of Chemistry and Chemical Engineering, Ningxia Normal University, Guyuan 756000, P. R. China.
Email: dingjh_nx@126.com
Fei Wang, BioBioPha Co., Ltd., Kunming 650201, P.R. China.
Email: f.wang@mail.biobiopha.com
Dong-Bao Hu, School of Chemical Biology and Environment, Yuxi Normal University, Yuxi 653100, P.R. China.
Email: hudongbao@126.com
Meng-Liang Wen, Key Laboratory for Microbial Resources, Ministry of Education, Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, P.R. China.
Email: mlwen@ynu.edu.cn

Table 1. ^{1}H and ^{13}C NMR Data for Compound **1**.

No.	δ_H	δ_C	No.	δ_H	δ_C
1a	1.69 (brt)	23.9 t	1'		112.5 s
1b	0.89 (brd)				
2a	1.79 (m)	25.1 t	2'		156.5 s
2b	1.36 (m)				
3	3.15 (m)	73.4 d	3'	6.31 (s)	101.4 d
4		37 .4 s	4'		144.2 s
5	2.05 (dd, 12.2, 1.5)	39.3 d	5'		105.9 s
6a	1.45 (m)	20.6 d	6'		158.2 s
6b	1.38 (m)				
7a	1.47 (m)	30.5 t	7'	4.22	49.3 t
8	1.75 (m)	36.7 d	8'		166.1 s
9		98.3 s	1"	3.35 (m)	40.8 t
10		41.8 s	2"	1.79 (m)	23.3 t
11a	3.00 (d,16.1)	30.9 t	3"	2.31 (m)	30.9 t
11b	2.63 (d,16.1)				
12	0.59 (d, 6.6)	15.5 q	4"		173.1 s
13	0.87(s)	28.7 q	5"	3.54 (s)	51.4 q
14	0.78 (s)	22.6 q	3-OH	4.09 (s)	
15	0.92 (s)	16.0 q	3'-OH	9.94 (s)	

with H-1" and H-3" (δ_H 2.31). Its relative configuration was determined by the Rotating frame overhauser enhancement spectroscopy (ROESY) correlations of H-3/H_3-14, H_3-14/H_3-15, H_3-15/H-8, and H_3-13/H-5, H-5/H*á*-7, H*á*-7/H_3-12 determined that H-3, H_3-14, and H_3-15 as being in *β*-orientations, while H_3-13, H-5, and H_3-12 as being in *á*-orientations (Figure 2). Consequently, compound **1** was established as stachartin F. The absolute configuration of **1** was determined by means of electronic circular dichroism (ECD). As shown in Figure 3, the calculated ECD spectrum of **1** matched well with the experimental one, suggesting the absolute configuration of **1** to be 3*R*, 5*S*, 8*R*, 9*R*, 10*S*.

Compound **1** was evaluated for its cytotoxicity against 5 human cancer cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method as reported previously.[8] Cisplation (Sigma, United States) was used as the positive control. The compound was inactive (IC_{50} values >40 μM).

Experimental

General

Optical rotations, a Horiba SEPA-300 polarimeter; NMR, Avance III 600, Bruker DRX-500, and Bruker AM-400 spectrometers; HR-ESI-MS, an API-Qstar-Pulsar-1 spectrometer.

Fungal Material and Cultivation Conditions

Stachybotrys chartarum was isolated from a soil sample collected from the Datun tin mine tailings area, Yunnan, P.R. China. A voucher specimen (No. YIM DT 10079) was deposited at Yunnan Institute of Microbiology, Yunnan University. The culture medium consisted of glucose (1.0%), peptone from porcine meat (0.5%), yeast powder (0.5%), KH_2PO_4 (0.1%), and $MgSO_4 \cdot 7H_2O$ (0.02%). Fermentation was carried out on a shaker at 200 rpm for 15 days.

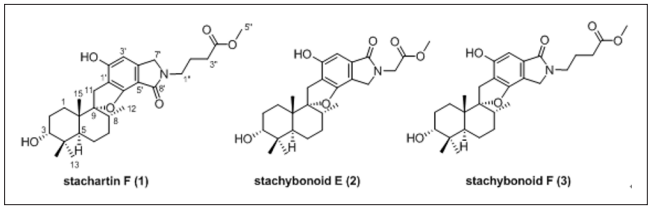

Figure 1. Chemical structures of **1** to **3** from *Stachybotrys chartarum* YIM DT 10079.

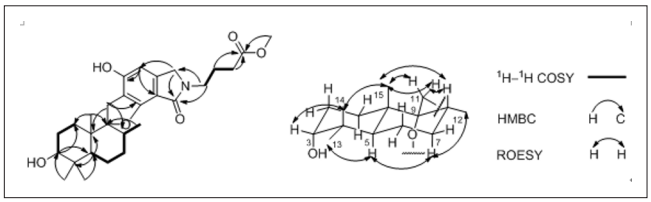

Figure 2. Selected 2D NMR correlations of **1**.

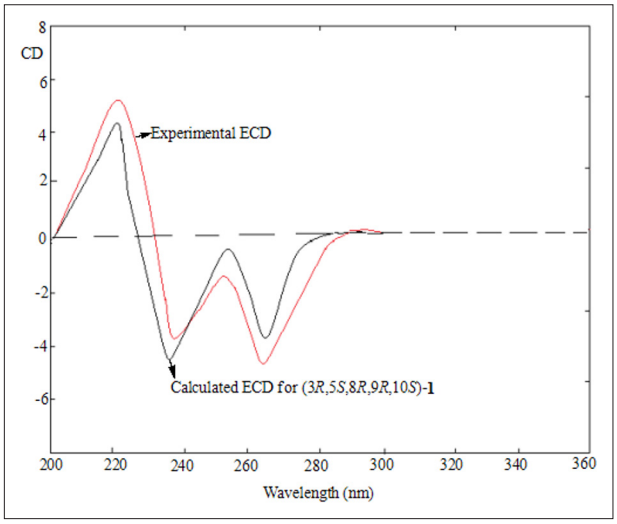

Figure 3. Experimental and calculated electronic circular dichroism for compound **1**.

Extraction and Isolation

The culture broth (100 L) of *S. chartarum* YIM DT 10079 was filtered, and the filtrate was extracted 3 times with EtOAc, while the mycelium was extracted 3 times with $CHCl_3$/MeOH (1:1). The EtOAc layer together with the mycelium extraction was concentrated under reduced pressure to give a crude extract. The extract was subjected to column chromatography over silica gel (200-300 mesh) eluted with a gradient of $CHCl_3$/MeOH (1:0⟶0:1) to obtain 6 fractions (1-6). Fraction 3 eluted with $CHCl_3$/MeOH (80:1) was separated repeatedly by Sephadex LH-20 ($CHCl_3$/MeOH 1:1) to afford **1** (16.0 mg), **2** (25.0 mg), and **3** (200.0 mg).

Cytotoxicity Assay

Five human cancer cell lines, breast cancer SK-BR-3, hepatocellular carcinoma SMMC-7721, human myeloid leukemia HL-60, pancreatic cancer PANC-1, and lung cancer A-549 cells, were used in the cytotoxic assay. Cells were cultured in RPMI-1640 or in DMEM medium (Hyclone, United States), supplemented with 10% fetal bovine serum (Hyclone, United States) in 5% CO_2 at 37°C. The cytotoxicity assay was performed according to the MTT method in 96-well microplates.[8] Briefly, 100 μL of adherent cells were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 hours before addition of test compounds, while suspended cells were seeded just before drug addition with initial density of 1×10^5 cells/mL. Each tumor cell line was exposed to the test compound at concentrations of 0.0625, 0.32, 1.6, and 8 ìM in triplicates for 48 hours, with cisplatin (Sigma, United States) as positive control. After compound treatment, cell viability was detected and a cell growth curve was graphed. IC_{50} values were calculated by Reed and Muench's method.[9]

Computational Methods

All Discrete fourier transformation (DFT) and Time dependent density functional theory (TD-DFT) calculations were carried out at 298 K in the gas phase with Gaussian 09. Conformational searches were carried out at the molecular mechanics level of theory employing MMFF force fields.[10,11] The conformers with relative energy within 10 kcal/mol of the lowest-energy conformer were selected and further geometry optimized at the B3LYP/6-311++G(2d,p) level. All the lowest-energy conformers, which correspond to 99% of the total Boltzmann distribution, were selected for ECD spectra calculation. The Boltzmann factor for each conformer was calculated based on Gibbs free energy. Vibrational analysis at the B3LYP/6-311++G(2d,p) level of theory resulted in no imaginary frequencies, confirming the considered conformers as real minima. TD-DFT was employed to calculate excitation energy (in nm) and rotatory strength *R* in dipole velocity form, at the B3LYP/6-311++G(2d,p) level.

Stachartin F (1)

White powder.

$[\alpha]_D^{26}$: −6.0 (*c* 0.35, MeOH).

^1H and ^{13}C NMR: Table 1.

HR-ESI-MS: *m/z* 508.2671 $[M+Na]^+$ (calcd for $C_{28}H_{39}NNaO_6$, 508.2670)

Declaration of Conflicting Interests

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

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Supplemental Material

Supplemental material for this article is available online.

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