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A new phenylspirodrimane dimer from the fungus *Stachybotrys chartarum*

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ABSTRACT

A new phenylspirodrimane dimer, named stachartarin A (**1**), was isolated from cultures of the tin mine tailings-associated fungus *Stachybotrys chartarum*. Its structures were elucidated by means of spectroscopic methods. At the same time, the compound was tested for its cytotoxicity against HL-60, SMMC-7721, A-549, MCF-7, and SW480 cells.

1. Introduction

The fungus *Stachybotrys chartarum* is a black mold, which is sometimes found in damp soil and grain [1]. This fungus has a long history of causing problems for animals [2]. It produces a new class of phenylspirodrimanes, a group of fungal secondray metabolites with a wide range of biological activities, such as antihyperlipidemic effects [3], and anti-HIV activity [4]. Our previous investigations of this fungus have reported a series of phenylspirodrimanes [5]. In a continuing phytochemical study of this fungus, a new phenylspirodrimane dimer, stachartarin A(**1**) was isolated from its scale-up cultures. The isolation, structural elucidation, and its cytotoxicity of the new compound are described herein.

2. Experimental

2.1. General experimental procedures

UV data were obtained on a Shimadzu UV-2401A spectrophotometer. Infrared spectroscopy (IR) spectra were obtained on a Bruker Tensor27 FT-IR spectrometer with KBr pellets. Nuclear Magnetic Resonance (NMR) spectra were obtained on Bruker AV-400 MHz spectrometer with tetramethylsilane (TMS) as an internal standard at room temperature. Mass spectra were recorded on a VG Autospec-3000 mass spectrometer and an API QSTAR Pulsar I spectrometer. Silica gel (200–300 mesh, Qingdao Marine Chemical Ltd., China) and MPLC was performed on a BUCHI Sepacore system (BUCHI Labortechnik AG, Switzerland), and columns packed with RP-18 (40–75 μm, Fuji Silysia Chemical Ltd., Japan). An Agilent 1100 series instrument equipped with Agilent ZORBAX SB-C18 column (5 μm, 4.6 mm × 150 mm) was used for HPLC analysis, and a semi-preparative Agilent ZORBAX SB-C18 column (5 μm, 9.4 mm × 150 mm) was used for the sample preparation. Fractions were monitored by TLC (GF 254, Qingdao Haiyang Chemical Co. Ltd), and spots were visualized by 10% H_2SO_4 in ethanol.

2.2. 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

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Table 1
^{1}H and ^{13}C NMR (400 and 100 MHz, respectively) data for compound **1** in CD_3SOCD_3.

No.	δ_H	δ_C	No.	δ_H	δ_C
1a	1.88 (m)	24.0 t	1″a	1.29 (m)	23.6 t
1b	0.95 (m)		1″b	0.88 (m)	
2a	1.82 (m)	25.0 t	2″a	1.82 (m)	24.7 t
2b	1.40 (m)		2″b	1.40 (m)	
3	3.16 (m)	73.7 d	3″	3.06 (dt, 11.8, 2.8)	74.8 d
4		37 0.4 s	4″		37.4 s
5	2.05 (dd, 11.8, 2.8)	39.3 d	5″	1.07 (m)	39.2 d
6a	1.44 (m)	20.5 d	6″a	1.05 (m)	20.3 t
6b	1.38 (m)		6″b	0.89 (m)	
7a	1.47 (m)	30.7 t	7″a	1.02 (m)	30.5 t
7b	1.37 (m)		7″b	0.40 (m)	
8	1.73 (m)	36.7 d	8″	1.50 (m)	35.8 d
9		97.4 s	9″		96.5 s
10		41.7 s	10″		41.5 s
11a	3.10 (d,17.3)	32.0 t	11″a	2.92 (d, 16.3)	31.4 t
11b	2.73 (d,17.3)		11″b	2.60 (d, 16.3)	
12	0.60 (d, 6.5)	15.3 q	12″	0.48 (d, 6.4)	15.5 q
13	0.85 (s)	28.5 q	13″	0.75 (s)	28.5 q
14	0.78 (s)	22.5 q	14″	0.69 (s)	22.7 q
15	0.93 (s)	15.9 q	15″	0.77 (s)	15.4 q
1′		120.7 s	1‴		110.4 s
2′		154.3 s	2‴		151.9 s
3′	6.66 (s)	100.5 d	3‴	6.33 (s)	106.5 d
4′		137.7 s	4‴		141.2 s
5′		126.0 s	5‴		108.7 s
6′		158.5 s	6‴		159.0 s
7′		204.6 s	7‴a	4.49 (dd, 13.0, 6.3)	61.3 t
			7‴b	4.42 (dd, 13.0, 6.3)	
8′	4.89 (dd, 6.8, 3.2)	73.7 d	8‴	3.71 (d, 3.2)	57.1 d
2′-OH	9.75 (s)		2‴-OH	9.21 (s)	
3′-OH	3.85 (d, 4.6)		3″-OH	3.42 (d, 11.8)	
8′-OH	5.44 (d, 6.8)		7‴-OH	5.03 (dd, 6.3, 4.9)	

(0.5%), KH_2PO4 (0.1%) and $MgSO4{\cdot}7H_2O$ (0.02%). Fermentation was carried out on a shaker at 200 RPM for 15 days.

2.3. Extraction and isolation

The culture broth (150 L) of *Stachybotrys chartarum* was filtered, and the filtrate was extracted three times with EtOAc, while the mycelium was extracted three 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 2 fractions (1–2). Fraction 2 was applied to MPLC (MeOH/H_2O, eluting from 1:9 to 1:1) to give subfractions (A-B). Subfraction B was separated by silica gel column chromatography ($CHCl_3$/MeOH, 15:1 → 14:1), to **1** (232 mg).

Stachartarin A (1): a white powder; $[\alpha]_D^{21.6}$-37.5 (c 0.001, $CHCl_3$/MeOH, 1:1); UV (MeOH) λ_{max}: 230, 280 nm; IR (KBr) ν_{max}: 3441, 3265, 2938, 2877, 1705, 1621, 1458, 1386, 1346, 1071 cm^{-1}; ^{1}H and ^{13}C NMR data (see Table 1); Positive ion HRESIMS: *m/z* 781.4282 ($[M + Na]^+$, $C_{11}H_{18}O_3N_2Na$; calcd 781.4292).

3. Results and discussion

Compound **1** possessed a molecular formula of $C_{46}H_{62}O_9$, as evienced by HRESIMS (observed at *m/z* 781.4282, calcd at *m/z* 781.4292, $[M + Na]^+$) and appropriate for 16 degrees of unsaturation. The ^{1}H and ^{13}C NMR spectra of compound **1** displayed 46 carbon signals due to 16 quaternary carbons including six oxygenated ones, one carbonyl carbon, ten methines including three oxygen-occurring ones, eleven methylenes including oxygenated one, and eight methyls (Table 1).

Detailed of its NMR spectra suggested two phenylspirodrimane fragments (1a and 1b), which were also supported by the $^{1}H{-}^{1}H$ COSY and HMBC spectra (Fig.. 1). Compared to the known phenylspirodrimane, stachartin B [5], differences between **1a** and *stachartin* B were the absence of a lactone functional group (δ_C 68.9, CH_2, C-7′ and δ_C 168.0, C, C-8′) in stachartin B and the presence of a ketone carbonyl (δ_C 204.6, C, C-7′) and an a oxygenated methane (δ_C 73.7, CH, C-8′) in (Table 1) in **1a**. This was further confirmed by HMBC correlations from H-3′ to C-7′, and from H-8′ to C-5′ and C-7′; In **1b**, the presence of hydromethyl (δ_C 61.3, CH_2, C-7‴) and a methane (δ_C 571, CH, C-8‴) was further confirmed by the HMBC correlations (Fig. . 1) from H-3‴ to C-7‴, from H-7‴ to C-3‴, C-4‴ and C-5‴, H-8‴ to C-4‴, C-5‴ and C-6‴. Finally, the presence of key HMBC correlations from H-8‴ to C-7′ and C-8′, and of $^{1}H{-}^{1}H$ COSY correlation of H-8‴/H-8′ indicated that the