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A New Compound Along With Seven Known Compounds from an Endophytic Fungus *Aspergillus* sp. HS-05

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Abstract: Investigation of EtOAc extract from the fermentation broth of the endophytic fungus *Asperilligus* sp. HS-05 led to the isolation of a new compound (**1**) of spiro moiety named aspergispiroketal and seven known compounds (**2-8**). Their structures were elucidated mainly by NMR and HR-TOF-MS, as well as on comparison with the reported data. The absolute configuration of **1** was defined by comparison of quantum chemical TDDFT calculated and experimental ECD spectra.

Keywords: *Asperilligus* sp.; endophytic fungus; ECD

1. Introduction

Endophytic microorganisms are microbes that colonize living, internal tissues of plants without causing any immediate, overt negative effects [1]. The existence of endophytes has been known for over one hundred years. However, it was until the past decade that endophytes have been studied for their potential as novel sources of effective new drugs. Now novel antibiotics, antimycotics, immunosuppressants and anticancer compounds have been found after the purification and characterization of some of their natural products [2]. Containing about 180 recognized species, the genus *Aspergillus* has been proven to be a rich source of bioactive metabolites. In recent years, many new compounds were isolated from the genus *Aspergillus* as endophytic microorganisms [3-7] and some of them contain novel skeletons, such as asperterpenol A and B, two novel sesterterpenoids with an unusual 5/8/6/6 tetracyclic ring skeleton isolated from a mangrove endophytic fungus *Aspergillus* sp. 085242 [8]. Therefore, the prospects are great to find new compounds for drugs candidates from endophytic fungus *Aspergillus* sp. We focused on the constituents of the endophyte fungus *Aspergillus* sp.HS-05 which was isolated from the leaves of *Huperzia serrata* collected in Changbai Mountain, Jilin province of China in August 2008. Herein, we mainly report the isolation and structural elucidation of the novel compound **1**.

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2. Materials and Methods

2.1. Microorganism Material

The strain HS-05 was isolated from the leaves of *Huperzia serrata*. The strain HS-05 was identified as *Aspergillus* sp. by Prof. Yi-xuan Zhang and has been deposited in School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University.

2.2 Fermentation and Isolation

The strain HS-05 was incubated in liquid medium (2% glucose, 2% maltose, 1% monosodium glutamate, 0.05% KH_2PO_4, 0.03% $MgSO_4 \cdot 7H_2O$, 0.3% yeast extract, tap water, pH 6.5), on rotary shaker at 28 °C at 180 rpm for 8 days.

The fermentation broth of the strain HS-05 (about 80 L) was concentrated and extracted with ethyl acetate and *n*-butanol, successively. The EtOAc crude extract (24 g) was applied on a silica gel column, eluted with $CHCl_3$-CH_3OH gradient (from 100:1 to 0:1) to afford 12 fractions. Fr. 2 ($CHCl_3$-CH_3OH 100:0, 3.7 g) was re-crystallized with $CHCl_3$-CH_3OH to obtain compound **2** (3.3 g). Fr. 3 ($CHCl_3$-CH_3OH 100:3, 6.0 g) was isolated by CC on silica gel using petroleum ether-acetone gradient (from 100:1 to 0:1) to give four fractions (Fr. 3-1, 3-2, 3-3 and 3-4). The Fr. 3-1 (petroleum ether-acetone 100:10) was isolated by CC on silica gel, elucidated by $CHCl_3$ to yield compound **3** (12.5 mg). The Fr. 3-2 (petroleum ether-acetone 100:15, 100:20 and 100:30) gave compound **1** (1.7 mg) and Fr. 3-2-1 by HPLC (MeOH-H_2O 54:45). The Fr. 3-2-1 was further isolated to obtain compound **4** (300 mg) by HPLC (MeOH-H_2O 45:55). The Fr. 3-3 (petroleum ether-acetone 50:50) was then submitted to semi-preparative reverse-phase HPLC (C-18) by eluting with MeOH-H_2O (45:55) to get compound **5** (5.5 mg). The Fr. 3-4 (petroleum ether-acetone 100:50) gave compound **6** (7.7 mg). The Fr. 4 ($CHCl_3$-CH_3OH 100:3) gave compounds **7** (6.5 mg) and **8** (5.0 mg) by silica gel chromategraphy, Sephadex LH-20 and HPLC.

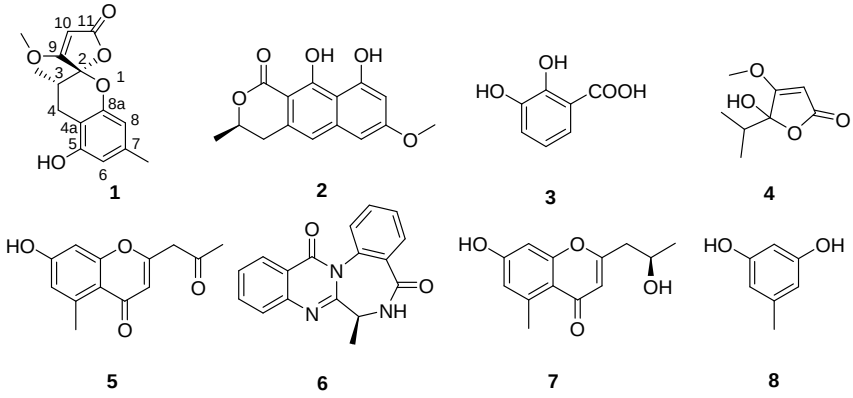

Figure 1. Structures of compounds **1**-**8**.

3. Results and Discussion

3.1. Structure elucidation

Investigation of EtOAc extract from the fermentation broth of the endophytic fungus *Asperilligus* sp. HS-05 led to the isolation of a new compound (**1**) of spiro moiety named aspergispiroketal and seven known compounds semivioxanthin (**2**) [9], 2,3-dihydroxybenzoic acid (**3**) [10], dihydropenicillic acid (**4**) [11], *R*-2-acetonyl-7-hydroxy-5-methylchromone (**5**) [12], circumadatin F (**6**) [13], 2-(2´-

hydroxypropyl)-5-methyl-7-hydroxychromone (**7**) [14], orcinol (**8)** [15]. All of them were isolated for the first time from the strain.

Compound **1** was obtained as a white amorphous solid, with a negative specific rotation value. The molecular formula of $C_{15}H_{16}O_5$ was deduced from the HR-FAB-MS (*m/z* 275.0923 $[M-H]^-$). IR absorptions were observed at 3410, 1735 (C=O), 1638, 1580, 1450 cm^{-1}, which indicated the presence of hydroxyl group, carbonyl group and benzyl moitey. In the ^{13}C NMR spectrum, fifteen carbon signals were observed. Among them, nine sp^2 carbon signals at δc 90.6, 100.6, 110.6, 111.1, 137.5, 151.6, 156.3, 169.5 and 177.3, two methyl groups at δc 14.6, 18.8 with corresponding proton signals at δ_H 0.90 (d, J = 6.0 Hz), 2.10 (s) determined by HSQC spectrum and one methoxyl group at δc 60.5 were given. The left three carbon signals in the ^{13}C NMR spectrum at δc 25.9, 30.1, 103 were assigned to be secondary, tertiary, quaternary carbon signals respectively for their corresponding proton signals at δ_H 2.31, 2.71 (δc 25.9), and δ_H 2.31 (δc 30.1) according to the HSQC spectrum.

Among the fifteen carbon signals, three sp^2 carbon signals at δc 90.6, 169.5 and 177.3, one methoxyl group at δc 60.5 were almost the same as those of the carbon data in compound **4**. The HMBC correlations from the methoxly group at δ_H 3.98 to δc 177.3 (C-9), from the methyl group at δ_H 0.90 to δc 103.0 (C-2), 30.1 (C-3) and 25.9 (C-4), and from δ_H 5.62 (H-10) to δc 103.0 (C-2), 177.3 (C-9), 169.5 (C-11) were observed. The extensively analysis of the HMBC correlations and the comparison of the NMR data above with those of compound **4** led to the determination of fragment **1b**, which was a double substituted substructure at the methyl and the hydroxyl groups of compound **4**.

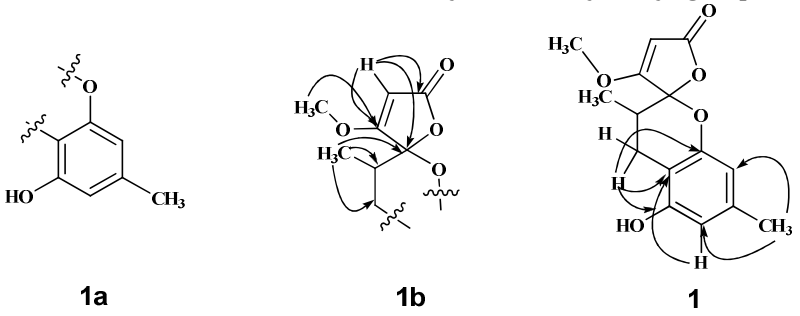

Figure 2. Fragments and key HMBC correlations of compound **1**.

So far eight carbon signals were assigned and the left six sp^2 carbon signals were considered to be a 1,3,4,5-tetrasubstituted benzyl moiety (fragment **1a**) for the coupling constant between the two benzyl proton signals at δ_H 6.08 (1H, d, J = 2.0 Hz), 6.29 (1H, d, J = 2.0 Hz). So the fragment **1b** and the left one hydroxyl and one methyl group at δ_H 2.10 were considered to be connected to the benzyl moiety to content the substructure of 1,3,4,5-tetrasubstituted benzyl moiety. The location of these groups were determined by the correlations from δ_H 6.08 (3H, s, 7-CH_3) to δc 111.1 (C-6), 100.6 (C-8), from δ_H 2.71 (H-4) to δc 110.6 (C-4a), 156.3 (C-5) and 151.6 (C-8a), from δ_H 6.29 (H-6) to δc 110.6 (C-4a), 100.6 (C-8) in the HMBC spectrum and compound **1** was elucidated as shown in figure 1.

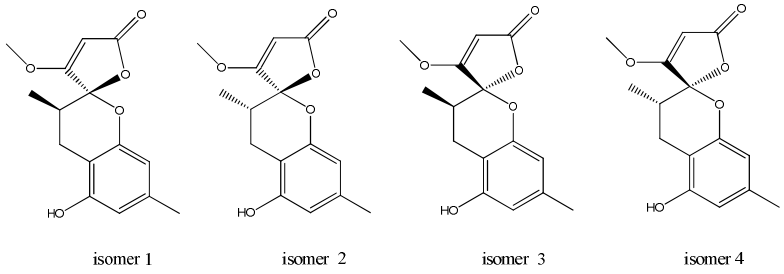

Figure 3. Four stereo-ismoers of compound **1**.

The absolute configuration of C-2 and C-3 was determined by comparison of quantum chemical TDDFT calculated and experimental ECD spectra. There are four possible isomers of compound **1**, each isomer was optimized using DFT at the B3LYP/6-31G (d) level in the GAUSSIAN

09 program. The optimized isomer was calculated using DFT at the B3LYP/6-311G (d, p) in the GAUSSIAN 09 program to generate its ECD property. The ECD spectrum of the isomer **4** was the most similar one compared with the experimental CD spectra of compound **1**. So the absolute configurations of C-2 and C-3 were determined to be *S, R* respectively.

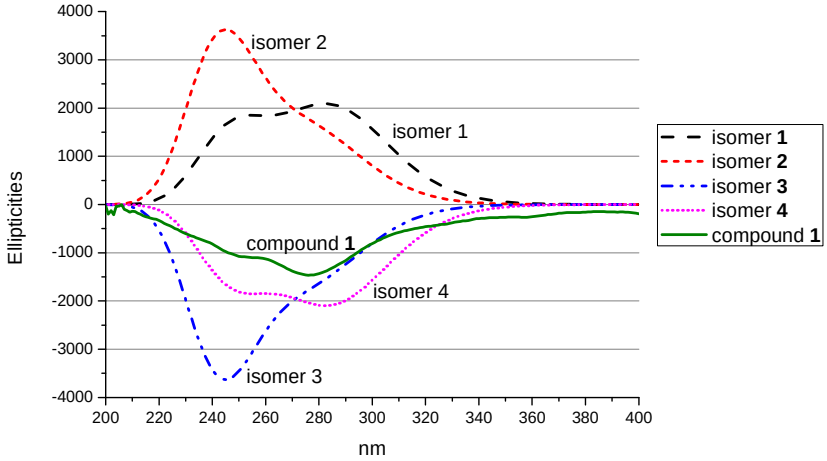

Figure 4. Quantum chemical TDDFT calculated ECD spectra of the four stereo-isomers and experimental ECD spectra of compound **1**.

Table 1. ^{1}H and ^{13}C NMR data for compound **1** (at 600 MHz in DMSO-d_6, δ in ppm, J in Hz).

Position (H)	δ_H	δ_C
2	/	103.0
3	2.31 (1H, m)	30.1
4	2.71 (1H, m) 2.32 (1H, m)	25.9 t
4a	/	110.6
5	/	156.3
6	6.29 (1H, d, J = 2.0)	111.1
7	/	137.5
8	6.08 (1H, d, J = 2.0)	100.6
8a	/	151.6
9	/	177.3
10	5.62 (1H, s)	90.6
11	/	169.5
C-3 Me	0.90 (3H, d, J = 6.0)	14.6
C-7 Me	2.10 (3H, s)	18.8
C-9 OMe	3.98 (3H, s)	60.5
-OH	9.26 (1H, br. s)	/

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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