Secondary Metabolites of *Aspergillus fumigatus* var. *fumigatus*

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【**ABSTRACT**】 **AIM:** To study the secondary metabolites of the commensal microorganism (*Aspergillus fumigatus* var. *fumigatus*) of *Cephalotaxus mannii*. **METHOD:** Chemical constituents were isolated by column chromatography and structures were elucidated based on spectral analysis. **RESULT:** Two compounds were purified from the fermented extracts and identified to be trypacidin (**1**) and 1,2-*seco*-trypacidin (**2**), respectively. **CONCLUSION:** Compound **2** is a new compound, and the ^{13}C NMR assignments for the C-4 and C-7a of trypacidin were revised based on HMQC and HMBC experiments.

【**KEY WORDS**】 *Aspergillus fumigatus* var. *fumigatus*; Trypacidin; 1,2-*seco*-trypacidin

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1 Introduction

Since plant commensal microbes dwell in different circumstances and possess different biological properties compared with the soil ones, they are expected to produce novel and active chemical substances. Recently, increasing attention has been paid to plant commensal microorganisms as a source of new substances[1-3].

During the course of our searching for new bioactive microbial metabolites, two compounds [trypacidin (**1**) and 1,2-*seco*-trypacidin (**2**)] were isolated and identified from the fungus *Aspergillus fumigatus* var. *fumigatus* isolated from the stems of *Cephalotaxus mannii*, and **1** showed weak antimicrobial activities; **2** is a new compound. Their structures are elucidatied in this paper.

2 Apparatus and Material

2.1 *Apparatus and Material*

Column chromatography (CC): Qingdao silica gel (200-300 mesh); Sephades LH-20: Pharmacia products; TLC: Qingdao precoated plates, silica GF_{254} plates; NMR Spectra: Bruker AM-400 or DRX-500 spectrometer with TMS as internal standard; ESIMS: Thermo-Finnigen LCQ-Advantage spectrometer *m/z*. Malting points were measured on an X-4 melting point apparatus and were uncorrected.

2.2 *Cultural Conditions and Extraction*

Aspergillus fumigatus var. *fumigatus* was grown on PD agar plates at 28 ℃ for eight days under dark. Every plate (Diameter 90 mm) contained 20 mL of PDA media (potato 200 g/L; dextrose 20 g/L; Agar 15 g/L). Total 500 mL (25 plates) were cultivated under the above fermentation conditions.

The cultured agar was chopped, diced and extracted with EtOAc-MeOH-AcOH (80∶15∶5, 0.5 L) at room temperature for over night. The organic solution was collected through filtration, and the remaining agar residue was extracted exhaustively with EtOAc-MeOH-AcOH (80∶15∶5) until the filtrate was colorless. The combined filtrates were concentrated under vacuum to remove solvents and 1.5 g crude extracts were obtained. The antifungal assay against *Penicillium avellanceum* UC-4376 indicated that the extracts were active.

2.3 *Isolation*

The crude extract (1.5 g) was subjected to col-

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umn chromatography over reversed-phase C_{18} Si gel (130 g) eluted with methanol-water [H_2O 500 mL; 60 % methanol (*v*/*v*) 1500 mL (Fr. 1-5); 80 % methanol (*v*/*v*) 900 mL (Fr. 6-8); methanol (Fr. 9)], and 300 mL was collected for each fraction. Bioassay results indicated that only Fr. 2 showed antifungal activity, so this fraction was undertaken for further isolation.

The active fraction (Fr. 2, 30 mg) was chromatographed with Si gel (5 g) and eluted with chloform-methanol ($CHCl_3$, $CHCl_3$-MeOH 100∶1, 100∶2, methanol) to afford **1** (4 mg) and **2** (4 mg), and **1** showed modest antifungal activities against *Penicillium avellaneum* UC-4376.

3 Results

3.1 *Structure Elucidation*

Compound **1** was obtained as white powder, mp 188～190 ℃, UV λ_{max}^{MeOH} 293 nm, ESI-MS (*m*/*z*): 345 $[M+1]^+$, 711 $[2M+Na]^+$, determining the molecular weight to be 344. The ^1H NMR, ^{13}C NMR and DEPT spectra of **1** showed 18 carbon signals for one methyl, three methoxys, four methines, ten quaternary carbons including three carboxyls at δ 190.5, 185.6 and 163.5, respectively. The connectivities through quaternary carbons were determined based on HMBC experiments. The at δ 6.35 (H-5) was correlated with the carbons at δ 108.3 (C-3a), 158.3 (C-4), 23.1 (C-6a) and 152.1 (C-6); and the proton at δ 6.53 (H-7) was correlated with C-3a, C-6a, 105.3 (C-5), 174.3 (C-7a) and the methyl proton at δ 2.42 (H-6a) with 105.3 (C-5), 152.1 (C-6), 105.3 (C-7); and the methoxy protons at δ 3.93 with C-4. All these indicated a tetra-substituted benzene ring in the structure as shown in **1a** (Fig 1). The proton at δ 5.75 (H-4′) was correlated with the carbons at δ 138.4 (C-1′), 84.0 (C-2), 137.1 (C-2′), 185.6 (C-3′) and 169.5 (C-5′), and δ 7.09 (H-2′) with 163.5 (COO-C-1′) and 103.9 (C-4′). The methoxy protons at δ 3.67 (1′-COOMe) was correlated with C-1′ and the methoxy protons at δ 3.64 with C-5′, indicating that the presence of a cyclohexenone ring as shown in **1b** (Fig 1). Compared these data with those in ref. 4, the partial structure **1a** and **1b**, and that a quaternary oxygenated olefinic carbon (C-7a) in **1a** and an oxygenated olefinic carbon (C-2) in **1b** were connected through an oxygen. The carbonyl carbon at C-3 (δ 190.5) must be attached at C-3a as indicated by the chemical shift of the latter. Those results revealed the structure of **1** to be trypacidin[4-6] (Fig 1). The ^{13}C NMR assignments for C-4 and C-7a were revised based on HMQC and HMBC experiments.

Compound **2**, $[\alpha]_D^{18}$ +4.5 (*c* 0.001 in MeOH), was obtained as white powder, mp 173～174 ℃, UV λ_{max}^{MeOH} 287 nm. The HRESI-MS data determined the molecular formula to be $C_{18}H_{18}O_7Na$ (369,0948, *calc* 369.0950), and its molecular weight was two mass units more than that of **1**. The ^1H NMR was very similar to that of **1**. However, the ^{13}C NMR showed only two carbonyl carbons at δ 199.8 and 166.8, and that another olefinic carbon signal appeared at δ 127.0 (C-2) instead of at δ 84.0. Other carbon signals of **2** agreed with those of **1**. Upon comparing the molecular weight and unsaturation units and HMBC correlations with those of **1**, compound **2** was determined to be 1,2-*seco*-trypacidin (Fig 1). The most obvious discrepancy among the ^{13}C NMR assignments of compounds **1** and **2** was for C-7a, which may due to the opening of the five member spiro-cyclic oxygen ring to release the intramolecular stress and the formation of the free phenol functional group from the ether oxygen at C-1.

The NMR data of **1** and **2** are summarized in Table 1.

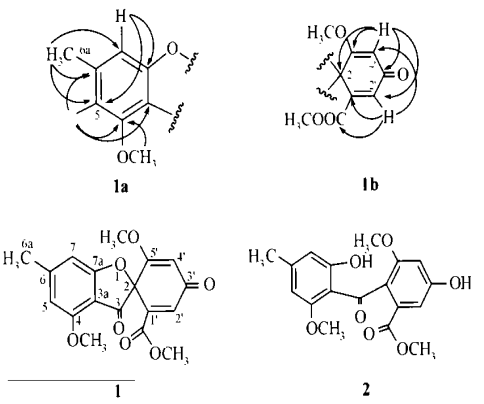

Fig 1 The selected HMBC correlations of 1 and the structures of 1 and 2

3.2 *Biological Activity*

The antifungal activity against *Penicillium avellaneum* UC-4376 was tested by paper-disc diffusion method. Compound **1** showed modest activities against *Penicillium avellaneum* UC-4376 and *Staphylococcus aureus*, *Mycobacterium tuberculosis* at the amount of 200 μg/disc, and **2** showed no inhibitory activities against the tested microorganism.

Table 1 The NMR spectral data of 1 and 2 (400 MHz for ^{1}H and 100 MHz for ^{13}C NMR, δ in $CDCl_3$, J = Hz)

Position	1			2		
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
2	-	84.0s	-	-	127.0s	-
3	-	190.5s	-	-	199.8s	-
3a	-	108.3s	-	-	110.3s	-
4	-	158.3s	-	-	160.9s	-
5	6.35 (s)	105.3 *d	C-3a, C-4, C-6a, C-6	6.37 (s)	110.7d	C-6, C-6a, C-7
6	-	152.1s	-	-	147.9s	-
7	6.53 (s)	105.5 *d	C-3a, C-6a, C-5, C-7a	6.55 (s)	103.2d	C-6, C-5, C-7a
7a	-	174.3s	-	-	163.7s	-
1′	-	138.4s	-	-	128.2s	-
2′	7.09 (d, 1.5)	137.1d	CO-C-1′, C-4′	6.91 (s)	107.7d	C-1′, CO- C-1′, C-3′C-4′
3′	-	185.6s	-	-	157.6s	-
4′	5.75 (d, 1.5)	103.9d	C-1′, C-2, C-2′, C-3′, C-5′	6.00 (s)	102.9d	C-2′, C-3′, C-5′
5′	-	169.5s	-	-	157.0s	-
6a	2.42 (s, 3H)	23.1q	C-5, C-6, C-7	2.06 (s, 3H)	22.3q	C-6, C-6a, C-7
4-OMe	3.93 (s, 3H)	56.0q	C-4	3.38 (s, 3H)	52.1q	C-4
1′COOMe	3.67 (s, 3H)	52.8q	1′COO	3.58 (s, 3H)	55.6q	1′COO
		163.5s			166.8s	
5′OMe	3.64 (s, 3H)	56.7q	C-5′	3.67 (s, 3H)	56.0q	C-5′

* exchangeable

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真菌 *Aspergillus fumigatus* var. *fumigatus* 的次生代谢产物

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【摘 要】 目的:研究西双版纳粗榧共生真菌 *Aspergillus fumigatus* var. *fumigatus* 的次生代谢产物。**方法**:通过柱层析法分离发酵产物的化学成分,并利用波谱数据和文献数据对照鉴定化合物的结构。**结果**:分离到 2 个化合物,分别鉴定为 trypacidin (**1**) 及其衍生物 1,2-*seco*-trypacidin (**2**)。**结论**:化合物 **2** 是新结构;并对文献中关于 trypacidin 的 C-4 和 C-7a 位碳谱数据的指定进行了更正。

【关键词】 *Aspergillus fumigatus* var. *fumigatus*; Trypacidin; 1,2-*seco*-trypacidin

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