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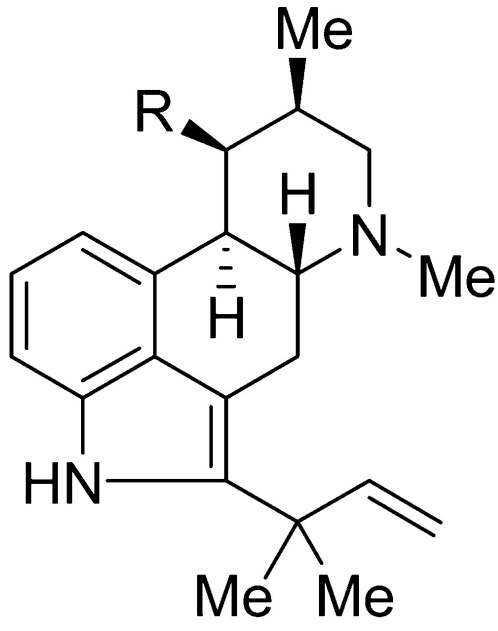

1 R=OH
2 R=H
3 R=OAc

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Bioactive Alkaloids from Endophytic *Aspergillus fumigatus*

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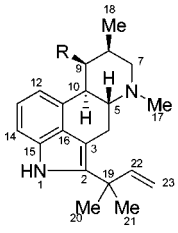
Two new alkaloids, named 9-deacetylfumigaclavine C (**1**) and 9-deacetoxyfumigaclavine C (**2**), along with 12 known compounds (**3**−**14**), were isolated from the culture of *Aspergillus fumigatus*. The structures of the new compounds were elucidated by comprehensive spectroscopic analyses. Compound **2** showed selectively potent cytotoxicity against human leukemia cells (K562) with an IC_{50} value of 3.1 μM, which was comparable to that of doxorubicin hydrochloride, a presently prescribed drug for the treatment of leukemia. Furthermore, 14-norpseurotin (**4**) significantly induced neurite outgrowth of rat pheochromocytoma cells (PC12) at a 10.0 μM concentration.

The genus *Aspergillus* (Moniliaceae), which contains about 180 recognized species,[1] has been proven to be a rich source of bioactive metabolites. Even after investigations spanning over two decades, this genus still continues to provide metabolites with new structures and interesting biological activities.[2−10] In our continuous search for chemically new and biologically potent metabolites produced by medicinal plants[11] or symbiotic fungi harbored in plant tissues[12] or animal organs,[13] the endophyte fungus *A. fumigatus* was isolated from a healthy stem of *Cynodon dactylon*. Subsequent culturing and fractionation of the EtOAc extract of the *A. fumigatus* culture led to the isolation of two new alkaloids, named 9-deacetylfumigaclavine C (**1**) and 9-deacetoxyfumigaclavine C (**2**), together with 12 known metabolites identified as fumigaclavine C (**3**),[2] 14-norpseurotin (**4**),[3] pseurotin A (**5**),[4] spirotryprostatin A (**6**),[5] 6-methoxyspirotryprostatin B (**7**),[3] fumitremorgin F (**8**),[6] dimethylgliotoxin (**9**),[7] 12α-fumitremorgin C (**10**),[2] demethoxyfumitremorgin C (**11**),[8] verruculogen (**12**),[9] and tryprostatins A (**13**)[10] and B (**14**).[10] The structures of the new compounds were determined by MS, NMR, and CD methods. We present herein the isolation, structural elucidation, and bioactivity assessment of these compounds.

The EtOAc extract of *A. fumigatus* culture was subjected to silica gel and Sephadex LH-20 chromatography, followed by further purification with reversed-phase HPLC if necessary, to give 14 alkaloids (**1**−**14**).



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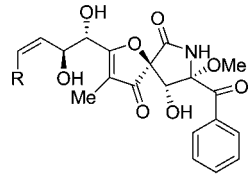
2 R=H
3 R=OAc



4 R=Me
5 R=Et

9-Deacetylfumigaclavine C (**1**) was isolated as a white, amorphous powder. Its molecular formula was determined as $C_{21}H_{28}N_2O$ according to the positive-ion mode HR-ESIMS, suggesting 42 amu less than that of fumigaclavine C (**3**). The ^1H and ^{13}C NMR spectra of compound **1** were very similar to those of **3**,[2] except for the 9-*O*-acetyl group in fumigaclavine C (Table 1). In addition, downfield shifts for C-8 and C-10 and upfield shifts for C-5, C-7, and C-9 were observed. The observation, together with its MS data, suggested that it was likely a deacetylated derivative of fumigaclavine C (**3**). This conclusion was further supported by discernible ^3J HMBC correlation of H-9 (δ 4.53) with C-5 (δ 60.8), C-7 (δ 57.3), C-11 (δ 128.7), and C-18 (δ 16.7) and $^1H-^1H$ COSY correlations from H-8 (δ 2.18) through H-10 (δ 3.33).

9-Deacetoxyfumigaclavine C (**2**) was isolated as a white, amorphous powder. Its HRESIMS data indicated that its molecular formula was $C_{21}H_{28}N_2$, 16 amu less than compound **1**. The ^1H and ^{13}C NMR data (Table 1) of **2** were similar to those of 9-deacetylfumigaclavine C (**1**) except for signals of the 9-methine group [δ_H 4.53 (brs), δ_C 68.9] in **1**, which was replaced by a methylene function [δ_H 2.69 (m), 1.13 (m); δ_C 36.1)] in **2**. The upfield chemical shifts of C-9 and H-9 in **2**, together with the loss of an oxygen atom (16 amu), suggested that compound **2** was likely the 9-deoxy derivative of **1**. This conclusion was further supported by the $^1H-^1H$ COSY correlations from H-8 (δ 2.28) through H-10 (δ 3.17) and also confirmed by ^3J HMBC correlation of C-9 with H-7 (δ 2.10/3.17) and H-18 (δ 1.02).

The relative configuration of **1** and **2** was determined by NOESY data and the magnitude of the $^1H-^1H$ coupling constants. Thus, the observed NOE correlations between proton pairs H-8/H-9 and H-9/H-10 in **1** and H-8/H-10 in **2** indicated a similar relative configuration to **3**. The absolute configuration of **1** and **2** was established from the CD curves (Supporting Information), in which the Cotton effects were comparable to those of **3**.

Compounds **1** and **2** were tested for cytotoxicity against KB (human nasopharynyeal epidermoid), MCF-7 (human breast adenocarcinoma), and K562 (human leukemia) cancer cell lines. They showed inhibition with an apparent selectivity for K562 cells, with IC_{50} values of 41.0 ± 4.6 and 3.1 ± 0.9 μM, respectively. It is noteworthy that the activity of metabolite **2** is close to that of doxorubicin hydrochloride (1.2 ± 0.2 μM), a presently prescribed drug for the treatment of leukemia. Moreover, 14-norpseurotin (**4**) significantly induced neurite outgrowth of PC12 cells at a concentration of 10.0 μM, more active than pseurotin A (**5**), which has been reported to induce neurite outgrowth (Figure 1), whereas other isolates were practically inactive.[14]

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Perkin-Elmer 341 digital polarimeter. UV spectra were recorded on a Hitachi U-3000 spectrophotometer. The IR spectra were measured on a Nexus 870 FT-IR spectrometer. HRESIMS spectra were recorded on an Agilent 6210 mass spectrometer. NMR data were acquired on Bruker AV500 and AV300 NMR spectrometers using TMS as the internal standard, and chemical shifts were recorded as δ values. Semipreparative HPLC was performed using an Allsphere ODS column (250 × 4.6 mm), Hitachi pump L-7100, and UV detector L-7400. Silica gel (200−300 mesh) for column chromatography was purchased from

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Table 1. NMR Data of Compounds **1**−**3**[a]

	1		**2**		fumigaclavin C (**3**)
no.	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1		7.81 (brs)		7.82 (brs)	
2	137.2 (s)		137.0 (s)		136.6 (s)
3	106.2 (s)		105.6 (s)		105.9 (s)
4	27.0 (t)	3.49 (dd, 13.5, 3.5) 2.71−2.73 (m)	27.2 (t)	3.52 (dd, 14.6, 4.4) 2.91 (dd, 14.6, 11.2)	27.9 (t)
5	60.8 (d)	2.73−2.76 (m)	67.0 (d)	2.42 (dt, 11.2, 4.4)	61.5 (d)
7	57.3 (t)	2.91 (11.6, 3.5) 2.72−2.74 (m)	64.5 (t)	3.17 (t, 11.3) 2.10 (t, 11.3)	57.5 (t)
8	33.9 (d)	2.16−2.18 (m)	29.5 (d)	2.23−2.28(m)	32.9 (d)
9	68.9 (d)	4.53 (brs)	36.1 (t)	2.63−2.69 (m) 1.07−1.19 (m)	71.3 (d)
10	41.0 (d)	3.33 (brd, 9.6)	39.4 (d)	3.17 (t, 11.3)	39.2 (d)
11	128.7 (s)		132.1 (s)		129.0 (s)
12	112.4 (d)	6.93 (d, 6.2)	113.2 (d)	6.88 (d, 7.0)	112.6 (d)
13	108.5 (d)	7.12 (t, 6.2)	108.1 (d)	7.09 (t, 7.0)	107.7 (d)
14	122.5 (d)	7.12 (d, 6.2)	122.2 (d)	7.09 (d, 7.0)	122.0 (d)
15	132.6 (s)		131.7 (s)		132.1 (s)
16	128.8 (s)		127.2 (s)		127.9 (s)
17	43.2 (q)	2.49 (s)	42.1 (q)	2.62 (s)	43.4 (s)
18	16.7 (q)	1.31 (d, 7.4)	19.3 (q)	1.02 (d, 6.4)	16.5 (s)
19	39.1 (s)		39.1 (s)		38.9 (s)
20	27.4 (q)	1.52 (s)	27.5 (q)	1.51 (s)	27.3 (s)
21	27.3 (q)	1.52 (s)	27.3 (q)	1.51 (s)	27.1 (s)
22	145.7 (d)	6.08 (dd, 17.8, 10.2)	145.7 (d)	6.08 (dd, 16.8, 10.5)	145.6 (d)
23	112.0 (t)	5.12 (d, 17.8) 5.11 (d, 10.2)	111.9 (t)	5.12 (d, 16.8) 5.11 (d, 10.5)	111.7 (t)
9-OAc					170.9 (s)
					21.1 (q)

[a] Spectra were recorded in $CDCl_3$ at 500 and 125 MHz for ^{1}H and ^{13}C NMR, respectively (TMS as internal standard).

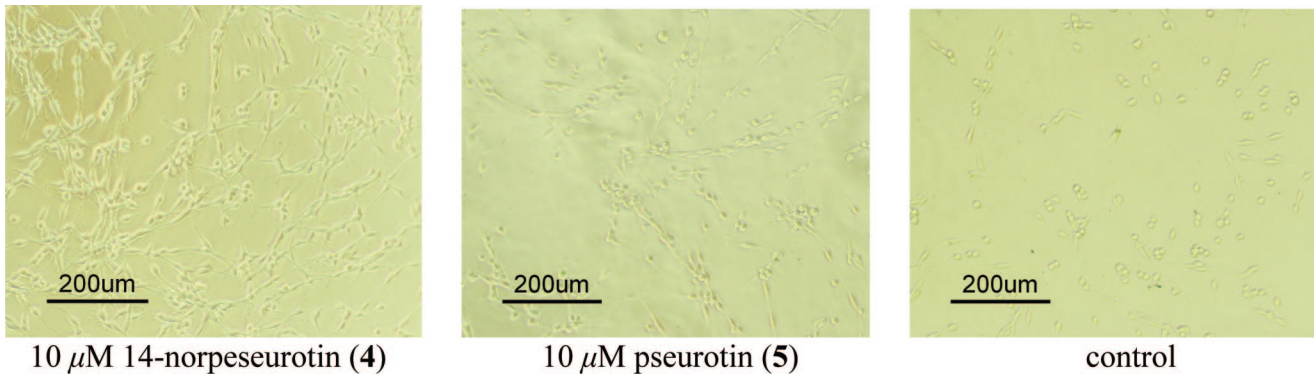

Figure 1. Effects of 14-norpseurotin (**4**) and pseurotin (**5**) on neurite outgrowth in PC-12 cells. PC-12 cells were incubated for 2 days without drug (control).

Qingdao Marine Chemical Factory, Qingdao, China. Sephadex LH-20 was purchased from Pharmacia Biotech, Sweden. All other chemicals were of analytical grade. All cell lines were supplied by Jiangsu Provincial Center for Disease Prevention and Control (Nanjing, China).

Fungal Material. The title strain of *A. fumigatus* (strain no. CY018) was isolated from the healthy stem of *C. dactylon* collected in November 2001 from Yancheng Biosphere Reserve, Jiangsu Province. The collected plants were authenticated by Prof. L. X. Zhang (Nanjing University), with a voucher specimen preserved in the herbarium of Nanjing University. The strain was identified by Dr. Y. C. Song. A voucher specimen is deposited in our laboratory at −80 °C. The working strain was preserved on potato dextrose agar slants containing 10% NaCl and stored at 4 °C.

Fermentation and Extraction. The fungal strain was cultured on slants of potato dextrose agar (PDA) at 25 °C for 5 days. The agar plugs were inoculated into Erlenmeyer flasks (1000 mL) each containing 300 mL of PDA medium. After 4 days, the seed culture was transferred into 1 L flasks, each preloaded with the Czapack medium containing sucrose (30.0 g/L), $NaNO_3$ (3.0 g/L), KCl (0.5 g/L), $MgSO_4 \cdot 7H_2O$ (0.5 g/L), $FeSO_4$ (0.01 g/L), K_2HPO_4 (1.0 g/L), and yeast extract (1.0 g/L). The culture was grown for 14 days at 28 °C. The harvested culture (30 L) was extracted at room temperature with EtOAc (3 × 30 L). Evaporation of the solvent under reduced pressure yielded a black oil (42 g), which was diluted with H_2O to give an aqueous suspension. The suspension was successively extracted with petroleum ether and EtOAc. The EtOAc fraction was shown to be cytotoxic, and it was concentrated in vacuo to give a residue (31 g) for further separation.

Isolation and Purification. The EtOAc extract (31 g) was separated on a silica gel column eluted with a $CHCl_3$−MeOH gradient (0−100%). The collected material was combined into 11 fractions based on TLC analysis. The bioactive fraction Fr-2 (2.3 g, IC_{50} of Fr-2 against KB cells is 76 μg/mL) was further separated on an RP C-18 column eluted with MeOH−H_2O mixtures (15:85, 30:70, 50:50, 60:40, 1:0) to give six subfractions, Fr-2-1−Fr-2-6. Fr-2-2 (112 mg) was further separated by HPLC with H_2O−MeOH (3:7) to give compounds **2** (7.1 mg) and **6** (2.5 mg). Fr-2-4 (32 mg) was purified with Sephadex LH-20 to give compound **5** (4.8 mg). Fr-2-5 (324 mg) was separated by HPLC eluted with MeOH−H_2O (3:1) to yield compounds **1** (17.0 mg), **4** (2.2 mg), **12** (1.2 mg), and **14** (9.3 mg). The fraction Fr-3 (1.1 g) was further separated into nine subfrations by Sephadex LH-20, eluted with MeOH−$CHCl_3$ (1:1). Compounds **3** (53 mg) and **9** (6 mg) were obtained from subfraction Fr-3-3 after HPLC separation with MeOH−H_2O (8:2). Compound **10** (13.9 mg) was recrystallized from Fr-3-5 using acetone−$CHCl_3$. Fr-5 (0.9 g) was subjected to a Si gel column eluted with petroleum ether−acetone (10:1) to afford three major components, each of which was purified with Sephadex LH-20 with MeOH−$CHCl_3$ (1:1) to give compounds **7** (13.7 mg), **8** (7.6 mg), and **11** (5.1 mg).

Cytotoxicity Test. The cytotoxicity of the new compounds was tested on three cell lines, human leukemia (K562), human nasopharynyeal

epidermoid tumor (KB), and human breast adenocarcinoma (MCF-7) cells. Their effects on the viability of these cells were assayed by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.[15] Briefly, cells at the exponential growth phase were collected and transferred into 96-well plates. After incubation for 24 h, compound dilutions were dispensed to the established culture plates for 48 h. The MTT solution was then added to each well (0.1 mg/well). After further incubation for 4 h, the supernatant was removed, the crystals were fully dissolved in 150 μL of DMSO, and the absorbance of each well was read at 570 nm. IC_{50} was determined as the concentration that inhibited cell growth by 50% using the MTT assay. The data represent the mean of three experiments performed in triplicate and are expressed as means ± SD.

Neurite Outgrowth Assay.[16] PC12 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum (Cell Culture Laboratory, Cleveland, OH), 5% horse serum (Invitrogen, Carlsbad, CA), penicillin (50 units/mL), and streptomycin (50 μg/mL) in an incubator maintained at 37 °C with 5% CO_2. The PC-12 cells were seeded onto 24-well multiplates (1 × 104 cells/mL) and cultivated for a day. The medium was replaced with fresh medium containing the test compounds, and then PC-12 cells were cultivated for 2 days and observed under a phase-contrast microscope.

9-Deacetylfumigaclavine C (1): white, amorphous powder; $[\alpha]_D^{20}$ +11.3 (*c* 0.18, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 203 (4.3), 212 (4.0), 228 (4.4), 252 (3.5), 282 (3.8); CD (MeOH) $\Delta\epsilon$ = 209 (−2.6), 217 (−4.2), 232 (+9.2), 284 (−2.0); IR (KBr) ν_{max} 3550, 3328, 3956, 2922, 2797, 1715, 1636, 1608, 1462, 1379, 1331, 1130, 1020, 914 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; HRESIMS *m/z* 325.2268 ($[M + H]^+$ calcd for $C_{21}H_{29}N_2O$ 325.2274, Δ 1.8 ppm).

9-Deacetoxyfumigaclavine C (2): white, amorphous powder; $[\alpha]_D^{20}$ −67.8 (*c* 0.11, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 203 (4.9), 226 (4.6), 252 (3.8), 281 (3.0); CD (MeOH) $\Delta\epsilon$ = 219 (−8.1), 242 (+2.1); IR (KBr) ν_{max} 3237, 2962, 2941, 2850, 2785, 1716, 1638, 1606, 1462, 1375, 1329, 1231, 1042, 916, cm^{-1}; ^1H and ^{13}C NMR data, Table 1; HRESIMS *m/z* 309.2320 ($[M + H]^+$ calcd for $C_{21}H_{29}N_2$ 309.2325, Δ 1.6 ppm).

Acknowledgment. This work was cofinanced by NSFC (30821006 and 90813036) and MOST (2007AA09Z446).

Supporting Information Available: The NMR and CD spectra of compounds **1**−**3** are available free of charge via the Internet at http://pubs.acs.org.

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