

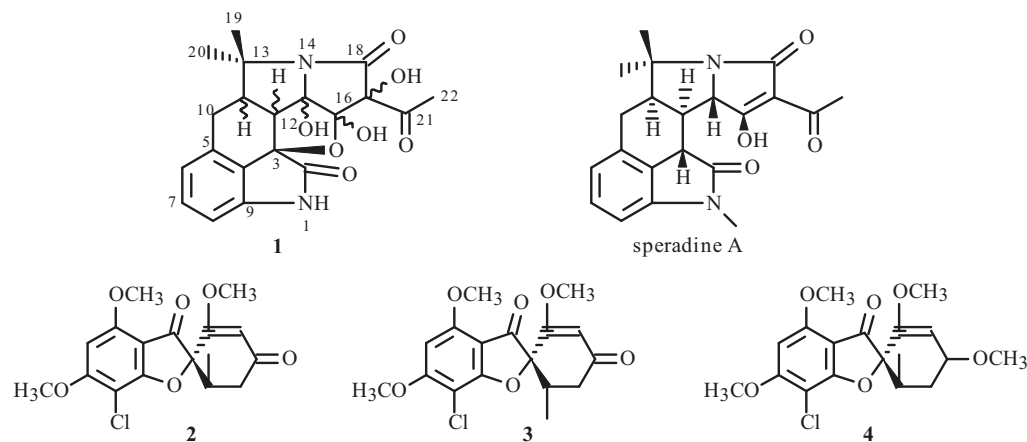
A NEW ALKALOID FROM *Penicillium dipodomyicola*

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A new alkaloid, speradine B (**1**), together with griseofulvin (**2**), epigriseofulvin (**3**), and isogriseofulvin (**4**), was purified from the extracts of *Penicillium dipodomyicola* isolated from *Clerodendrum inerme*, a tree from the intertidal zone of Nanhai. Their structures were elucidated on basis of extensive spectroscopic analysis.

Keywords: *Penicillium dipodomyicola*, *Clerodendrum inerme*, alkaloids.

Nowadays, marine natural products are attracting more and more attention from biologists and chemists all over the world. The second metabolites of marine microorganisms consists of compounds that repel predators by their toxicity and those that are attractive to make reproduction more probable. Among the diverse marine microorganisms, marine fungi have been proven to be a promising source of structurally diverse secondary metabolites possessing a broad range of biological activities [1]. *Penicillium dipodomyicola*, isolated from *Clerodendrum inerme*, a tree from the intertidal zone of Nanhai, belongs to marine fungi and was reported to possess many bioactivities such as inducing systematic antiviral resistance in susceptible plants and inhibiting gastroenteric motion [2]. In this paper, we report the isolation and structure elucidation of a new compound, speradine B (**1**), and three known compounds, griseofulvin (**2**) [3], epigriseofulvin (**3**) [4], and isogriseofulvin (**4**) [3], purified from the extracts of *Penicillium dipodomyicola*.

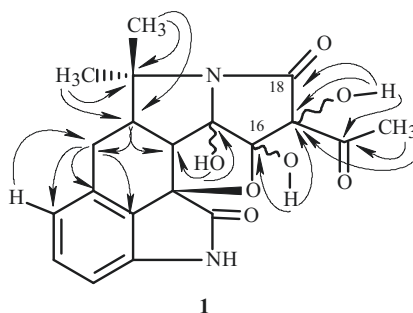


Compound **1** was isolated as a white amorphous powder, $[\alpha]_D^{20} -13.3^\circ$ (c 0.15, MeOH). The molecular formula was determined to be $C_{20}H_{20}O_7N_2$ with 12 degrees of unsaturation in the molecule by HR-ESI-MS (m/z 423.1187 $[M + Na]^+$, calcd 423.1163) and NMR data (Table 1). In the 1H NMR spectrum the proton signals at δ 7.26 (1H, dd, $J = 7.5, 7.8$ Hz), 6.92 (1H, d, $J = 7.5$ Hz), and 6.74 (1H, d, $J = 7.8$ Hz) suggested the existence of a 1,2,3-trisubstituted phenyl group.

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TABLE 1. ^1H (600 MHz) and ^{13}C NMR (150 MHz) Spectral Data of Compound **1** (DMSO- d_6 , δ , ppm, J/Hz)

C atom	δ_{H}	δ_{C}	C atom	δ_{H}	δ_{C}
1(NH)	10.95 (1H, br.s)	–	13	–	68.5
2	–	180.1	15	–	102.5
3	–	83.1	16	–	107.0
4	–	123.0	17	–	87.9
5	–	138.5	18	–	170.0
6	6.92 (1H, d, J = 7.5)	121.2	19	1.64 (3H, s)	21.3
7	7.26 (1H, dd, J = 7.5, 7.8)	131.7	20	1.45 (3H, s)	30.1
8	6.74 (1H, d, J = 7.8)	108.4	21	–	209.0
9	–	141.0	22	2.24 (3H, s)	27.1
10	2.63 (1H, dd, J = 4.0, 14.1) 2.39 (1H, dd, J = 12.0, 14.1)	26.9	15-OH	5.33 (1H, s)	–
11	2.37 (1H, m)	54.4	16-OH	6.21 (1H, s)	–
12	3.22 (1H, d, J = 7.5)	53.8	17-OH	6.51 (1H, s)	–

Fig. 1. HMBC correlations of compound **1**.

The proton signals at δ 10.95 (1H, br.s), 6.51 (1H, s), 6.21 (1H, s), and 5.33 (1H, s) represented four active hydrogens from the absence of correlations with any carbon signal in the HSQC spectrum, and the proton signals at δ 2.24 (3H, s), 1.64 (3H, s), and 1.45 (3H, s) were from three methyl groups. The ^{13}C NMR spectrum (Table 1) gave 20 carbon signals, including three carbonyl signals at δ 170.0, 180.1, and 209.0 and six aromatic signals at δ 108.4, 121.2, 123.0, 131.7, 138.5, and 141.0. All these NMR data resembled those of speradine A [5].

Compared to the NMR data of speradine A, the main part of the molecular structure was confirmed by the correlations in the HMBC spectrum from H-19, 20 at δ 1.64, 1.45 to C-13 at δ 68.5 and C-11 at δ 54.4; from the active proton signal at δ 5.33 to C-15 at δ 102.5 and C-12 at δ 53.8; from H-11 at δ 2.37 to C-10 at δ 26.9 and C-15 at δ 102.5; from H-10 at δ 2.63 to C-6 at δ 121.2, C-5 at δ 138.0, and C-4 at δ 123.0 (Fig. 1). Fragment C-16 to C-18 was elucidated in the same way by the correlations from 16-OH δ 6.21 to C-16 at δ 107.0 and C-17 at δ 87.9; from 17-OH at δ 6.51 to C-18 at δ 170.0 and C-21 at δ 209.0; from H-22 at δ 2.24 to C-17 at δ 87.9 and C-21 at δ 209.0.

EXPERIMENTAL

General Experimental Procedures. The NMR spectral data were recorded on a Bruker AV-600 spectrometer (600 MHz for ^1H NMR and 150 MHz for ^{13}C NMR; Bruker, Fallanden, Switzerland) in dimethyl sulfoxide DMSO- d_6 with TMS as an internal standard. The HR-FAB-MS data were obtained on a Micros Mass Autospec-UltimaE TOF mass spectrometer. Chromatography was performed on silica gel (200–300 mesh; Qingdao Haiyang Chemical Factory, Qingdao, China), Sephadex LH-20 (Pharmacia, Piscataway, NJ, USA), and reversed-phase HPLC on a Shimadzu LC-10 AVP chromatograph (Kyoto, Japan).

Microorganism Material. The strain Y26-02 was isolated from *C. inermis* collected in the intertidal zone of South China Sea in December 2006 and was identified as *Penicillium dipodomyicola* by comparing the ITS rDNA in GenBank. A voucher specimen has been deposited in the School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University.

Fermentation and Isolation. The supernatant of the fermentation broth of the strain Y26-02 (50 L) was concentrated to 5 L in vacuum and extracted with ethyl acetate and *n*-butanol. The EtOAc soluble fraction (20.7 g) was subjected to silica gel column chromatography and eluted with CHCl₃-CH₃OH (100:1-0:1), yielding 12 fractions. Fraction 2 (2.5 g) was then subjected to silica gel column chromatography again and eluted with petroleum ether-EtOAc (100:1-0:1), yielding 14 fractions. From these 14 fractions, fraction 2 (255 mg) was subjected to Sephadex LH-20 chromatography and eluted with CHCl₃-CH₃OH (1:1), preparative TLC developed with petroleum ether-acetone (2:1), and preparative HPLC eluted with CH₃OH-H₂O (65%) successively to obtain compound **1** (3 mg). Fraction 4 (201 mg) was subjected to preparative HPLC and eluted with CH₃OH-H₂O (75%) to obtain compounds **2** (5 mg), **3** (10 mg), and **4** (8 mg).

Speradine B (1). White amorphous powder, $[\alpha]_D^{20} -13.3^\circ$ (*c* 0.15, MeOH). IR (KBr, ν_{\max} , cm⁻¹): 3450, 2935, 1756. For ¹H NMR and ¹³C NMR spectral data, see Table 1. HR-ESI-MS *m/z* 423.1187 [M + Na]⁺ (calcd for C₂₀H₂₀O₇N₂Na, 423.1163).

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