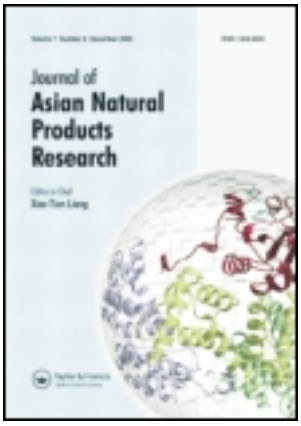

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Chemical examination of the endophytic fungus *Pestalotiopsis* sp., isolated from the leaves of the Chinese mangrove *Rhizophora mucronata*, yielded a new amide called pestalotiopamide E (**1**). The structure of the new compound was unambiguously elucidated on the basis of extensive spectroscopic data analysis.

Keywords: endophytic fungus; *Pestalotiopsis* sp.; amide; structure elucidation

1. Introduction

The fungus *Pestalotiopsis*, the causal pathogen of leaf spot disease or tea gray blight disease on a variety of plants, produces important secondary metabolites used as potential leads for the treatment of human diseases and control of plant diseases, such as acetogenins, antioxidants, immunosuppressants, and anticancer agents [1]. Our previous work reported on the genus *Pestalotiopsis* sp. residing in the leaves of the Chinese mangrove plant *Rhizophora mucronata*, and further demonstrated it to be a prolific source of new bioactive natural products [2–5]. As part of our ongoing investigation of the minor constituents obtained after the fermentation of the fungus on solid rice medium, a new amide pestalotiopamide E (**1**) was afforded. The structure of **1** was unequivocally determined by 1- and 2D NMR spectroscopy as well as by comparison with the literature data (Figure 1).

2. Results and discussion

The mycelia and culture medium of the endophytic fungus *Pestalotiopsis* sp. were extracted with ethyl acetate. This extract was concentrated, and then repeatedly chromatographed over silica gel and Sephadex LH-20, followed by preparative HPLC, to yield a new compound called pestalotiopamide E (**1**).

Pestalotiopamide E (**1**) was obtained as a colorless oil. Its molecular formula of $C_{11}H_{17}NO_5$ was established by HR-ESI-MS at *m/z* 244.1178. The ^1H and ^{13}C NMR spectral data of **1** (Table 1) indicated that four unsaturation degrees of the molecule are due to a double bond and three carbonyl groups. The NMR spectral data

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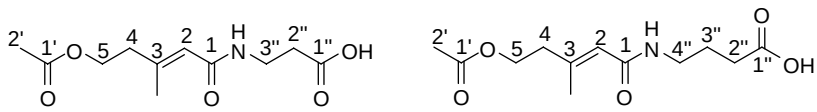

Figure 1. Structures of compounds **1** and **2**.

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data for pestalotiopamide E (**1**) in CD_3OD.

	1			Pestalotiopamide B	
Atom no.	δ_H (ppm), J in Hz	δ_C (ppm)	HMBC (H to C)	δ_H (ppm), J in Hz	δ_C (ppm)
1		169.3, s			170.2, s
2	5.69, d, $J = 1.3$	121.1, d	C-1,3,4	5.70, d, $J = 0.95$	121.1, d
3		150.8, s			150.5, s
4	2.41, t, $J = 6.6$	40.4, t	C-2,4,3-CH_3	2.42, t, $J = 6.66$	40.4, t
5	4.20, t, $J = 6.6$	63.2, t	C-3,4,1′	4.21, t, $J = 6.66$	63.2, t
1′		172.8, s	C-2′		172.8, s
2′	2.00, s	20.7, q	C-1′	2.01, s	20.7, q
1″		176.2, s			176.5, s
2″	2.50, t, $J = 6.8$	35.1, t	C-1″,3″	2.32, t, $J = 7.25$	32.6, t
3″	3.43, t, $J = 6.8$	36.3, t	C-1,1″,2″	1.89, m	26.2, t
4″				3.23, t, $J = 6.9$	39.5, t
3-CH_3	2.11, d, $J = 1.3$	18.4, q	C-2,3,4	2.12, d, $J = 0.95$	18.4, q

(Table 1) of **1** and the information from its ^{1}H–^{1}H COSY and HMQC spectra indicated the presence of an olefinic methine at δ_H 5.69 (d, $J = 1.3$ Hz) and δ_C 121.1; a 1,2-disubstituted ethoxyl group at δ_H 2.41 (t, $J = 6.6$ Hz) and δ_C 40.4 (4-CH_2), δ_H 4.20 (t, $J = 6.6$ Hz), and δ_C 63.2 (5-CH_2); a 1,2-disubstituted ethyl group at δ_H 2.50 (t, $J = 6.8$ Hz) and δ_C 35.1 (2″-CH_2), δ_H 3.43 (t, $J = 6.8$ Hz), and δ_C 36.3 (3″-CH_2); and two methyl substituents at δ_H 2.00 and δ_C 20.7 (2′-CH_3), δ_H 2.11 (d, $J = 1.3$ Hz) and δ_C 18.4 (3-CH_3). These NMR spectral data were very similar to those of pestalotiopamide B (**2**) [5], except for the replacement of a butyric acid group in **2** by the propanoic acid group in **1**. This assignment was further confirmed by HMBC correlations between H-3″/C-1 and H-5/C-1′ (Figure 2). The *E*-geometry of the double bond (Figure 3) in **1** was characterized by NOE. Based on the above results, the structure of pestalotiopamide E (**1**) was characterized as (*E*)-3-(5-acetoxy-3-methylpent-2-enamido)propanoic acid.

Compound **1** was subjected to antibiotic assay against several pathogenic bacteria, such as *Escherichia coli* and *Bacillus subtillis*, together with four fungal strains including *Staphylococcus aureus*, *Streptococcus pyrogenes*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*; however, **1** was proved to be devoid of significant activity in the bioassays used.

3. Experimental

3.1 General experimental procedures

UV spectral data were obtained from online UV spectra measured by photodiode array detection (Gynkotek, Germering, Germany). ^{1}H and ^{13}C NMR (chemical shifts in ppm) spectra were recorded on Bruker ARX 600 or DRX 500 NMR spectrometers in acetone-d_6,

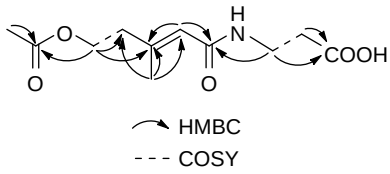

Figure 2. Key HMBC and $^{1}H-^{1}H$ COSY correlations of **1**.

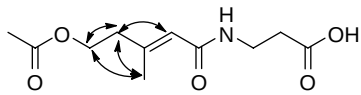

Figure 3. Key NOESY correlations of **1**.

methanol-d_4, or $CDCl_3$. High-resolution ESI-MS were recorded on a Micromass Q-Tof-2 mass spectrometer using peak matching.

3.2 Isolation and cultivation of the fungus

Pestalotiopsis sp. was isolated from the fresh, healthy leaf material of *R. mucronata* (Rhizophoraceae) collected in October 2005 in Dong Zhai Gang-Mangrove Garden on Hainan Island, China. The fungus (strain no. JCM2A4) was isolated under sterile conditions from the inner tissue of the leaf following an isolation protocol described previously [6] and identified using a molecular biological protocol by DNA amplification and sequencing of the ITS region [7]. The fungus was grown on solid rice medium at room temperature under static conditions for 39 days. The sequence data have been submitted to and deposited at GenBank (accession no. FJ465172). A voucher strain (code no. 2) has been deposited at the Institut für Pharmazeutische Biologie und Biotechnologie, Heinrich-Heine-Universität, Dusseldorf.

3.3 Extraction and isolation

The mycelia and the solid rice medium were extracted with EtOAc. The extract was evaporated under reduced pressure to yield 8.0 g of residue. This residue was subjected to vacuum liquid chromatography) on a silica gel column employing a step gradient of dichloromethane–methanol. Thirty-nine fractions were collected and examined by TLC on premade silica Si 60 F_{254} (Merck, Darmstadt, Germany), using a dichloromethane/methanol-based solvent system to yield eight fractions, F1–F8. Moreover, each fraction obtained was analyzed by HPLC using a reversed-phase column and employing a linear gradient of methanol and water (adjusted to pH 2.0 by addition of phosphoric acid). Promising fraction F6 (58 mg) was subjected to further chromatographic separation using Sephadex LH-20 with methanol as solvent. Subfraction F6-3 (8.09 mg) was further purified by semi-preparative reversed-phase HPLC ($MeOH/H_2O$ (3:10), 5 ml/min) to obtain compound **1** (3.39 mg).

*3.3.1 Pestalotiopamide E (**1**)*

A colorless oil (MeOH); UV (MeOH) λ_{max}: 219, 454, 558 nm; ^{1}H and ^{13}C NMR spectral data, see Table 1; HR-ESI-MS *m/z* 244.1178 $[M + H]^+$ (calcd for $C_{11}H_{18}NO_5$, 244.1185).

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