A New Glycine Derivative and a New Indole Alkaloid from the Fermentation Broth of the Plant Endophytic Fungus *Pestalotiopsis podocarpi* Isolated from the Chinese Podocarpaceae Plant *Podocarpus macrophyllus*

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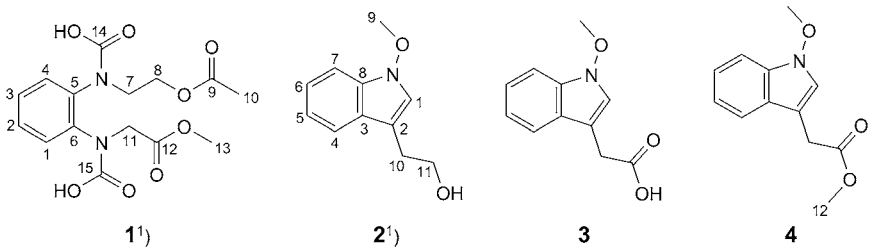
A new glycine derivative, podocarpiamide (**1**), a new indole alkaloid, 1-methoxy-1*H*-indol-3-ethanol (**2**), together with two known compounds, 1-methoxy-1*H*-indole-3-acetic acid (**3**) and methyl 1-methoxy-1*H*-indole-3-acetate (**4**), were isolated from the fermentation broth of the plant endophytic fungus *Pestalotiopsis podocarpi*. Their structures were elucidated by extensive spectroscopic analysis including 1D- and 2D-NMR (HSQC, HMBC, and COSY) and MS experiments. Compound **1** has an interesting unusual carbamic acid structure.

Introduction. – Fungi of the *Pestalotiopsis* belong to an anamorphic genus of the family Amphisphaeriaceae, and are widely distributed in tropical and subtropical plant species. Many of them are saprobes, while others are either pathogenic or endophytic to living plants [1][2]. Since discovery of the anticancer agent taxol from an endophytic fungal strain of the genus *Pestalotiopsis*, interest in searching for bioactive compounds from this fungal genus has increased considerably [3]. Recently, many alkaloids were isolated from the *Pestalotiopsis* spp. Two new heterodimeric diketopiperazine alkaloids, (+)-pestalazines A and B, and three new amides, pestalamides A – C [4], along with the known compounds aspernigrin A [5] and carbonarone A [6], have been isolated from the solid-substrate fermentation culture of the plant pathogenic fungus *Pestalotiopsis theae*. Among those compounds, pestalazine A and B displayed inhibitory effects on HIV-1 replication in C8166 cells with EC_{50} values of 47.6 and 98.9 μM, respectively, and pestalamide A displayed inhibitory effects on HIV-1 replication in C8166 cells with an EC_{50} value of 64.2 μM and potent antifungal activity against *Aspergillus fumigatus* with IC_{50}/*MIC* values of 1.50/57.8 μM [4]. Up to date, 28 N-containing compounds were isolated from the *Pestalotiopsis* spp. [7]. In the course of our research on bioactive metabolites of the genus *Pestalotiopsis* in China, many new compounds have been isolated from *Pestalotiopsis spp.* [8 – 11]. The present study was undertaken to investigate the chemical constituents of the culture broth of *Pestalotiopsis podocarpi* isolated from the branch of *Podocarpus macrophyllus* in Hainan, People's Republic of China, and have led to the isolation of the new carbamic acid derivative **1**[1]), the new indole alkaloid **2**[1]), and the two known compounds 1-methoxy-1*H*-indole-3-acetic acid

[1]) Arbitrary atom numbering; for systematic names, see *Exper. Part.*

(**3**) and methyl 1-methoxy-1*H*-indole-3-acetate (**4**; *Fig.1*). Details of the isolation and structural elucidation of the new metabolites are reported herein.



1[1]) **2**[1]) **3** **4**

Fig. 1. *Compounds* **1**–**4**, *isolated from* Pestalotiopsis podocarpi

Results and Discussion. – Compound **1** was obtained as an oil. The ESI-HR-MS of **1** indicated a molecular formula $C_{15}H_{19}N_2O_8$ (*m*/*z* 355.11436 ($[M+H]^+$)) with 8 degrees of unsaturation. Careful analysis of the ^{1}H- and ^{13}C-NMR data (*Table 1*) revealed that **1** consisted of two Me groups, four COOH or COOR groups, one disubstituted aromatic ring, and three CH_2 groups. In the NMR spectra, signals at δ(C) 129.9 (*d*), 114.8 (*d*), 114.9 (*d*), 129.5 (*d*), 128.6 (*s*), and 124.9 (*s*), and corresponding signals at δ(H) 7.07 (*d*, $J = 8.6$ Hz, 1 H), 6.74 (*dd*, $J = 7.2$, 8.6 Hz, 1 H), 6.75 (*dd*, $J = 7.2$, 8.6 Hz, 1 H), and 7.03 (*d*, $J = 8.6$ Hz, 1 H) indicated a typical *o*-disubstituted benzene moiety, which was further supported by the correlations H–C(1)/H–C(2)/H–C(3)/H–C(4) observed in the ^{1}H,^{1}H-COSY experiment (*Fig. 2*). The two substituting groups at the benzene ring were elucidated from the key HMBC cross-peaks (*Fig. 2*) CH_2(7)/C(5), C(14), and C(8), CH_2(8)/C(7) and C(9), Me(10)/C(9), CH_2(11)/C(6), C(12), and C(15), and Me(13)/C(12), along with the ^{1}H,^{1}H-COSY cross-peak CH_2(7)/CH_2(8). Finally, the structure of **1** was determined as shown in *Fig. 1* and named podocarpiamide.

Table 1. *^{1}H- and ^{13}C-NMR Data* (CD_3OD; 600 and 150 MHz, resp.) *of* **1**[1]). δ in ppm, *J* in Hz.

Position	δ(H)	δ(C)	Position	δ(H)	δ(C)
H–C(1)	7.07 (*d*, $J = 8.6$)	129.9 (*d*)	C(9)		171.6 (*s*)
H–C(2)	6.74 (*dd*, $J = 7.2$, 8.6)	114.8 (*d*)	Me(10)	1.98 (*s*)	19.4 (*q*)
H–C(3)	6.75 (*dd*, $J = 7.2$, 8.6)	114.9 (*d*)	CH_2(11)	3.50 (*s*)	39.5 (*t*)
H–C(4)	7.03 (*d*, $J = 8.6$)	129.5 (*d*)	C(12)		173.2 (*s*)
C(5)		128.6 (*s*)	Me(13)	3.63 (*s*)	51.1 (*q*)
C(6)		124.9 (*s*)	C(14)		155.6 (*s*)
CH_2(7)	2.80 (*t*, $J = 7.1$)	33.7 (*t*)	C(15)		156.1 (*s*)
CH_2(8)	4.17 (*s*)	65.2 (*t*)			

Compound **2** possessed a molecular formula $C_{11}H_{13}NO_2$, as deduced from the positive-mode HR-ESI-MS (*m*/*z* 214.08350 ($[M+Na]^+$, $C_{11}H_{13}NNaO_2^+$) with 6 degrees of unsaturation. The ^{13}C-NMR (DEPT) spectrum (*Table 2*) of **2** displayed the signals of three aromatic quaternary C-atoms and five aromatic CH, one MeO, and two CH_2 groups (including an oxygenated one). The ^{1}H-NMR spectrum showed five aromatic CH signals at δ(H) 7.56 (*d*, $J = 8.0$ Hz, 1 H), 7.38 (*d*, $J = 8.2$ Hz, 1 H), 7.18–7.21 (*m*,

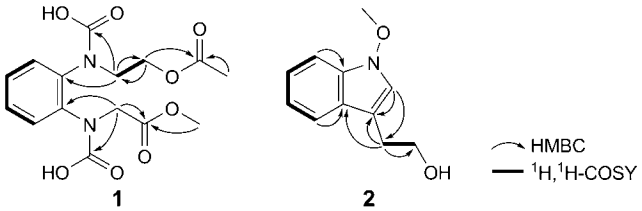


Fig. 2. *^{1}H,^{1}H-COSY and selected HMBC features of compounds* **1** *and* **2**

1 H), 7.24 (*s*), and 7.04–7.07 (*m*, 1 H), one MeO signal at δ(H) 4.05 (*s*), and two CH_2 signals at δ(H) 2.95 (*t*, $J = 7.1$ Hz) and 3.81 (*t*, $J = 7.1$ Hz). The ^{1}H- and ^{13}C-NMR data of **2** indicated that **2** was very similar to the known **3**, suggesting similar substitution patterns. The distinct difference between **2** and **3** was the δ(C) value of C(11) of **2** (δ(C) 61.9 (*t*)), which was absent in **3** (δ(C) 174.2 (*s*)), implying that the COOH group of **3** was reduced to a hydroxymethyl group in **2**. In addition, the chemical-shift value of C(10)) of **3** at δ(C) 31.9 (*t*) was shifted upfield to δ(C) 28.1 (*t*) in the case of **2**, due to the absence of the COOH group. The structure of **2** was further surpported by the key HMBC cross-peaks H–C(1)/C(2) and C(10), H–C(4)/C(3), H–C(7)/C(8), CH_2(10)/C(2), C(3), and C(11), and CH_2(11)/C(10), along with the ^{1}H,^{1}H-COSY cross-peak CH_2(10)/CH_2(11) (*Fig. 2*). Finally, the structure of **2** was established as shown in *Fig. 1* and named 1-methoxy-1*H*-indol-3-ethanol.

Table 2. *^{1}H- and ^{13}C-NMR Data* (CD_3OD; 600 and 150 MHz, resp.) *of* **2** *and* **3**[1]). δ in ppm, J in Hz

Position	**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	7.24 (*s*)	121.1 (*d*)	7.34 (*s*)	123.4 (*d*)
C(2)		108.7 (*s*)		106.2 (*s*)
C(3)		124.1 (*s*)		125.2 (*s*)
H–C(4)	7.56 (*d*, $J = 8.0$)	118.5 (*d*)	7.56 (*d*, $J = 8.0$)	120.1 (*d*)
H–C(5)	7.04–7.07 (*m*)	119.1 (*d*)	7.06–7.09 (*m*)	120.7 (*d*)
H–C(6)	7.18–7.21 (*m*)	121.0 (*d*)	7.20–7.22 (*m*)	123.4 (*d*)
H–C(7)	7.38 (*d*, $J = 8.2$)	107.7 (*d*)	7.40 (*d*, $J = 8.2$)	109.1 (*d*)
C(8)		132.6 (*s*)		133.8 (*s*)
Me(9)	4.05 (*s*)	64.4 (*q*)	4.06 (*s*)	66.1 (*q*)
CH_2(10)	2.95 (*t*, $J = 7.1$)	28.1 (*t*)	3.71 (*s*)	31.9 (*t*)
CH_2(11) or C(11)	3.81 (*t*, $J = 7.1$)	61.9 (*t*)		174.2 (*s*)

Comparison of the physicochemical properties with reported data allowed the identification of compound **3** and **4** as 1-methoxy-1*H*-indole-3-acetic acid and methyl 1-methoxy-1*H*-indole-3-acetate, respectively [12][13].

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 200–300 mesh; *Yantai ZhiFu Chemical Co. Ltd.*, P. R. China). TLC: silica gel *GF254* plates (SiO_2, *Yantai Zhi Fu Chemical Co. Ltd.*, P. R. China) and *Sephadex LH-20* gel (25–100 μm, *GE Healthcare Co. Ltd.*, Sweden). UV Spectra: *UV-210* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Perkin–Elmer*-577 spectrometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker-AM-600* spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. FT-MS: *Bruker*-A*pex-Ultra-7.0-T* spectrometer; in *m*/*z*.

Fungal Material and Cultivation Conditions. Pestalotiopsis podocarpi was isolated from the branch of *Podocarpus macrophyllus* in Hainan, P. R. China, in April 2008, identified by Prof. *Jing-Ze Zhang*, Institute of Biotechnology, Zhejiang University, and assigned the accession number L381 in the culture collection at the College of Life Science, Hebei University. The fungal strain was cultured on slants of potato dextrose agar (modified PDA) at 28° for 7 d, and then inoculated in 500 ml *Erlenmeyer* flasks containing 100 ml of modified PDA medium (glucose (20 g), potato (peeled; 200 g), KH_2PO_4 (3 g), $MgSO_4$ (1.5 g), citric acid (0.1 g), and thiamin hydrochloride (10 mg) in deionized H_2O (1.0 l)). The final pH of the media was adjusted to 6.5 before sterilization. After 7 d of incubation at 28° on rotary shakers at 150 rpm, 25 ml of culture liquid were transferred as seed into each 500 ml *Erlenmeyer* flask containing rice medium (200.0 g), and the fermentation was carried out on an incubator for 40 d.

Extraction and Isolation. The culture broth (30 l) was extracted three times with AcOEt (each time soaking for 2 d), and the org. layer was concentrated to yield a brown oily residue (60.0 g). This residue was subjected to CC (SiO_2, petroleum ether/AcOEt 100:0, 98:2, 95:5, 9:1, 6:1, 3:1, and 1:1): *Fractions 1–7. Fr. 3* (500 mg; eluted with petroleum ether/AcOEt 95:5) was repeatedly purified by CC (SiO_2, petroleum ether/acetone 50:1; and *Sephadex LH-20*, MeOH): **1** (8.0 mg; R_f (petroleum ether/AcOEt 30:1) 0.5). *Fr. 6* (3.0 g; eluted with petroleum ether/AcOEt 3:1) was repeatedly purified by CC (SiO_2, petroleum ether/acetone 5:1; *Sephadex LH-20*, MeOH): **3** (6 mg; R_f ($CHCl_3$/MeOH 60:1) 0.6), **2** (7 mg; R_f ($CHCl_3$/MeOH 60:1) 0.5), and **4** (15 mg; R_f ($CHCl_3$ /MeOH 60:1) 0.6), resp.

Podocarpiamide (= N-*{2-{[2-(Acetyloxy)ethyl]carboxyamino}phenyl}*-N-*carboxyglycine Methyl Ester*; **1**)*:* Yellow oil. UV (MeOH): 245 (3.10), 264 (4.36), 282 (3.72). IR (KBr): 3394, 1614, 1516, 1442, 707. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-HR-MS (pos.): 355.11436 ($[M+H]^+$, $C_{15}H_{19}N_2O_8^+$; calc. 355.11359).

*1-Methoxy-1*H-*indol-3-ethanol* (**2**)*:* Oil. UV (MeOH): 223 (4.50), 276 (3.36), 289 (3.79). IR (KBr): 3336, 2929, 1720, 1658, 1449, 772. ^{1}H- and ^{13}C-NMR: *Table 2*. ESI-HR-MS (pos.): 214.08350 ($[M+Na]^+$, $C_{11}H_{13}NNaO_2^+$; calc. 214.08385).

REFERENCES

[1] T. S. Suryanarayanan, G. Senthilarasu, V. Muruganandam, *Fungal Diversity* **2000**, *4*, 117.
[2] S. B. Toofanee, R. Dulymamode, *Fungal Diversity* **2002**, *11*, 169.
[3] J.-Y. Li, G. Strobel, R. Sidhu, W. M. Hess, E. J. Ford, *Microbiology* **1996**, *142*, 2223.
[4] G. Ding, L. Jiang, L. Guo, X. Chen, H. Zhang, Y. Che, *J. Nat. Prod.* **2008**, *71*, 1861.
[5] Y. H. Ye, H. L. Zhu, Y. C. Song, J. Y. Liu, R. X. Tan, *J. Nat. Prod.* **2005**, *68*, 1106.
[6] Y. Zhang, T. Zhu, Y. Fang, H. Liu, Q. Gu, W. Zhu, *J. Antibiot.* **2007**, *60*, 153.
[7] X.-L.Yang, J.-Z. Zhang, D.-Q. Luo, *Nat. Prod. Rep.* **2012**, *29*, 622.
[8] D.-Q. Luo, H.-Y. Deng, X.-L.Yang, B.-Z. Shi, J.-Z. Zhang, *Helv. Chim. Acta* **2011**, *94*, 1041.
[9] X.-L. Yang, S. Zhang, H-J. Zhu, D.-Q. Luo, *Molecules* **2011**, *16*, 1910.
[10] X.-L.Yang, S. Zhang, H.-J. Zhu, D.-Q. Luo, X.-Y. Gao, *Helv. Chim. Acta* **2011**, *94*, 1463.
[11] X.-L.Yang, S. Zhang, Q.-B. Hu, D.-Q. Luo, Y. Zhang, *J. Antibiot.* **2011**, *64*, 723.
[12] T. Kawasaki, A. Kodama, T. Nishida, K. Shimizu, M. Somei, *Heterocycles* **1991**, *32*, 221.
[13] M. Somei, T. Kawasaki, K. Shimizu,Y. Fukui, T. Ohta, *Chem. Pharm. Bull.* **1991**, *39*, 1905.

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