The Journal of Antibiotics (2012) 65, 143–145

www.nature.com/ja

NOTE

Cytochalasans with different amino-acid origin from the plant endophytic fungus *Trichoderma gamsii*

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The Journal of Antibiotics (2012) **65,** 143–145; doi:10.1038/ja.2011.124; published online 11 January 2012

Keywords: cytochalasans; cytotoxicity; endophytes; structure elucidation; *Trichoderma gamsii*

Endophytes, as members of special niche of fungi inhabiting unique environments, namely, the normal plant tissues or organs, have acquired genetic and species diversities, which could transform into chemo-structural and bioactive diversities.[1] Thus, endophytes are considered an outstanding source of bioactive natural products, and many different secondary metabolites with diverse structural features have been isolated from endophytes.[1–6] During an ongoing search for new bioactive secondary metabolites from endophytic fungi, the extract of one endophytic fungus *Trichoderma gamsii* inhabiting the traditional Chinese medicinal plant *Panax notoginseng* (BurK.) F.H.Chen displayed cytotoxic activity against HeLa cells. Bioactivity-guided separation of this extract, resulted in two new cytochalasans, trichalasin A (**1**) originated from valine, and trichalasin B (**2**) together with aspochalasins I (**3**)[7] and J (**4**)[7] derived from leucine (Figure 1). In this note, we present the isolation, structural elucidation and bioactivity of these compounds.

The culture of *T. gamsii* was isolated from traditional Chinese medicinal plant *P. notoginseng* (BurK.) F.H.Chen. The isolate was identified based on the sequence (Genbank Accession No. JF964996) obtained the by analysis of ITS region of the rDNA. The fungal strain was cultured on the slants of potato dextrose agar at 25 °C for 10 days. The agar plugs were used to inoculate 250-ml Erlenmeyer flasks, each containing 40 ml of media (0.4% glucose, 1% malt extract and 0.4% yeast extract), and the final pH of the media was adjusted to 6.5 before sterilization. Flask cultures were incubated at 25 °C on a rotary shaker at 170 r.p.m. for 5 days. Fermentation was carried out in Fernbach flasks (500 ml) each containing 80 g of rice. Spore inoculum was prepared by suspension in sterile, distilled H_2O to give a final spore/cell suspension of 1×10^6 per ml. Distilled H_2O (100 ml) was added to each flask, and the contents were soaked overnight before autoclaving at 15 lb in^{-2} for 30 min. After cooling to room temperature, each flask was inoculated with 5 ml of the spore inoculum and incubated at 25 °C for 40 days.

The fermented material was extracted with ethyl acetate (20 l), and the organic solvent was evaporated to dryness under vacuum to afford a crude extract (100 g), which was fractionated by silica gel column chromatography (10×100 cm) using CH_2Cl_2–CH_3OH gradient elution. The fraction (0.8 g) which eluted with 100:2 CH_2Cl_2–CH_3OH was separated by column chromatography, and further purification by reversed-phase HPLC (Lumtech, Berlin, Germany; YMC-Pack ODS-A column; 10 μm; 250×10 mm; 2 ml min^{-1}, 75% MeOH in H_2O for 5 min, and followed by 75–90% for 60 min) afforded trichalasin A (**1**; 1.5 mg, t_R 21.5 min) and aspochalasin J (**4**; 5 mg, t_R 29.3 min). Other fraction (0.3 g) eluted with 100:2 CH_2Cl_2–CH_3OH was separated by Sephadex LH-20 CC repeatedly to obtain trichalasin B (**2**; 2 mg). One fraction eluted with 100:8 CH_2Cl_2–CH_3OH was separated by Sephadex LH-20 and reversed-phase HPLC (Lumtech; YMC-Pack ODS-A column; 10 μm; 250×10 mm; 2 ml min^{-1}, 70% MeOH in H_2O for 5 min, and followed by 70–85% for 60 min) to give aspochalasin I (**3**; 5 mg, t_R 35.9 min). UV data were recorded on a Shimadzu UV-2550 UV-vis spectrophotometer (Shimadzu, Kyoto, Japan). IR data were recorded using a Shimadzu FTIR-8400S spectrophotometer. ^{1}H and ^{13}C NMR data were acquired with a Bruker 600 (Bruker, Billerica, MA, USA) and Varian Inova 600 spectrometer (Varian Inova, Salt Lake City, UT, USA) using solvent signals ($CDCl_3$; δ_H 7.26/δ_C 77.6, DMSO-d_6; δ_H 2.49/δ_C 39.5) as references. The HMQC and HMBC experiments were optimized for 145 and 8 Hz, respectively. HRESIMS data were acquired using a LTQ Orbitrap XL mass spectrometer (Thermo, Waltham, MA, USA).

Trichalasin A (**1**) was isolated as a white amorphous powder with UV (CH_3OH) max (log ε): 216 (1.70), 234 nm (0.53). The IR spectrum of **1** displayed absorption bands at 3390 (br, -OH and *N*H) and 1705 (br, C=O). The molecular formula of trichalasin A (**1**) was established as $C_{23}H_{33}NO_4$ on the basis of HRESI MS (*m/z* 410.2297 $[M + Na]^+$; Δ + 0.1 mmu). Analysis of the ^{1}H, ^{13}C, and

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Received 27 October 2011; revised 23 November 2011; accepted 29 November 2011; published online 11 January 2012

HMQC NMR data of **1** (Table 1) revealed the presence of five methyl groups, three methylene units, six methines (one oxygenated), one oxygenated quaternary carbon, six olefinic carbons (four of which are protonated) and two carbonyl groups. These data accounted for all the ^{1}H and ^{13}C NMR resonances (Supplementary Figures S1 and S2) and required **1** to be a tricyclic secondary metabolite. Interpretation of the ^{1}H–^{1}H COSY NMR data of **1** identified three isolated proton spin-systems corresponding to the C-15–C-16–C-17–C-18(18-OH)–

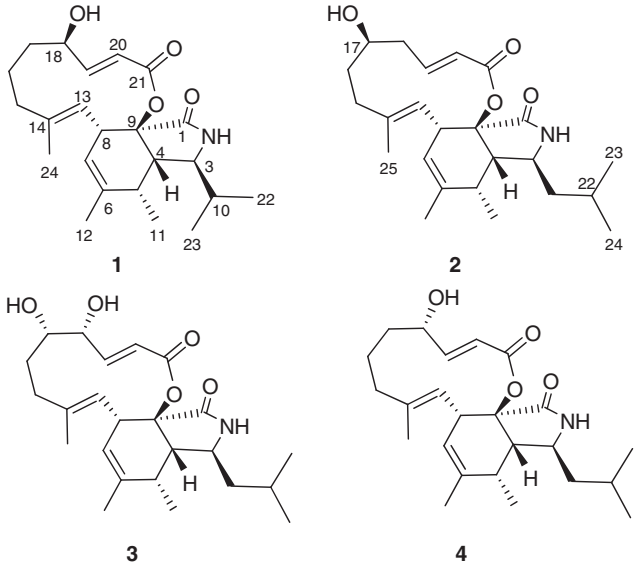

Figure 1 Structures for compounds **1**–**4**.

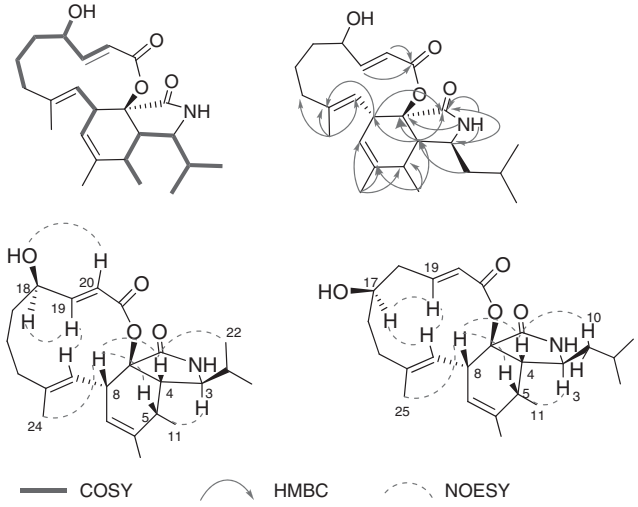

Figure 2 ^{1}H–^{1}H COSY, HMBC and NOESY for compounds **1** and **2**. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

Table 1 NMR data for Compounds 1 (in DMSO-d_6) and 2 (in $CDCl_3$)

	*Trichalasin A (**1**)*		*Trichalasin B (**2**)*	
Pos	δ_H[a] *(J in Hz)*	δ_C[b], *multiple*	δ_H[a] *(J in Hz)*	δ_C[b], *multiple*
1		172.2, s		173.3, s
2-*N*H-	8.19, s	—	6.07, s	
3	2.90, br.t (2.7)	58.2, d	3.13, dt (3.6, 10.2)	52.1, d
4	2.83, dd (2.7, 4.8)	47.3, d	2.90, dd (3.6, 4.8)	52.2, d
5	2.91, m	33.8, d	3.04, br.s	34.4, d
6	—	139.8, s	—	140.4, s
7	5.18, br.s	123.9, d	5.30, br.s	124.3, d
8	3.70, d (10.8)	39.0, d	3.75, d (10.2)	40.1, d
9	—	87.9, q	—	88.3, q
10	1.55, m	33.2, d	1.55, m; 1.30 m	48.5, t
11	1.15, d (7.2)	13.6, q	1.21, d (7.2)	14.0, q
12	1.68, s	19.4, q	1.73. s	19.9, q
13	6.22, d (10.8)	122.9, d	6.22, d (11.4)	123.6, d
14	—	138.2, s	—	138.5, q
15a15b	1.85, dd (9.0, 13.2) 2.16, t (13.2)	42.1, t	2.25, m; 2.35, m	39.5, t
16a 16b	1.38, m; 1.55, m	17.3, t	1.78, m	36.2, t
17a 17b	1.55, m 1.99, m	38.1, t	3.88, m	75.5, d
18	4.36, br.s	67.2, d	2.20, m; 2.70m	42.8, d
19	7.17, dd (1.8, 15.0)	158.2, d	7.17, ddd (4.2, 10.8, 15.0)	150.4, d
20	5.73, dd (1.8, 15.0)	117.9, d	5.73, dd (1.2, 15.0)	122.8, d
21	—	167.2, s	—	167.7
22	0.75, d (6.6)	16.4, q	1.60, m	25.4, d
23	0.85, d (6.6)	19.1, q	0.93, d (6.6)	23.8, q
24	1.31, s	15.6, q	0.92, d (6.6)	21.5, q
25			1.43, s	15.3, q
18-OH	5.02, d (4.2)	—	—	—

[a]Recorded at 600 MHz.
[b]Recorded at 150 MHz.

C-19–C-20, C-11–C-5–C-4–C-3–C-10–C-22/C-23, and C-7–C-8–C-13 fragments (Figure 2), and the remaining connection was completely established by Heteronuclear multiple-bond correlations (Figure 2). The correlations from H-3 to C-1, H-4 to C-1 and C-9, and 2-*N*H- to C-1, C-3, C-4 and C-9 established a pyrrolidin-2-one ring system with one isobutyl connected at C-3. Those correlations between 12-Me with C-5, C-6 and C-7 indicated that C-6 were connected with C-5, C-7 and C-12, and those from H-4 to C-8 and C-9 completed one cyclohexene ring fused to the pyrrolidin-2-one at C-4 and C-9. The cross-peaks from 24-Me to C-13, C-14 and C-15 confirmed that C-14 was connected with C-13, C-15 and C-24. Accounting for the chemical shift value for C-9, and unsaturation degree, the remaining oxygen atom was met by the ester linkage formed between C-9 and C-21. Thus, the planar structure for **1** was characterized.

The relative configuration was determined by NOESY correlations. The correlations between H-4 and Me-22 indicated that those protons were on the same side of pyrrolidin-2-one ring. The cross-peaks from H-8 to H-4 and H-5 put these protons on the same orientation on the cyclohexene ring system. Those correlations of 18-OH with H-20, H-13 with H-18 and H-19, H-8 with Me-24, and H-3 with Me-11 revealed that those protons were close to each other in space. Thus, the relative configuration of **1** was determined, which displayed that **1** possessed similar relative configuration as compound **4** except that the 18-hydroxyl group in **1** was β instead of α-configuration present in **4** (Figure 2).

Trichalasin B (**2**) was isolated as a brown gum with UV (CH_3OH) maxim (log ε): 216 (1.71), and 234 nm (0.53). The IR spectrum of **2** displayed similar absorption wavelength with those of **1**. Compound **2** gave a molecular formula of $C_{24}H_{35}NO_4$ by analysis of its HRESIMS (*m*/*z* 402.2639 $[M + 1]^+$; Δ −0.6 mmu) same as that of **4**. The NMR spectra of **2** (Supplementary Figures S3 and S4) was nearly the same as those of **4** except that the H-20 (dd, *J*=15.3, 2.6) in **4** was changed to be three doublets (ddd, *J*=15, 10.8, 4.2) in **2** implying that C-21 must be connected to one methylene instead of the oxymethine unit as in **4**. Analysis of ^1H–^1H COSY and HMBC confirmed that the 18-hydroxyl group in **4** was replaced by the one at C-17 in **2**. Thus, the planar structure of **2** was established. The relative configuration for **2** was determined by NOESY correlations revealing the same configuration as that of **4** except that the 17-hydroxyl group possessed β-configuration in **2** (Figure 2).

The absolute configurations for **1** and **2** were tentatively postulated to be same as that of **3** and **4** considering the congeneric biosynthesis.

Compounds **1**–**4** were evaluated for cytotoxic activity against the HeLa cancer cell line with only aspochalasin J (**4**) displaying weak inhibitory activity with IC_{50} value 27.8 μM, whereas the IC_{50} values for compounds **1**–**3** were more than 40 μM.

This is the first report in which cytochalasans have been found biosynthesized from different amino acid by the same fungus. Thus, further supporting the thesis that endophytes, as fungi from a unique environment, can produce diverse secondary metabolites with a wide range of bioactivities.

ACKNOWLEDGEMENTS

We gratefully acknowledge financial support from the National Natural Science Foundation of China (31000011), and the Chinese National S&T Special Project on Major New Drug Innovation (2011ZX09307-002-01).

1 Zhang, H. W., Song, Y. C. & Tan, R. X. Biology and chemistry of endophytes. *Nat. Prod. Rep.* **23,** 753–771 (2006).
2 Ding, G. *et al.* Chaetoglobosin U, a cytochalasan alkaloid from endophytic *Chaetomium globosum* IFB-E019. *J. Nat. Prod.* **69,** 302–304 (2006).
3 Ding, G. *et al.* Antifungal metabolites from the plant endophytic fungus *Pestalotiopsis foedan*. *J. Nat. Prod.* **71,** 615–618 (2008).
4 Ge, H. M. *et al.* Chaetoglobins A and B, two unusual alkaloids from endophytic *Chaetomium globosum* culture. *Chem. Commun.* 5978–5980 (2008).
5 Ding, G. *et al.* Photinides A-F, cytotoxic benzofuranone-derived γ-lactones from the plant endophytic fungus *Pestalotiopsis photiniae*. *J. Nat. Prod.* **72,** 942–945 (2009).
6 Ding, G. *et al.* Pestaloquinols A and B, isoprenylated epoxyquinols from *Pestalotiopsis* sp. *J. Nat. Prod.* **74,** 286–291 (2011).
7 Zhou, G. X. *et al.* Aspochalasins I, J, and K: three new cytotoxic cytochalasans of *Aspergillus flavipes* from the Rhizosphere of *Ericameria laricifolia* of the Sonoran desert. *J. Nat. Prod.* **67,** 328–332 (2004).

Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)