The Journal of Antibiotics (2014) 67, 593–595

www.nature.com/ja

NOTE

# Terretonin G, a new sesterterpenoid antibiotic from marine-derived *Aspergillus* sp. OPMF00272

Takashi Fukuda[1], Yuko Kurihara[2], Akihiko Kanamoto[2] and Hiroshi Tomoda[1]

*The Journal of Antibiotics* (2014) **67,** 593–595; doi:10.1038/ja.2014.46; published online 7 May 2014

Marine-derived microorganisms are known as producers of many structurally unique and strong-bioactive compounds, including clinical medicines and research reagents.[1–4] During our screening for new metabolites from marine-derived fungi, one new sesterterpenoid, named terretonin G (**1**), was isolated from the culture broth of *Aspergillus* sp. OPMF00272 along with eight known natural products, terretonin (**2**),[5–7] LL-S490β (**3**),[8] methyl-3,4,5-trimethoxyl-2-[2-(nicotinamide)benzamido]benzoate (**4**),[9] territrem B(**5**),[10] aspernolid A (**6**),[11] butyrolactones I (**7**)[12] and V (**8**),[13] and aspulvinone J (**9**)[14] (Figure 1). This study describes the fermentation, isolation, structural elucidation and biological activity of **1**.

The fungus *Aspergillus* sp. OPMF00272 was isolated from poriferan collected on Ishigaki island in Okinawa, Japan, in 2008. The strain was inoculated into a 500-ml Erlenmeyer flask containing 100 ml seed medium (2.0% glucose, 0.2% yeast extract, 0.05% $MgSO_4 \cdot 7H_2O$, 0.5% polypeptone, 0.1% $KH_2PO_4$ and 0.1% agar, pH 6.0). The flask was shaken on a rotary shaker at 27 °C for 3 days. The seed culture (2.0 ml) was transferred in a 1000-ml culture box containing 150 ml production medium (5% oat meal, 0.2% yeast extract, 0.1% Na tartrate, 0.1% $KH_2PO_4$, 0.8% Daigo authentic seawater, Nihon Pharmaceutical Co., Ltd., Tokyo, Japan). The fermentation was carried out at 27 °C for 7 days under static conditions. The culture broth (150 ml × 3) was extracted with ethanol (450 ml) for 2 h. After this extract had been evaporated to an aqueous solution, the residue was partitioned between water and EtOAc to yield the crude extract (684 mg) after evaporation of the EtOAc fraction. This extract was dissolved in a small volume of chloroform, applied to a silica gel column (40 g, 3.4 × 10 cm, 0.04–0.063 mm; Merck, Darmstadt, Germany), and eluted stepwise with 100% chloroform, 50:1, 10:1, 5:1 and 1:1 (v/v) chloroform–methanol and 100% methanol (300 ml each). Terretonin G was observed in the fraction eluted with 50:1 chloroform–methanol. This fraction was further purified by reversed-phase C-18 HPLC (20 × 250 mm; PEGASIL ODS, Senshu Scientific Co., Tokyo, Japan) under the following conditions: solvent, 53% $CH_3CN$ water isocratic condition; flow rate of 8.0 ml $min^{-1}$; UV detection at 210 nm. Under these conditions, terretonin G was eluted as a peak with a retention time of 20.1 min. This fraction was collected and concentrated to yield pure terretonin G (1.3 mg) as a colorless solid.

The physico-chemical properties of terretonin G (**1**) are summarized in Table 1. The molecular formula of **1** was established as $C_{27}H_{38}O_9$ ($[M+Na]^+$ *m/z* 529.2411) on the basis of HI-ESI-MS measurement, indicating that terretonin G contained nine degrees of unsaturation. The IR spectrum of **1** showed characteristic absorption at 3467, 1738 and 1713 $cm^{-1}$, suggesting the presence of hydoxy and carbonyl moieties. The structure of terretonin G (**1**) was mainly elucidated by analysis of NMR spectra including 2D NMR. The $^{13}C$ NMR spectrum (in $CD_3OD$) showed 27 resolved signals, which were classified into eight $sp^3$ methyl carbons, three $sp^3$ methylene carbons, one $sp^2$ methylene carbon, four $sp^3$ methine carbons and 11 quaternary carbons including four carbonyl carbons (C-3, C-6, C-16 and C-18) (Table 2). Analysis of the $^1H$–$^1H$ COSY spectra revealed two partial structures C-1 to C-2 and C-9 to C-11 (Figure 2). The $^{13}C$–$^1H$ long range couplings of 2*J* and 3*J* observed in the $^{13}C$–$^1H$ HMBC experiments (Figure 2) gave the following information: (1) The cross peaks from 1-$H_2$ (δ 1.75, 2.20) to C-3 (δ 215.9), C-5 (δ 63.8) and C-10 (δ 44.5), from 2-$H_2$ (δ 2.23, 2.85) to C-3, from 5-H (δ 2.81) to C-4 (δ 47.8), C-6 (δ 210.4), C-9 (δ 54.4), C-10, C-19 (δ 22.6), C-20 (δ 24.5) and C-22 (δ 16.3), from 7-H (δ 4.12) to C-6, C-8 (δ 50.9), C-14 (δ 57.6) and C-21 (δ 12.4), from 9-H (δ 2.05) to C-8 and C-10, from 11-$H_2$ (δ 2.45, 2.61) to C-8, C-12 (δ 150.7), C-13 (δ 58.4) and C-23 (δ 111.5), from 14-H (δ 3.75) to C-7 (δ 85.9), C-8, C-9, C-12, C-13, C-16 (δ 209.7), C-21 and C-24 (δ 22.6), from 19-$H_3$ (δ 1.50) to C-3, C-4, C-5 and C-20, from 20-$H_3$ (δ 1.07) to C-3, C-4, C-5 and C-19, from 21-$H_3$ (δ 1.17) to C-7, C-8, C-9 and C-14, from 22-$H_3$ (δ 1.15) to C-1, C-5, C-9 and C-10, from 23-$H_2$ (δ 4.32, 4.81) to C-11(δ 29.1), C-12 and C-13, and from 24-$H_3$ (δ 1.52) to C-12, C-13 and C-14 supported the partial structure I. (2) The cross peaks from 25-$H_3$ (δ 1.58) to C-16, C-17 (δ 83.3) and C-18 (δ 174.5) and from 18-$OCH_3$ (δ 3.73) to C-18 supported the partial structure II. (3) The cross peak from 15-$OCH_3$ (δ 3.52) to C-15 (δ 173.0) supported the partial structure III. (4) The cross peaks from 14-H to C-15 and from 24-$H_3$ to C-16 indicated that the partial structures I, II and III are connected as shown in Figure 2. Taking into consideration the

[1]Graduate School of Pharmaceutical Sciences, Kitasato University, Tokyo, Japan and [2]OP BIO FACTORY Co., Ltd., Uruma Sandpit, Okinawa, Japan
Correspondence: Professor H Tomoda, Graduate School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan.
E-mail: tomodah@pharm.kitasato-u.ac.jp
Received 23 October 2013; revised 22 March 2014; accepted 1 April 2014; published online 7 May 2014

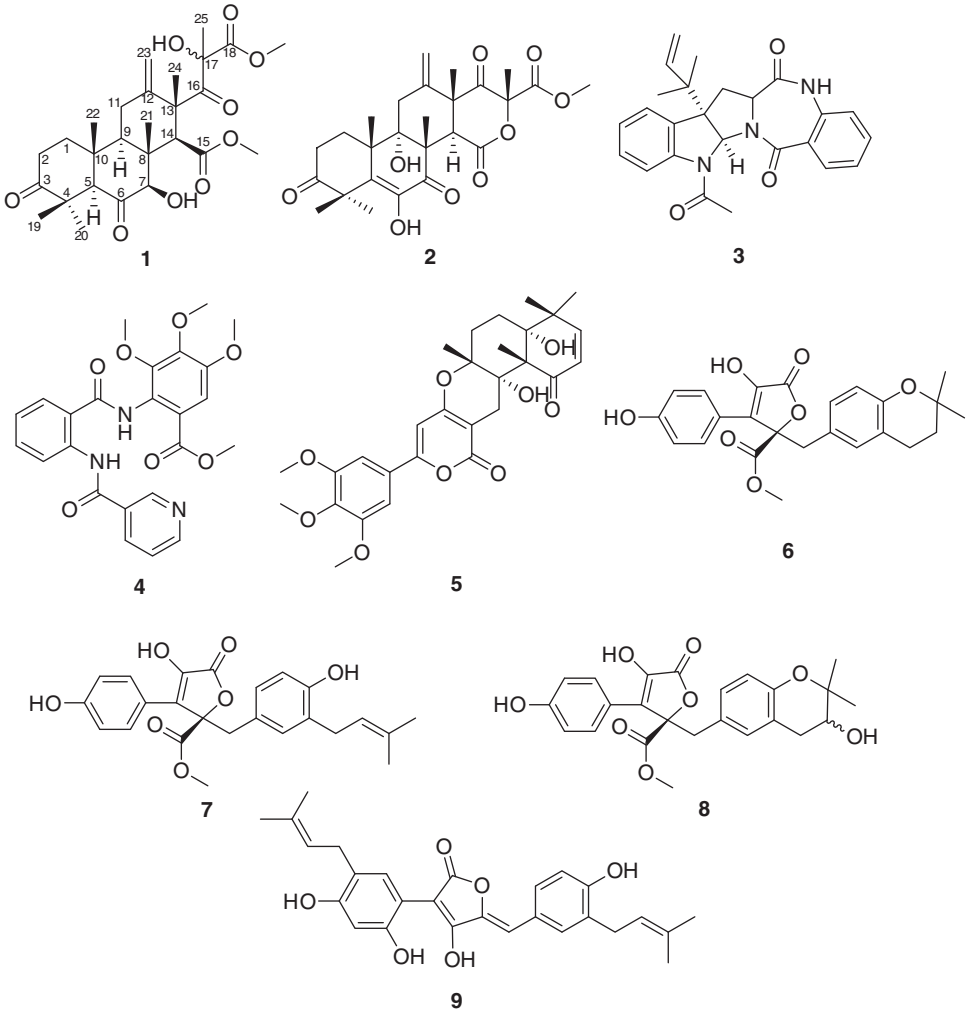

**Figure 1** Structures of nine compounds produced by *Aspergillus* sp. OPMF00272. Terretonin G (**1**), terretonin (**2**), LL-S490β (**3**), methyl-3,4,5-trimethoxyl-2-[2-(nicotinamide)benzamido]benzoate (**4**), territrem B (**5**), aspernolid A (**6**), butyrolactones I (**7**) and V (**8**), and aspulvinone J (**9**).

**Table 1 Physico-chemical properties of terretonin G**

| | *Terretonin G* |
|---|---|
| Appearance | Colorless solid |
| $[\alpha]_D^{26}$ | 4.2 (*c* 0.1, MeOH) |
| Molecular formula | $C_{27}H_{38}O_9$ |
| Molecular weight | 506 |
| HR ESI MS *m/z* | $[M+Na]^+$ |
| Calcd | 529.2413 (for $C_{27}H_{38}O_9Na$) |
| Found | 529.2411 |
| UV (MeOH) | End absorption |
| IR $\nu_{max}^{KBr}$ ($cm^{-1}$) | 3467, 1738, 1713 |

molecular formula, the IR data and chemical shifts of C-7 (δ 85.9) and C-17 (δ 83.3), a hydroxy moiety should be bound to both C-7 and C-17. Thus, the planar structure of terretonin G was elucidated as shown in Figure 1.

The relative configurations of C-5, C-7, C-8, C-9, C-10, C-13 and C-14 were determined by NOE experiments. Observation of NOEs from 5-H to 7-H, 9-H and 20-$H_3$, from 9-H to 14-H, from 22-$H_3$ to 19-$H_3$ and 21-$H_3$, and from 21-$H_3$ to 24-$H_3$ (Figure 3) indicated that they were assigned as 5*R**, 7*R**, 8*R**, 9*R**, 10*R**, 13*R** and 14*S**. Stereochemistry of C-17, however, was not determined by NOE experiments.

The antimicrobial activity of **1** and **2** was investigated using our routine in-house assay system.[15] From the antimicrobial assay using paper disk, **1** (20 μg per 6 mm disk) showed antimicrobial activity with an inhibitory zone (10, 8 and 8 mm) against Gram-positive bacteria (*Staphylococcus aureus* FDA209P, *Bacillus subtillis* PCI219 and *Micrococus luteus* ATCC9341), but not against Gram-negative bacteria (*Pseudomonas aeruginosa* IFO12689 and *Escherichia coli* JM109) and fungi (*Candida albicans* ATCC64548 and *Saccharomyces cerevisiae* S288c). Interestingly, **2** (20 μg per 6 mm disk) showed no inhibitory activity against all microorganisms we tested.

Terretonin and its structurally related terretonins A–F have been reported; terretonin was reported as a mycotoxin[5] and terretonins E and F were reported as inhibitors of the mammalian mitochondrial respiratory chain.[7] Unfortunately, there has been no report about the biological activity of terretonins A–D.[6] Accordingly, other biological activities of **1** in our in-house assays such as effect on cell cycle,[16] lipid metabolites[17] and alkaline phosphatase expression in myoblasts[18] were investigated. Unfortunately, **1** showed no

**Table 2 NMR spectroscopic data for terretonin G in $CD_3OD$**

| Position | $\delta_H$[a] | $\delta_C$[b] | HMBC |
|---|---|---|---|
| 1 | 1.75, m<br>2.20, m | 40.5 | C-2, 3, 5, 10 |
| 2 | 2.23, m<br>2.85, m | 34.6 | C-1, 3 |
| 3 | | 215.9 | |
| 4 | | 47.8 | |
| 5 | 2.81, s | 63.8 | C-4, 6, 9, 10, 19, 20, 22 |
| 6 | | 210.4 | |
| 7 | 4.12, s | 85.9 | C-6, 8, 14, 21 |
| 8 | | 50.9 | |
| 9 | 2.05, dd ($J$=14.0, 3.0) | 54.4 | C-8, 10 |
| 10 | | 44.5 | |
| 11 | 2.45, dd ($J$=15.0, 3.0)<br>2.61, m | 29.1 | C-8, 9, 12, 13, 23 |
| 12 | | 150.7 | |
| 13 | | 58.4 | |
| 14 | 3.75, s | 57.6 | C-7, 8, 9, 12, 13, 15, 16, 21, 24 |
| 15 | | 173.0 | |
| 16 | | 209.7 | |
| 17 | | 83.3 | |
| 18 | | 174.5 | |
| 19 | 1.50, s | 22.6 | C-3, 4, 5, 20 |
| 20 | 1.07, s | 24.5 | C-3, 4, 5, 19 |
| 21 | 1.17, s | 12.4 | C-7, 8, 9, 14 |
| 22 | 1.15, s | 16.3 | C-1, 5, 9, 10 |
| 23 | 4.32, d ($J$=2.0)<br>4.81, d ($J$=2.0) | 111.5 | C-11, 12, 13 |
| 24 | 1.52, s | 22.6 | C-12, 13, 14, 16 |
| 25 | 1.58, s | 27.0 | C-16, 17, 18 |
| 15-$OCH_3$ | 3.52, s | 51.7 | C-15 |
| 18-$OCH_3$ | 3.73, s | 53.6 | C-18 |

[a]Chemical shifts are shown with reference to $CD_3OD$ as δ 3.30.
[b]Chemical shifts are shown with reference to $CD_3OD$ as δ 49.0.

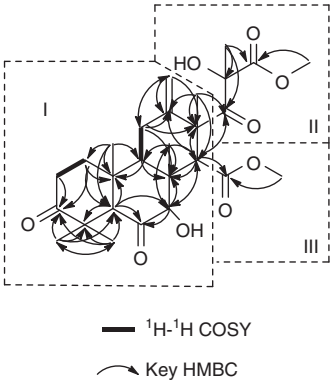


**Figure 2** Key cross peaks observed in $^1$H–$^1$H COSY and $^{13}$C–$^1$H HMBC experiments of **1**.

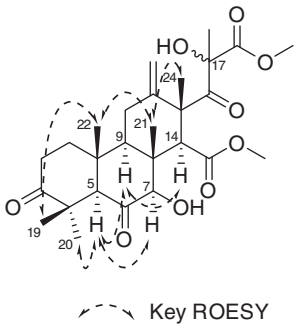


**Figure 3** NOE experiments of **1**.

significant activity in these assays even at 25 μg ml$^{-1}$ (data not shown). To our knowledge, this is the first report that terretonins showed antimicrobial activity.

## ACKNOWLEDGEMENTS

We wish to thank Ms Nozomi Sasaki for excellent assistance throughout this work, and Ms Noriko Sato and Dr Kenichiro Nagai (School of Pharmaceutical Sciences, Kitasato University) for measurements of NMR spectra and MS data. This work was supported by JSPS KAKENHI Grant Number 25870704.

1 Blunt, J. W., Copp, B. R., Keyzers, R. A., Munro, M. H. G. & Prinsep, M. R. Marine natural products. *Nat. Prod. Rep.* **30,** 237–323 (2013).
2 Fenical, W. & Jensen, P. R. Developing a new resource for drug discovery: marine actinomycete bacteria. *Nat. Chem. Biol.* **2,** 666–673 (2006).
3 Feling, R. H. *et al.* Salinosporamide A: a highly cytotoxic proteasome inhibitor from a novel microbial source, a marine bacterium of the new genus *Salinospora*. *Angew. Chem. Int. Ed. Engl.* **42,** 355–357 (2003).
4 Fukuda, T. *et al.* Structures and biosynthesis of the pyridinopyrones, polyenepyrones from a marine-derived *Streptomyces* Species. *J. Nat. Prod.* **74,** 1773–1778 (2011).
5 Springer, J. P., Dorner, J. W., Cole, R. J. & Cox, R. H. Terretonin, a toxic compound from *Aspergillus terreus*. *J. Org. Chem.* **44,** 4852–4854 (1979).
6 Li, G. Y. *et al.* Sesterterpenoids, terretonins A-D, and an alkaloid, asterrelenin, from *Aspergillus terreus*. *J. Nat. Prod.* **68,** 1243–1246 (2005).
7 López-Gresa, M. P. *et al.* Terretonins E and F, inhibitors of the mitochondrial respiratory chain from the marine-derived fungus *Aspergillus insuetus*. *J. Nat. Prod.* **72,** 1348–1351 (2009).
8 He, J. *et al.* Cytotoxic and other metabolites of *Aspergillus* inhabiting the rhizosphere of Sonoran desert plants. *J. Nat. Prod.* **67,** 1985–1991 (2004).
9 Wang, Y., Zheng, J., Liu, P., Wang, W. & Zhu, W. Three new compounds from *Aspergillus terreus* PT06-2 grown in a high salt medium. *Mar. Drugs* **9,** 1368–1378 (2011).
10 Ling, K. H., Yang, C. K., Kuo, C. A. & Kuo, M. D. Solvent systems for improved isolation and separation of territrems A and B. *Appl. Environ. Microbiol.* **44,** 860–863 (1982).
11 Parvatkar, R. R., D'Souza, C., Tripathi, A. & Naik, C. G. Aspernolides A and B, butenolides from a marine-derived fungus *Aspergillus terreus*. *Phytochemistry* **70,** 128–132 (2009).
12 Kitagawa, M. *et al.* Butyrolactone I, a selective inhibitor of cdk2 and cdc2 kinase. *Oncogene* **8,** 2425–2432 (1993).
13 Lin, T., Lu, C. & Shen, Y. Secondary metabolites of *Aspergillus* sp. F1, a commensal fungal strain of *Trewia nudiflora*. *Nat. Prod. Res.* **23,** 77–85 (2009).
14 Haug-Schifferdecker, E., Arican, D., Brückner, R. & Heide, L. A new group of aromatic prenyltransferases in fungi, catalyzing a 2,7-dihydroxynaphthalene 3-dimethylallyl-transferase reaction. *J. Biol. Chem.* **285,** 16487–16494 (2010).
15 Iwatsuki, M. *et al.* Lariatins, novel anti-mycobacterial peptides with a lasso structure, produced by *Rhodococcus jostii* K01-B0171. *J. Antibiot.* **60,** 357–363 (2007).
16 Hagimori, K., Fukuda, T., Hasegawa, Y., Omura, S. & Tomoda, H. Fungal malformins inhibit bleomycin-induced G2 checkpoint in Jurkat cells. *Biol. Pharm. Bull.* **30,** 1379–1383 (2007).
17 Ohshiro, T. & Tomoda, H. Isoform-specific inhibitors of ACATs: recent advances and promising developments. *Future Med. Chem.* **3,** 2039–2061 (2011).
18 Fukuda, T. *et al.* Trichocyalides A and B, new inhibitors of alkaline phosphatase activity in bone morphogenetic protein-stimulated myoblasts, produced by *Trichoderma* sp. FKI-5513. *J. Antibiot. (Tokyo)* **65,** 565–569 (2012).