

Article

## Chemical Constituents of a Marine-Derived Endophytic Fungus *Penicillium commune* G2M

Hui-Jiao Yan<sup>1,2</sup>, Shu-Shan Gao<sup>1,2</sup>, Chun-Shun Li<sup>1</sup>, Xiao-Ming Li<sup>1</sup> and Bin-Gui Wang<sup>1,\*</sup>

<sup>1</sup> Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China; E-Mails: yanhuijiao@yahoo.com.cn (H.-J.Y.); xisea01@126.com (S.-S.G.); lichunshun@ms.qdio.ac.cn (C.-S.L.); lixmqd@yahoo.com.cn (X.-M.L.)

<sup>2</sup> Graduate School of the Chinese Academy of Sciences, Beijing 100049, China

\* Author to whom correspondence should be addressed; E-Mail: wangbg@ms.qdio.ac.cn; Tel.: +86-532-82898553; Fax: +86-532-82880645.

Received: 30 November 2009; in revised form: 8 April 2010 / Accepted: 20 April 2010 /

Published: 4 May 2010

---

**Abstract:** Cultivation of the endophytic fungus *Penicillium commune*, which was isolated from the semi-mangrove plant *Hibiscus tiliaceus*, afforded one new compound 1-*O*-(2,4-dihydroxy-6-methylbenzoyl)-glycerol (**1**) along with thirteen known products, including 1-*O*-acetyl-glycerol (**2**), *N*-acetyltryptophan (**3**), 3-indolylacetic acid methyl ester (**4**), 1-(2,4-dihydroxy-3,5-dimethylphenyl)ethanone (**5**), 2-(2,5-dihydroxyphenyl)acetic acid (**6**), (4*R*,5*S*)-5-hydroxyhexan-4-olide (**7**), thymidine (**8**), uracil (**9**), thymine (**10**), ergosterol (**11**),  $\beta$ -sitosterol (**12**),  $\beta$ -daucosterol (**13**), and ergosta-7,22-dien-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol (**14**). The structures of these compounds were established by detailed NMR spectroscopic analysis, as well as by comparison with literature data or with authentic samples.

**Keywords:** chemical constituents; endophytic fungus; *Hibiscus tiliaceus*; *Penicillium commune*

---

### 1. Introduction

Mangrove and semi-mangrove plants are mostly distributed in the tropical and subtropical coastal regions of the world. Because of their special ecosystems that straddle the land and the sea, mangrove

plants are found to be a rich source of mutualistic fungal endophyte microorganism species [1–2]. Our previous investigations on the mangrove-derived fungi *Eurotium rubrum* have led to the isolation of a variety of structurally unique metabolites with different biological activities [3–5]. During our ongoing search for structurally interesting metabolites from mangrove-derived fungi, a strain of *Penicillium commune* G2M, which was isolated from the semi-mangrove plant *Hibiscus tiliaceus* collected from Hainan Island of China, was investigated. As a result, one new and 13 known metabolites with diverse molecular structures were isolated and identified from the rice fermentation culture of the strain. This paper describes the isolation and structure elucidation of these compounds.

## 2. Results and Discussion

Compound **1** was obtained as pale yellow oil. The IR absorptions at 3,440 and 3,274, 1,716, and 1,623  $\text{cm}^{-1}$  indicated the presence of hydroxyl, carbonyl, and aromatic groups in the molecule, respectively. The molecular formula was determined to be  $\text{C}_{11}\text{H}_{14}\text{O}_6$  on the basis of HRESIMS ( $m/z$  265.0689  $[\text{M} + \text{Na}]^+$ , calcd. for  $\text{C}_{11}\text{H}_{14}\text{O}_6\text{Na}^+$ , 265.0688), which was in agreement with the  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectral data (Table 1).

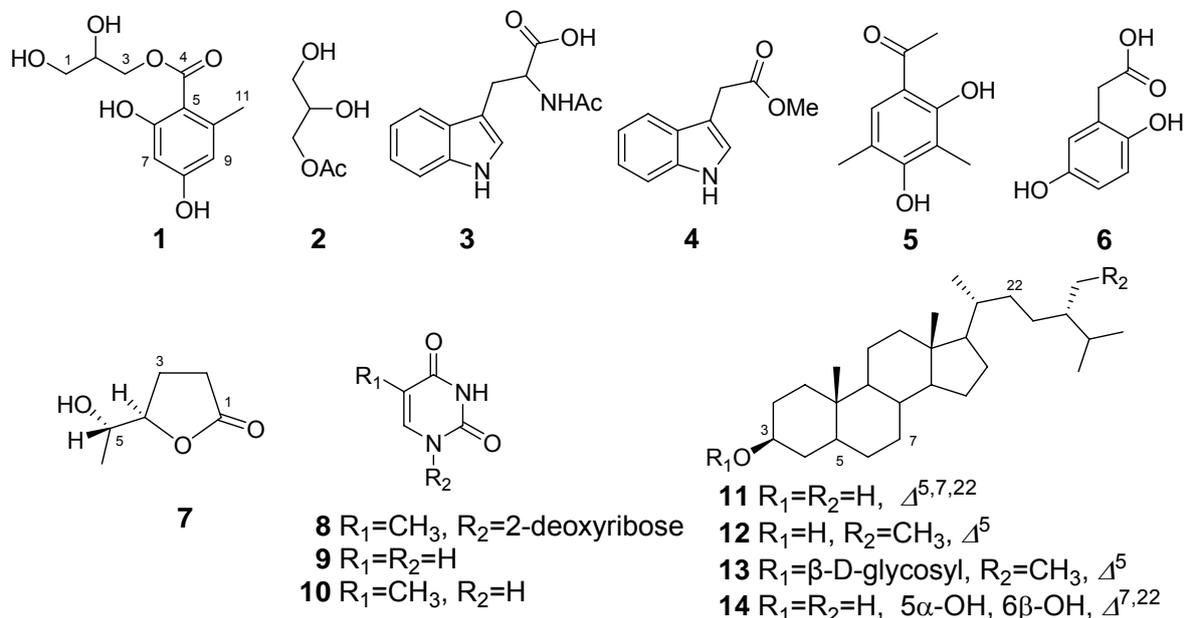
**Table 1.** 1D and 2D NMR data of **1** (in  $\text{CD}_3\text{OD}$ ,  $\delta$  in ppm,  $J$  in Hz).

	$^{13}\text{C}$ (DEPT)	$^1\text{H}$ (mult, $J$ in Hz)	$^1\text{H}$ - $^1\text{H}$ COSY	HMBC
1	64.4 (t)	3.63 (m)	H-2	C-2, 3
2	71.1 (d)	3.97 (m)	H-1, H <sub>2</sub> -3	C-1, 3
3	67.1 (t)	3a: 4.31 (dd, 11.5, 6.0)	H-2	C-1, 2, 4
		3b: 4.42 (dd, 11.5, 4.5)	H-2	C-1, 4
4	172.8 (s)	-	-	-
5	106.0 (s)	-	-	-
6	166.1 (s)	-	-	-
7	101.8 (d)	6.15 (d, 2.5)	H-9	C-5, 6, 8, 9
8	163.8 (s)	-	-	-
9	112.5 (d)	6.21 (d, 2.5)	H-7	C-5, 7, 8, 11
10	144.7 (s)	-	-	-
11	24.4 (q)	2.50 (s)	-	C-5, 9, 10

The  $^{13}\text{C}$ -NMR and DEPT spectra displayed the presence of one methyl, two oxymethylenes, one oxymethine, one carbonyl, and six aromatic carbon groups in the molecules. The characteristic  $^1\text{H}$ -NMR resonances clearly indicated the presence of two aromatic *meta*-protons at  $\delta_{\text{H}}$  6.15 (1H, d,  $J = 2.5$  Hz, H-7) and 6.21 (1H, d,  $J = 2.5$  Hz, H-9). The HMBC correlations from the methyl H<sub>3</sub>-11 ( $\delta_{\text{H}}$  2.50) to C-5 ( $\delta_{\text{C}}$  106.0), C-9 ( $\delta_{\text{C}}$  112.5), and C-10 ( $\delta_{\text{C}}$  144.7) located the methyl group at C-10. In addition, the observed HMBC cross peaks from H-7 to C-5, C-6 ( $\delta_{\text{C}}$  166.1), C-8 ( $\delta_{\text{C}}$  163.8), and C-9, and from H-9 to C-5, C-7 ( $\delta_{\text{C}}$  101.8), and C-11 ( $\delta_{\text{C}}$  24.4) unambiguously suggested the substitution mode of the benzene ring. On the other hand, the  $^1\text{H}$ - $^1\text{H}$  COSY spectrum indicated that all aliphatic oxymethylene and oxymethine protons were part of a contiguous spin system comprising H<sub>2</sub>-1 ( $\delta_{\text{H}}$  3.63), H-2 ( $\delta_{\text{H}}$  3.97), and H<sub>2</sub>-3 ( $\delta_{\text{H}}$  4.31 and 4.42). Finally, the observed HMBC correlations from H<sub>2</sub>-3 to the carbonyl carbon C-4 ( $\delta_{\text{C}}$  172.8) established the connection of glyceryl and 2,4-dihydroxy-6-

methylbenzoyl units via ester linkage. Thus, the structure of **1** was determined as 1-*O*-(2,4-dihydroxy-6-methylbenzoyl)-glycerol, as shown in Figure 1.

**Figure 1.** Chemical structures of compounds **1–14**.



Besides the new compound **1**, another 13 known metabolites, including 1-*O*-acetyl-glycerol (**2**) [6], *N*-acetyltryptophan (**3**) (determined by comparison with spectral data of *N*-acetyltryptophan methyl ester) [7], 3-indolylacetic acid methyl ester (**4**) [8], 1-(2,4-dihydroxy-3,5-dimethylphenyl)ethanone (**5**, known as clavacol) [9], 2-(2,5-dihydroxyphenyl)acetic acid (**6**) [10], (4*R*,5*S*)-5-hydroxyhexan-4-olide (**7**) [11], thymidine (**8**) [12], uracil (**9**) [12], thymine (**10**) [12], ergosterol (**11**) [13],  $\beta$ -sitosterol (**12**),  $\beta$ -daucosterol (**13**), and ergosta-7,22-dien-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol (**14**) [14], were also isolated and identified from the rice fermentation culture of *Penicillium commune*. Their structures are shown in Figure 1.

### 3. Conclusions

In summary, we have identified a new glycerol derivative 1-*O*-(2,4-dihydroxy-6-methylbenzoyl)-glycerol (**1**), together with thirteen known products **2–14** of diverse molecular types from the solid cultures of the endophytic fungal strain *P. commune* that was isolated from the semi-mangrove plant *Hibiscus tiliaceus*. The fungal species *P. commune* are widely spread in nature, being isolated usually from foods and plant rhizospheres. The earlier studies showed that strains of *P. commune* mainly produce indole alkaloids such as cyclopiazonic acid [15,16], fumigaclavines A and B [17], penitrem A and roquefortine [18]. Up to the present, there are no reports of the chemical constituents of mangrove-derived *P. commune* and this is the first report of the isolation of glycerol derivative such as compound **1** from the fungal genus *Penicillium*. Our chemical study of the mangrove-derived endophyte *P. commune* enriches the chemical diversity of this fungal species.

## 4. Experimental

### 4.1. General

Optical rotations were measured on a AA-55 polarimeter (Optical Activity Ltd). IR spectra were performed on a JASCO FT/IR-4100 Fourier Transform infrared spectrometer. UV spectra were measured on a Lengguang Gold spectrumlab 54. NMR spectra were recorded on a Bruker Avance 500 MHz spectrometer (500 MHz for  $^1\text{H}$  and 125 MHz for  $^{13}\text{C}$ ) and chemical shifts were recorded as  $\delta$  values. Mass spectra were performed on a VG Autospec 3000 mass spectrometer. Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co., Qingdao, China), reversed-phase silica gel C18 (40–75 mm, Fuji Silysia Chemical Ltd.) and Sephadex LH-20 (18–110 mm, Merck, Darmstadt, Germany) were used for open column chromatography (CC).

### 4.2. Fungal material

*Penicillium commune* G2M was isolated from a sample of the mangrove plant *Hibiscus tiliaceus* Linn. that was collected from Hainan Island, China, in August, 2004, by using the procedures described in our previous report [3–5]. Fungal identification was carried out using a molecular biological protocol of DNA amplification and sequencing of the ITS region as described previously [3–5]. The sequence data obtained from the fungal strain has been submitted to and deposited at GenBank under accession no. HM064435. A BLAST search result indicated that the sequence was the most similar (98%) to the sequence of *Penicillium commune* (compared to GQ 340555.1, GI 260986214). The strain is preserved at 4 °C at the Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences. The fungal strain was statically fermented at room temperature on sterilized solid medium containing 100 g rice, 0.6 g peptone, and 100 mL sea water, in 1 L Fernbach flasks ( $\times 100$ ) for 42 days.

### 4.3. Extraction and isolation

The fermented rice substrate was extracted repeatedly with EtOAc, and the organic solvent was evaporated to dryness under vacuum to afford 20 g of crude extract. The extract was subjected to CC over silica gel eluted with petroleum ether (PE)–EtOAc (from 1:0 to 1:1) and  $\text{CHCl}_3$ –MeOH (from 20:1 to 0:1) to yield seven fractions (Frs. 1–7). Fr. 2 was further purified by Sephadex LH-20 ( $\text{CHCl}_3$ –MeOH 1:1) to afford **5** (15.8 mg). Fr. 3 was fractionated by CC on silica gel eluted with PE–EtOAc (from 100:1 to 10:1) to obtain **11** (20.0 mg) and **12** (8.9 mg). Fr. 4 was subjected to CC on silica gel eluted with PE–EtOAc (from 50:1 to 10:1), and RP-18 (MeOH) to yield **7** (5.4 mg). Fr. 5 was subjected to Sephadex LH-20 ( $\text{CHCl}_3$ –MeOH 1:1) and RP-18 eluted with MeOH– $\text{H}_2\text{O}$  (0–100) to give **3** (4.2 mg), **8** (32.6 mg), **13** (19.3 mg), and **14** (6.1 mg). Fr. 6 was further purified by Sephadex LH-20 ( $\text{CHCl}_3$ –MeOH 1:1), Sephadex LH-20 (MeOH), and RP-18 with MeOH– $\text{H}_2\text{O}$  (0–100) as an eluent to afford **1** (4.1 mg), **2** (19.3 mg), **6** (31.1 mg), **9** (6.1 mg), and **10** (7.3 mg). Fr. 7 was further purified by Sephadex LH-20 ( $\text{CHCl}_3$ –MeOH 1:1) to yield **4** (3.4 mg).

*1-O-(2,4-Dihydroxy-6-methylbenzoyl)-glycerol (1)*: pale yellow oil;  $[\alpha]_D^{25}$ : + 15° (c 0.4, MeOH); IR (KBr)  $\text{cm}^{-1}$ : 3,440, 3,274, 3,070, 2,939, 1,716, 1,623, 1,450, 1,381, 1,323, 1,261, 1,192, 1,115; UV  $\lambda_{\text{max}}$  (MeOH) nm (log  $\epsilon$ ): 215 (4.63), 264 (4.40);  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$ : see Table 1; ESI-MS: 265  $[\text{M} + \text{Na}]^+$ , 507  $[2\text{M} + \text{Na}]^+$ ; HR-ESI-MS:  $m/z$  265.0689  $[\text{M} + \text{Na}]^+$ , calcd. for  $\text{C}_{11}\text{H}_{14}\text{O}_6\text{Na}^+$ , 265.0688.

## Acknowledgements

This work was financially supported by the Chinese Academy of Sciences (KZCX2-YW-211-04), by the National High-Tech R & D Program (2007AA09Z446), and by the Natural Science Foundation of China (30910103914 and 30770234).

## References and Notes

1. Lin, P. A review on mangrove research in China. *J. Xiamen Univ. (Nat. Sci.)* **2001**, *40*, 592–603.
2. Liu, A.R.; Wu, X.P.; Xu, T. Research advances in endophytic fungi of mangrove. *Chin. J. Appl. Ecol.* **2007**, *18*, 912–918.
3. Li, D.L.; Li, X.M.; Wang, B.G. Natural anthraquinone derivatives from a marine mangrove plant-derived endophytic fungus *Eurotium rubrum*: structural elucidation and DPPH radical scavenging activity. *J. Microbiol. Biotechnol.* **2009**, *19*, 675–680.
4. Li, D.L.; Li, X.M.; Li, T.G.; Dang, H.Y.; Wang, B.G. Alkaloids produced by the marine mangrove derived endophytic fungus *Eurotium rubrum*. *Helv. Chim. Acta* **2008**, *91*, 1888–1893.
5. Li, D.L.; Li, X.M.; Li, T.G.; Dang, H.Y.; Proksch, P.; Wang, B.G. Benzaldehyde derivatives from *Eurotium rubrum*, an endophytic fungus derived from the mangrove plant *Hibiscus tiliaceus*. *Chem. Pharm. Bull.* **2008**, *56*, 1282–1285.
6. Suemune, H.; Mizuhara, Y.; Akita, H.; Sakai, K. Enzymatic synthesis of 2,3-*O*-isopropylidene-*sn*-glycerol, a chiral building block for platelet-activating factor. *Chem. Pharm. Bull.* **1986**, *34*, 3440–3444.
7. Amir-Heidari, B.; Micklefield, J. NMR confirmation that tryptophan dehydrogenation occurs with *syn* stereochemistry during the biosynthesis of CDA in *Streptomyces coelicolor*. *J. Org. Chem.* **2007**, *72*, 8950–8953.
8. Fukuyama, T.; Chen, X.Q.; Peng, G. A novel tin-mediated indole synthesis. *J. Am. Chem. Soc.* **1994**, *116*, 3127–3128.
9. Zhang, C.L.; Zheng, B.Q.; Lao, J.P.; Mao, L.J.; Chen, S.Y.; Kubicek, C.P.; Lin, F.C. Clavatul and patulin formation as the antagonistic principle of *Aspergillus clavatonanicus*, an endophytic fungus of *Taxus mairei*. *Appl. Microbiol. Biotechnol.* **2008**, *78*, 833–840.
10. Dai, J.R.; Kardono, L.B.S.; Tsauri, S.; Padmawinata, K.; Pezzuto, J.M.; Kinghorn, A.D. Phenylacetic acid derivatives and a thioamide glycoside from *Entada phaseoloides*. *Phytochemistry* **1991**, *30*, 3749–3752.
11. Buchanan, M.S.; Hashimoto, T.; Takaoka, S.; Asakawa, Y. (+)-Osmundalactone,  $\nu$ -lactones and spiromentins from the fungus *Paxillus atrotomentosus*. *Phytochemistry* **1995**, *40*, 1251–1257.
12. Rong, G.B.; Zhu, S.Z. *Structure Determination of Organic Compounds: Tables of Spectral Data*; ECUST Press: Shanghai, China, 2002; pp.154, 237.

13. Xu, X.Q.; Hong, W.R.; Chen, D.J. Studies on ergosterol production by *Claviceps purpurea*. *Chin. J. Antibiot.* **2006**, *31*, 379–381.
14. Cafieri, F.; Fattorusso, E.; Gavagnin, M.; Santacroce, C.  $3\beta,5\alpha,6\beta$ -Trihydroxysterols from the Mediterranean bryozoan *Myriapora truncata*. *J. Nat. Prod.* **1985**, *48*, 944–947.
15. Gqaleni, N.; Smith, J.E.; Lacey, J.; Gettinby, G. The production of cyclopiazonic acid by *Penicillium commune* and cyclopiazonic acid and aflatoxins by *Aspergillus flavus* as affected by water activity and temperature on maize grains. *Mycopathologia* **1996**, *136*, 103–108.
16. Larsen, T.O.; Gareis, M.; Frisvad, J.C. Cell cytotoxicity and mycotoxin and secondary metabolite production by common penicillia on cheese agar. *J. Agric. Food Chem.* **2002**, *50*, 6148–6152.
17. Vinokurova, N.G.; Ozerskaya, S.M.; Baskunov, B.P.; Arinbasarov, M.U. The *Penicillium commune* Thom and *Penicillium clavigerum* Demelius fungi producing fumigaclavines A and B. *Microbiol.* **2003**, *72*, 149–151.
18. Wagener, R.E.; Davis, N.D.; Diener, U.L. Penitrem A and roquefortine production by *Penicillium Commune*. *Appl. Environ. Microbiol.* **1980**, *39*, 882–887.

*Sample Availability:* Samples of the compounds **1-14** are available from the authors.

© 2010 by the authors; licensee MDPI, Basel, Switzerland. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/3.0/>).