The Journal of Antibiotics (2018) 71:658–661
https://doi.org/10.1038/s41429-018-0046-y

JARA Japan Antibiotics Research Association
The Society for Actinomycetes Japan

BRIEF COMMUNICATION

Roquefortine J, a novel roquefortine alkaloid, from the deep-sea-derived fungus *Penicillium granulatum* MCCC 3A00475

Siwen Niu[1] · Ning Wang[2] · Chun-Lan Xie[3] · Zuowang Fan[1] · Zhuhua Luo[1] · Hai-Feng Chen[3] · Xian-Wen Yang[1]

Received: 14 January 2018 / Revised: 5 March 2018 / Accepted: 7 March 2018 / Published online: 4 April 2018

Abstract

Chemical investigation on the deep-sea-derived fungus *Penicillium granulatum* MCCC 3A00475 led to the isolation of a previously undescribed (roquefortine J, **1**) and four known (**2**−**5**) roquefortine alkaloids, along with six ergosterol analogues (**6**−**11**). The planar structure of **1** was established mainly on the basis of extensive analysis of its 1D, 2D NMR, and HRESIMS spectra. The absolute configuration of **1** was determined by comparison of the calculated and experimental electronic circular dichroism spectra. Compounds **5**, **6**, and **7** exhibited potent anti-proliferative effects against HepG2 tumor cells with IC_{50} values of 7.0, 8.6, and 8.2 μM, respectively.

Marine fungi have been recognized as a new source for discovery of structurally fascinating and pharmaceutically useful secondary metabolites in recent years [1–4]. The deep-sea-derived fungi, which inhabit extreme environments, are a relatively untapped source because of the limitations of sampling and culturing technologies [5, 6]. Therefore, fewer investigations have been conducted on the secondary metabolites from marine-derived fungi living below 1000 m [6]. In our current study to search for novel bioactive secondary metabolites from deep-sea sediment-derived microorganisms [7–9], the fungus *Penicillium granulatum* MCCC 3A00475 was chosen for chemical investigation because its fermentation extract exhibited significant anti-allergic and antitumor effects. Previous bio-guided isolation had provided three anti-allergic diterpenoids [10]. A further investigation on the strain resulted in the isolation of 11 compounds (**1**−**11**) with cytotoxic activity (Fig. 1). This paper reports the isolation, structure elucidation, and cytotoxic activity of these compounds.

The fungus *Penicillium granulatum* MCCC 3A00475 was isolated from the deep-sea sediment at the depth of 2284 m. Its EtOAc extract of the fermented cultures was fractionated by column chromatography on Sephadex LH-20, silica gel, and ODS to yield a novel roquefortine alkaloid (**1**) and 10 known compounds (**2**−**11**). By comparison of the NMR data with those reported in the literature, 10 known compounds were identified as roquefortine C (**2**) [11], 16-hydroxyroquefortine C (**3**) [12], roquefortine F (**4**) [13], meleagrin (**5**) [14], isonuatigenin I (**6**) [15], penicisteroid A (**7**) [16], anicequol (**8**) [17], 24ε-ethylcholest-5-en-3β-ol (**9**) [18], ergosterol (**10**) [19], and 5α,8α-epidioxyergosta-6,22-dien-3β-ol (**11**) [20].

Compound **1** was obtained as a yellow powder. Its molecular formula was determined to be $C_{22}H_{21}N_5O_2$ on the basis of the HRESIMS at *m/z* 388.1765 $[M+H]^+$ (calcd for $C_{22}H_{22}N_5O_2$, 388.1773), indicating 15 degrees of unsaturation. The ^1H NMR spectrum exhibited signals characteristic of ortho-substituted benzene moiety (δ_H 6.66, d, $J = 7.6$ Hz, H-7; 7.04, t, $J = 7.6$ Hz, H-8; 6.67, t, $J = 7.6$ Hz, H-9; and 7.19, d, $J = 7.6$ Hz, H-10), as well as five olefinic protons (δ_H 5.94, dd, $J = 17.2$, 10.7 Hz, H-19; 6.42, s, H-11; 6.43, br s, H-12; 7.35, br s, H-17; and 7.73, br s, H-15), one exomethylene (δ_H 5.10, d, $J = 17.2$ Hz and

Electronic supplementary material The online version of this article (https://doi.org/10.1038/s41429-018-0046-y) contains supplementary material, which is available to authorized users.

✉ Xian-Wen Yang
yangxianwen@tio.org.cn

[1] State Key Laboratory Breeding Base of Marine Genetic Resources, Key Laboratory of Marine Genetic Resources, Fujian Key Laboratory of Marine Genetic Resources, Third Institute of Oceanography, State Oceanic Administration, 184 Daxue Road, Xiamen 361005, China

[2] Department of Oncology, Luxembourg Institute of Health, 84 Val Fleuri, L-1526 Luxembourg, Luxembourg

[3] Fujian Provincial Key Laboratory of Innovative Drug Target, School of Pharmaceutical Sciences, Xiamen University, Xiamen 361005, China

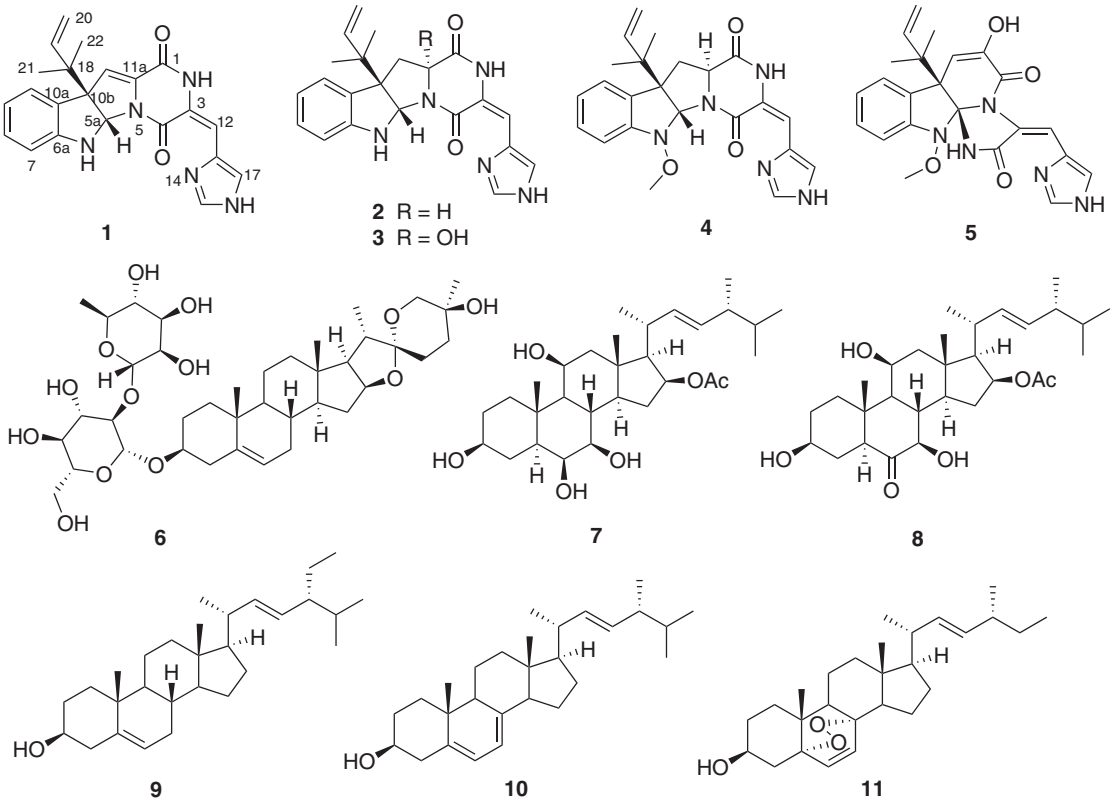

Fig. 1 Chemical structures of **1**–**11** from *Penicillium granulatum* MCCC 3A00475

5.14, d, $J = 10.7$ Hz, H_2-20), and two methyl singlets (δ_H 1.09 and 1.15). The ^{13}C NMR spectrum showed 22 carbon signals including 17 sp^2 carbons with six for one phenyl unit (δ_C 110.6, 119.4, 126.3, 129.8, 130.1, and 151.1), eight for four double bonds (δ_C 110.6, 114.5, 121.7, 125.0, 128.2, 133.7, 124.8, and 145.0), two for carbonyl carbons (δ_C 156.6 and 157.5), and one for nitrogen-bearing carbon (δ_C 137.9), in addition to 5 sp^3 carbons comprising two quaternary carbons (δ_C 43.0 and 69.1), one nitrogen-bearing methine (δ_C 81.7), and two methyls (δ_C 22.8 and 22.9) (Table 1).

The HMBC correlations from H-10 to C-6a (δ_C 151.1)/C-10a (δ_C 130.1)/C-10b (δ_C 69.1) and from H-5a (δ_H 6.08) to C-6a/C-10a/C-10b, together with the COSY correlations of H-7/H-8/H-9/H-10 deduced the presence of an indoline moiety. The HMBC correlations from H_3-21 (δ_H 1.09) and H_3-22 (δ_H 1.15) to C-10b/C-18 (δ_C 43.0)/C-19 (δ_C 145.0), and COSY correlations between H-19 and H_2-20 suggested the presence of an isoprenyl group on C-10b. The HMBC correlations from H-5a to C-10b, C-11 (δ_C 121.7), and C-11a (δ_C 134.8) confirmed the presence of a dihydropyrrole ring, while those from H-12 to C-3 (δ_C 125.0)/C-4 (δ_C 157.7)/C-13 (δ_C 128.2), from H-15 to C-13 and C-17 (δ_C 133.7), and from H-17 to C-12 (δ_C 110.6)/C-13/C-15 assigned a dehydrohistidine unit (Fig. 2). On the basis of the above evidence, the gross structure of **1** was established as 11,11a-dehydrogenated derivative of roquefortine C (**2**) [11].

The NOESY correlations from H-5a to H_3-21 and H_3-22 revealed the same orientation of H-5a and the isoprenyl group (Fig. 2). The absolute configuration of **1** was assigned by comparison of the calculated and experimental electronic circular dichroism (ECD) spectra. The model molecules of (5a*S*,10b*R*)-**1** (**1a**) and its enantiomer (**1b**) were calculated by the time-dependent density functional theory (TD-DFT) method at the B3LYP/6-311G (d,p) level in MeOH with the IEFPCM model using the B3LYP/6-311G (d,p)-optimized geometries after systematic conformational searches by Confab program at the MMFF94 force field. The experimental ECD spectrum of **1** matched well with the calculated curve of **1a**, indicating the absolute configuration of **1** to be 5a*S* and 10b*R* (Fig. 3). Therefore, the structure of **1** was elucidated to be 11(11a)-en-roquefortine C, and named roquefortine J.

All the isolated compounds were evaluated for their cytotoxic activities against HepG2 tumor cells using the MTT method [21]. Compound **1** showed weak growth inhibitory effect against HepG2 tumor cells with a IC_{50} value of 19.5 μM, while **5**, **6**, and **7** exhibited potent growth inhibitory effects with IC_{50} values of 7.0, 8.6, and 8.2 μM, respectively (Table 2), suggesting potential application of

Table 1 ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopic data for **1** in CD_3OD

Position	δ_C, type	δ_H, mult (*J* in Hz)
1	156.6, C	
3	125.0, C	
4	157.7, C	
5a	81.7, CH	6.08, s
6a	151.1, C	
7	110.6, CH	6.66, d (7.6)
8	129.8, CH	7.04, t (7.6)
9	119.4, CH	6.67, t (7.6)
10	126.3, CH	7.19, d (7.6)
10a	130.1, C	
10b	69.1, C	
11	121.7, CH	6.42, s
11a	134.8, C	
12	110.6, CH	6.43, br s
13	128.2, C	
15	137.9, CH	7.73, br s
17	133.7, CH	7.35, br s
18	43.0, C	
19	145.0, CH	5.94, dd (17.2, 10.7)
20	114.5, CH_2	5.14, d (10.7)
		5.10, d (17.2)
21	22.8, CH_3	1.09, s
22	22.9, CH_3	1.15, s

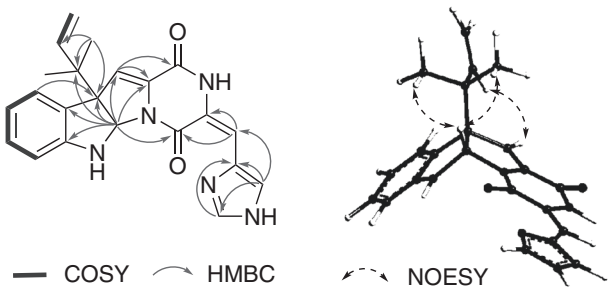

Fig. 2 Key COSY, HMBC and NOESY correlations of **1**

these compounds for further development as antitumor agents.

The present work reports a novel roquefortine alkaloid, roquefortine J (**1**), from the deep-sea sediment-derived fungus *Penicillium granulatum* MCCC 3A00475, together with four known roquefortine alkaloids (**2**–**5**) and six known ergosterol analogues (**6**–**11**). Biogenetically, the roquefortine alkaloids were assembled by condensation of tryptophan and histidine, which was different to the previously reported diterpenoids from the same fungus [10], indicating this fungus has multiple biogenetic pathways to produce structurally diverse secondary metabolites. The

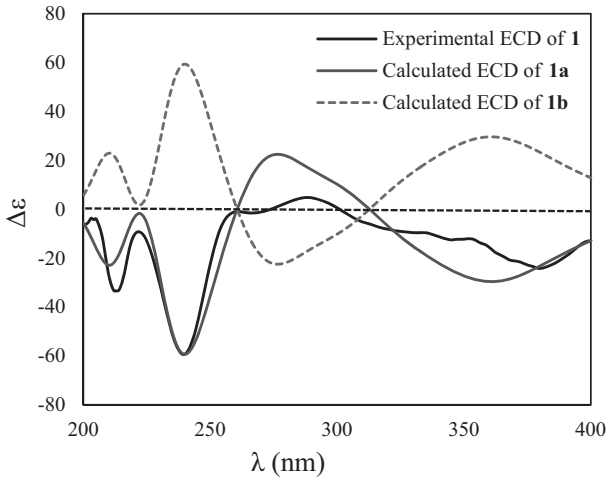

Fig. 3 Calculated and experimental ECD spectra of **1** in MeOH

Table 2 Growth inhibitory effects of **1**−**11** against HepG2 tumor cells

Compounds	IC_{50} (μM)
1	19.5
2	>20
3	>20
4	>20
5	7.0
6	8.6
7	8.2
8	>20
9	>20
10	>20
11	>20

absolute configuration of **1** was determined by the calculated ECD spectra. All compounds were evaluated for their cytotoxic activities against HepG2 tumor cells. Compounds **5**, **6**, and **7** exhibited potent inhibitory effects with IC_{50} values of 7.0, 8.6, and 8.2 μM, respectively, indicating their potential applications for further development as antitumor agents.

Roquefortine J (1): Yellow powder; $[\alpha]_D^{26}$ + 2 (*c* 0.26, MeOH), $[\alpha]_D^{23}$ −78 (*c* 0.28, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 241 (3.48), 359 (3.75) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 204 (−4.26), 213 (−33.40), 222 (−9.25), 240 (−59.30), 260 (−1.42), 289 (+4.78); ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 388.1765 $[M+H]^+$ (calcd for $C_{22}H_{22}N_5O_2$, 388.1773), 410.1586 $[M+Na]^+$ (calcd for $C_{22}H_{21}N_5O_2Na$, 410.1593), 386.1619 $[M-H]^-$ (calcd for $C_{22}H_{20}N_5O_2$, 386.1617), 422.1385 $[M+Cl]^-$ (calcd for $C_{22}H_{21}N_5O_2Cl$, 422.1384).

Acknowledgements This study was supported by grants from the Science and Technology Research Program of Fujian Province, China

(2017Y0060), the Fundamental Research for the Central University (2072016117), and the National Natural Science Foundation of China (41606185, 41676130, and 21372233).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Soldatou S, Baker BJ. Cold-water marine natural products, 2006 to 2016. Nat Prod Rep. 2017;34:585–626.
2. Rateb ME, Ebel R. Secondary metabolites of fungi from marine habitats. Nat Prod Rep. 2011;28:290–344.
3. Elissawy AM, et al. Bioactive terpenes from marine-derived fungi. Mar Drugs. 2015;13:1966–92.
4. Agrawal S, et al. Marine fungi: an untapped bioresource for future cosmeceuticals. Phytochem Lett. 2018;23:15–20.
5. Navarri M, et al. Deep subseafloor fungi as an untapped reservoir of amphipathic antimicrobial compounds. Mar Drugs. 2016;14:50.
6. Skropeta D, Wei L. Recent advances in deep-sea natural products. Nat Prod Rep. 2014;31:999–1025.
7. Niu S, et al. Spiromastilactones: a new class of influenza virus inhibitors from deep-sea fungus. Eur J Med Chem. 2016;108:229–44.
8. Niu S, et al. Sesquiterpenes from a deep-sea-derived fungus *Graphostroma* sp. MCCC 3A00421. Tetrahedron. 2017;73:7267–73.
9. Niu S, et al. Cyclopiane-type diterpenes from the deep-sea-derived fungus *Penicillium commune* MCCC 3A00940. Tetrahedron Lett. 2018;59:375–8.
10. Niu S, et al. Spirograterpene A, a tetracyclic spiro-diterpene with a fused 5/5/5/5 ring system from the deep-sea-derived fungus *Penicillium granulatum* MCCC 3A00475. J Nat Prod. 2017;80:2174–7.
11. Scott PM, Polonsky J, Merrien MA. Configuration of the 3,12 double bond of roquefortine. J Agric Food Chem. 1979;27:201–2.
12. Trimble LA, et al. Characterization of (16*R*) and (16*S*)-hydroxyroquefortine C; diastereomeric metabolites from *Penicillium crustosum* DAOM 215343. Tetrahedron Lett. 2012;53:956–8.
13. Du L, et al. New alkaloids and diterpenes from a deep ocean sediment derived fungus *Penicillium* sp. Tetrahedron. 2009;65:1033–9.
14. Kawai K, et al. Studies on fungal products. VII. The structures of meleagrin and 9-O-*p*-bromobenzoylmeleagrin. Chem Pharm Bull. 1984;32:94–8.
15. Chen S, Snyder JK. Diosgenin-bearing, molluscicidal saponins from *Allium vineale*: an NMR approach for the structural assignment of oligosaccharide units. J Org Chem. 1989;54:3679–89.
16. Gao S, et al. Penicisteroids A and B, antifungal and cytotoxic polyoxygenated steroids from the marine alga-derived endophytic fungus *Penicillium chrysogenum* QEN-24S. Bioorg Med Chem Lett. 2011;21:2894–7.
17. Igarashi Y, et al. Anicequol, a novel inhibitor for anchorage-independent growth of tumor cells from *Penicillium aurantiogriseum* Dierckx TP-F0213. J Antibiot. 2002;55:371–6.
18. Hu J, Kelly M, Hamann MT. 26-Nor-25-isopropyl-ergosta-5,7,22*E*-trien-3β-ol: a new C_{29} sterol from the sponge *Agelas sceptrum* from Jamaica. Steroids. 2002;67:743–7.
19. Kang J, Wang H, Chen R. Studies on the constituents of the mycelium produced by fermenting culture of *Flammulina velutipes* (W. Curt.:Fr.) Singer (Agaricomycetideae). Int J Med Mushrooms. 2003;5:391–6.
20. Sgarbi DBDG, et al. Isolation of ergosterol peroxide and its reversion to ergosterol in the pathogenic fungus *Sporothrix schenckii*. Mycopathologia. 1997;139:9–14.
21. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65:55–63.