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New alkaloids and diterpenes from a deep ocean sediment derived fungus *Penicillium* sp.

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ABSTRACT

Four new alkaloids, including two new meleagrin analogs, meleagrins B (**2**) and C (**3**), and two new diketopiperazines, roquefortines F (**5**) and G (**6**), together with six new diterpenes, conidiogenones B–G (**7**–**12**), were isolated from a deep ocean sediment derived fungus *Penicillium* sp. The structures and stereochemistry of the new compounds were elucidated by spectroscopic methods. The cytotoxicity of the new compounds against the HL-60, A-549, BEL-7402, and MOLT-4 cell lines was evaluated.

1. Introduction

The meleagrin group and the roquefortine group are biogenetically interrelated alkaloids including not more than 10 natural analogs in each group.[1a–l] Most of the two classes of alkaloids were isolated from *Penicillium* species except for roquefortine E, which was from an isolate of *Gymnoascus reessii*.[1g] Among the metabolites, meleagrin, a common mycotoxin, was first isolated from a *Penicillium meleagrinum* in 1984[1h] and its structure and absolute configuration were established by X-ray crystallographic analysis of its *p*-bromobenzoyl monohydrate. The 9-*O*-methyl analog of meleagrin, oxaline,[1i,2] was reported to inhibit tubulin polymerization, resulting in cell cycle arrest at the M phase in Jurkat cells.[3] Roquefortine C, the biosynthetic precursor of meleagrin,[1f] was a relatively common fungal metabolite, of which over 130 papers have been published on its occurrence, biosynthesis, and biological activity.[1g] It was reported to possess neurotoxic properties in mice,[1a] inhibiting action of Gram-positive bacteria growth,[4a] and the mechanisms responsible for its toxicity and metabolism were related to inhibition of cytochromes P450s.[4b] Its biosynthesis from histidine, tryptophan, and mevalonic acid has been well established,[1f,5] and it was also known to be a precursor in the biosynthesis of the meleagrin group.[1f] In our search for novel antitumor compounds from marine-derived microorganisms, a fungal strain F23-2, authenticated as *Penicillium* sp., was obtained from an underwater sample (depth 5080 m). Its extract exhibited cytotoxicity against the K562 cell line. Investigation of the active constituents of this fungus led to the isolation of four new alkaloids, meleagrin B (**2**), meleagrin C (**3**), roquefortine F (**5**), and roquefortine G (**6**), together with the two known compounds meleagrin (**1**) and roquefortine C (**4**). Meleagrin B (**2**) is a novel complex compound composed of a meleagrin alkaloid moiety and a rare diterpene moiety. Six analogs of the diterpene moiety, conidiogenones B–G (**7**–**12**) were also isolated. They all belong to an unusual fungal diterpene class comprising only two examples, conidiogenol and conidiogenone, which showed potent conidiation inducing activity. [6] In this paper, we describe the isolation, structure, and sterochemistry elucidation, and cytotoxicity against HL-60, A-549, BEL-7402, and MOLT-4 cell lines of the new compounds.

2. Results and discussion

Compound **2** was obtained as a white solid. Its molecular formula $C_{43}H_{53}N_5O_6$ was determined by HRESIMS (*m*/*z* 736.4066 $[M+H]^+$, calcd for 736.4074), indicating 20 degrees of unsaturation. Analysis of the 1D NMR data revealed 15 quaternary carbons, 14 methines, 6 methylenes, and 8 methyls. Comparison of the ^1H and ^{13}C NMR data (Table 1) with those of the two known compounds meleagrin (**1**)[1h] and oxaline[7] indicated the structure of **2** includes

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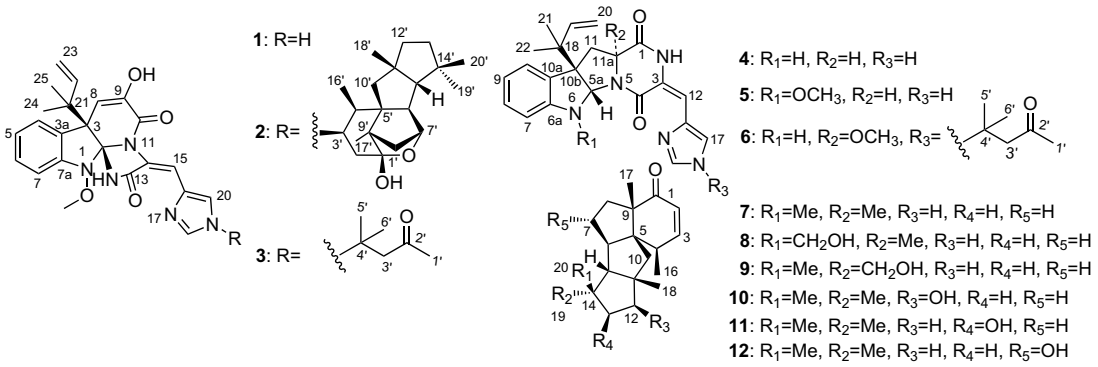


a meleagrin moiety. This assumption was supported by the ^{1}H–^{1}H COSY and HMBC correlations (Fig. 1). Comparing the ^{1}H NMR data with those of **1**, the chemical shift of H-20 moved to downfield significantly (δ 8.35 in **2**, δ 7.34 in **1**). Variations of the chemical shifts for C-15, C-16, and C-20 were also observed between compound **2** and oxaline, especially for C-20 (δ 122.3 in **2**, δ 133.8 in oxaline). All the data seemed to support that a substitution existed on N-19. Further examination of the ^{1}H–^{1}H COSY correlations between H-2′ and H-3′, H-3′ and H-4′, H-4′ and Me-16′, H-7′ and H-8′a and the key HMBC correlations from HO-1′ to C-1′, C-2′, and C-9′; from Me-16′ to C-5′; from Me-17′ to C-1′, C-5′, C-8′, and C-9′; from H-7′ to C-1′ afforded a substructure A. The chemical shift of C-1′ (δ 102.0) accounted for the existence of a hemiacetal group. A substructure B was obtained by analyzing the ^{1}H–^{1}H COSY correlations from H-6′ to H-15′, H-12′ to H-13′ together with the key HMBC correlations from Me-18′ to C-10′, C-11′, C-12′, and C-15′; from Me-19′ to C-13′, C-14′, C-15′, and C-20′. The connectivity of the two substructures was established by the key HMBC correlations from H-10′ to C-4′, C-5′, C-6′, and C-9′; from H-15′ to C-7′; from H-7′ to C-5′; from H-8′ to C-6′. Then a diterpene moiety was obtained with C-3′ substituted. The linkage between C-1′ and C-7′ via an oxygen atom gave a distinct hexa-cycle skeleton comparing with conidiogenol and conidiogenone, the only two penta-cycle analogs.[6] No HMBC correlation was observed between the diterpene moiety to the meleagrin moiety. Analyzing the chemical shifts of C-3′ (δ 53.9) and H-3′(δ 4.63) together with the NOESY correlation between H-20 and H-3′, the two moieties were concluded to attach to each other by a single bond between N-19 and C-3′. Then the planar structure of **2** was determined as a novel complex compound comprising an alkaloid moiety and a diterpene moiety, named meleagrin B.

The absolute configurations of C-2, C-3, and the C-12/C-15 double bond of meleagrin B were deduced as *S*, *R*, and *E*, respectively, on biogenetic grounds.[1h] This assumption was supported by the similar cotton effects in the CD spectra of meleagrin B (**2**) and meleagrin (**1**).[1h] The relative configuration of the diterpene moiety was established by analyzing the NOESY spectrum. Me-17′ correlated with Me-16′, H-2′a, and H-10′a, indicating that C-10, C-16, and C-17 were on the same side of the ring system. H-6′ correlated with H-3′ and H-4′, indicating that H-3′, H-4′, and C-6′ were on the other side of the ring system. The correlations of H-7′with H-15′ and H-8′a, those of Me-17′ with H-8′a and H-10′a, and that between H-10′a and Me-18′ could determine that H-15′, Me-18′, and C-8′had the same orientation. The correlations between H-6′ and Me-19′, and between H-4′ and H-13′a could further confirm this. Although the remaining configuration of C-1′ could not be determined directly, a molecular model (Chembio3D Ultra 11.0 software, using the MM2 minimizing method) afforded the only stable structure that was consistent with the NOESY spectrum (Fig. 1). The molecular model also indicated that the cage skeleton was rigid. Dihedral angles between the bridgehead proton (H-7′) and its neighborhood protons in structure were nearly 90° suggesting that the vicinal coupling constants between the protons should be very small. This was observed in the ^{1}H NMR spectrum where H-7′ appeared as a broad singlet. The relative configuration of the diterpene moiety was consistent with those of the two known diterpenes, conidiogenol and conidiogenone,[6] indicating that they had similar biosynthetic origin.

Compound **3** was obtained as a white solid. The HRESIMS (*m*/*z* 532.2571 $[M+H]^+$, calcd for 532.2560) established the molecular formula $C_{29}H_{33}N_5O_5$. Careful comparison of the ^{1}H and ^{13}C NMR data with those of compound **2** indicated that they had the same meleagrin moiety and a substitution also occurred on N-19. The structure of the other moiety was established as 4-methylpentan-2-one by examining the HMBC correlations from Me-1′ to C-2′ and C-3′; from H-3′ to C-2′, C-4′, C-5′, and C-6′; from Me-5′ to C-3′, C-4′, and C-6′. Analysis of the NOESY correlations from H-20 to H-3′, H-5′, and H-6′, and from H-18 to H-5′ and H-6′ concluded that C-4′ was attached to N-19. The planar structure was then established, named meleagrin C, and its absolute configuration was proposed the same as meleagrin (**1**) on biogenetic grounds and by comparison of their CD spectra.[1h]

Compound **5** was a white solid, which had the molecular formula $C_{23}H_{25}N_5O_3$ established by the HRESIMS (*m*/*z* 420.2055 $[M+H]^+$, calcd for 420.2036). Its 1D NMR spectra were different from meleagrin but quite similar to the biogenetic precursor of meleagrin, roquefortine C (**4**).[1a] The HMBC spectrum (Fig. 1) confirmed that compound **5** had the same planar structure as roquefortine C except for minor substitution variation on N-6. The NH-6 (δ 5.10 in **4**)[1a] seemed to be replaced by an OCH_3 (δ_H 4.06, δ_C 64.2), which had no HMBC correlation to any other carbon. It was confirmed by the significant differences of the chemical shifts for the neighboring carbons C-5a (δ 85.5 in **5**, δ 78.3 in **4**[1a]) and C-7 (δ 116.1 in **5**, δ 110.9 in **4**[1a]). Then the planar structure of **5** was established, named roquefortine F, and its absolute configurations of C-5a, C-10b, C-11a, and the C-3/C-12 double bond were proposed as *S*, *R*, *S*, and *E*, the same as roquefortine C (**4**) whose absolute configuration has been established.[8]

Another analog of roquefortine C, compound **6**, had the molecular formula $C_{29}H_{35}N_5O_4$, established by the HRESIMS (*m*/*z* 518.2761 $[M+H]^+$, calcd for 518.2767). Comparison of its 1D NMR spectra with those of roquefortine C (**4**)[1a] and compound **5** indicated that they had the same skeleton and N-6 in **6** was not substituted (NH-6, δ 5.04), but the H-11a (δ 4.06 in **5**, Table 2) was replaced by an OCH_3 (δ_H 2.84, δ_C 51.8). It was supported by the HMBC correlation from OCH_3-11a to C-11a (δ 91.4) and the doublet peaks of H-11α and H-11β. The upfield chemical shift of OCH_3-11a was due to the strong shielding effect of the benzene group. Further comparing the 1D NMR spectra with those of compound **3** indicated that they had same 4-methylpentan-2-one group attached to N-16. It was confirmed by their similar NOESY correlations (Fig. 1). The planar structure of **6** was established, named roquefortine G. The NOESY correlations between OCH_3-11a and H-11α,

Table 1
^{1}H and ^{13}C NMR data of compounds **2** and **3** (600 and 150 MHz, TMS, δ ppm)

No.	**2**[a]		**3**[b]	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1-OCH_3	3.64, s	64.7	3.70, s	65.4
2		100.9		100.9
3		52.1		52.1
3a		126.1		125.5
4	7.52, d (7.8)	124.6	7.54, d (7.8)	124.9
5	7.03, t (7.8)	123.0	7.04, t (7.8)	123.5
6	7.25, t (7.8)	127.8	7.25, t (7.8)	128.5
7	6.95, d (7.8)	111.4	6.95, d (7.8)	112.1
7a		146.3		146.3
8	5.20, s	108.9	5.44, s	107.2
9		142.8		142.1
10		158.5		158.8
12		124.3		123.3
13		163.8		163.5
15	8.17, s	114.3	8.48, s	117.2
16		134.5		135.4
18	7.84, s	136.2	7.63, s	134.4
20	8.35, s	122.3	8.57, s	121.8
21		41.9		42.8
22	6.07, br s	142.8	6.23, br s	143.3
23a	5.03, br d (16.5)	113.0	5.13, br d (18.8)	114.3
23b	4.98, br s		5.07, br s	
24	1.18, s	24.1	1.22, s	n.d.[c]
25	1.18, s	23.1	1.34, s	n.d.[c]
9-OH	9.15, s		6.24, br s	
14-NH	9.56, s			
17 or 19-NH				
1′		102.0	2.01, s	31.5
2′a	2.36, t (13.3, 12.8)	30.9		205.0
2′b	1.90, dd (13.3, 3.7)			
3′	4.63, dt (12.3, 4.0)	53.9	2.92, s	54.9
4′	2.02–2.05, m	41.2		56.5
5′		55.3	1.71, s	27.9
6′	2.27, br d (5.5)	60.0	1.71, s	27.9
7′	3.80, br s	77.8		
8′a	1.85, br d (10.5)	39.8		
8′b	1.54, br d (10.5)			
9′		51.0		
10′a	2.00, d (15.1)	50.7		
10′b	1.60, d (15.1)			
11′		52.1		
12′a	1.63–1.66, m	40.6		
12′b	1.56–1.59, m			
13′a	1.55–1.58, m	39.9		
13′b	1.43–1.46, m			
14′		42.5		
15′	1.14, d (5.5)	65.1		
16′	0.71, d (7.2)	10.5		
17′	1.05, s	13.1		
18′	1.23, s	31.5		
19′	1.10, s	26.9		
20′	1.03, s	30.0		
1′-OH	5.81, s			

[a] Measured in DMSO-d_6.
[b] Measured in $CDCl_3$.
[c] Signals not detected.

H-11α and H-10, H-11β and Me-21, H-11β and Me-22, H-5a and Me-21, and H-5a and Me-22 concluded that OCH_3-11a was at the opposite side of the ring system against C-18 and H-5a. Since the absolute configurations of C-5a and C-10b were proposed as *S* and *R*, the same as roquefortine C (**4**),[8] the absolute configuration of C-11a was deduced as *R*.

Interested in the novel complex skeleton of meleagrin B, especially in the origin of the diterpene moiety, we went on to look for the diterpene precursors or analogs from the low-polarity fractions. Fortunately, six diterpene analogs, conidiogenones B–G (**7**–**12**), were isolated.

Compound **7** was colorless oil with the molecular formula $C_{20}H_{30}O$, established by the HRESIMS (m/z 287.2376 $[M+H]^+$, calcd for 287.2375). Its 1D NMR spectra were similar to those of the known diterpenes conidiogenol and conidiogenone,[6] and the conjugated tetra-ring skeleton was easily established by interpretation of the ^{1}H–^{1}H COSY and HMBC spectra (Fig. 2). The ^{1}H–^{1}H COSY correlations between H-2 and H-3, H-3 and H-4, and H-4 and Me-16 together with the HMBC correlations from Me-16 to C-3, C-4, and C-5, from Me-17 to C-1, C-5, and C-9, and from H-3 to C-1 indicated the existence of an α,β-unsaturated ketone system on ring A (Fig. 2). The relative configuration of **7**, established by the NOESY spectra, was also consistent with the known compounds conidiogenol and conidiogenone.[6] The correlations between Me-16 and H-10a, Me-17, and H-10b indicated Me-16, Me-17, and C-10 had the same orientation on ring A. The correlations between Me-18 and H-10b, Me-18 and H-15, H-15 and H-7b, H-15 and Me-20, and Me-19 and H-6 indicated Me-18, H-15, and C-7 had the same orientation on ring C (Fig. 2). Interpretation of the critical NOESY correlations between Me-17 and H-10b, Me-16 and H-10a, and H-4 and H-12b concluded that the cyclohexenone ring had a sofa confirmation and both Me-16 and Me-17 were axial (Fig. 3).

Compounds **8**–**12** all had the same molecular formula of $C_{20}H_{30}O_2$, established by their HRESIMS (Section 3). Their 1D NMR data were similar to those of compound **7** and each had a hydroxyl group in structure. Comparison of the 1D NMR data of **8** and **7** indicated that the Me-20 (δ_H 1.06, δ_C 31.0) in **7** was hydroxylated in **8** and it was confirmed by the NOESY correlations between H-20 (δ 3.41, 3.38) and H-15, H-20 and Me-18, H-20 and Me-19, and Me-19 and H-6 (Fig. 2). Similar analysis of the 1D NMR and NOESY data of compound **9** indicated that the hydroxylation occurred on C-19 (δ_C 70.7) (Fig. 2). ^{1}H–^{1}H COSY spectrum indicated that the hydroxyl group in **10** was attached to C-12 or C-13 (Fig. 2). Based on the significant upfield shift of the carbon chemical shift of Me-18 (δ 32.5 in **7**, δ 22.3 in **10**) (Table 4) and the NOESY correlation between the hydrogen proton (δ 4.07) and H-4 (Fig. 2), the hydroxyl group was definitely attached to C-12 with the same orientation as Me-18 on ring D. The HMBC correlation between Me-18 and C-12 (δ 78.5) confirmed the location of the hydroxyl group. The structure of **11** was established as shown based on the similar elucidation of its ^{13}C NMR data (Table 4) and 2D NMR correlations (Fig. 2). Comparison of the 1D NMR data of **12** and **7** indicated that the hydroxyl group was located at C-7 in **12** (Tables 3 and 4). The ^{1}H–^{1}H COSY correlation between H-7 (δ 3.81, d, 4.3) and H-8b (δ 1.67, dd, 13.7, 4.9) and the NOESY correlations between H-8b and Me-17, and between H-7 and H-15 (Fig. 2) further confirmed that H-7 had the same orientation as Me-17 on ring B. In the ^{1}H NMR spectrum, H-7 appeared as a doublet. It indicated that the dihedral angle between H-7 and its neighborhood protons H-6 and H-8a was nearly 90°.

Due to the shortage of the pure samples, chemical methods such as modified Mosher's method were not feasible to study the absolute configurations of the diterpenes. Considering the existence of the α,β-unsaturated ketone chromophore in the structures, the CD spectra of the diterpenes were recorded. The cotton effects were consistent with those of 6*S*,9*S*,10*S*-6-acetoxy-9-methy-Δ^2-octalin-1-one [λ_{max} ($\Delta\varepsilon$) 195 (−10.6), 234 (0.25), 338 (0.30)].[9] It indicated that the cyclohexenone ring was preponderantly in a sofa conformation with nearly planar chromophore and the signs of the strong cotton effect below 200 nm resulted from the CO n→σ* transition mainly correlated with the presence of the α′-axial Me group.[9] So the absolute configuration of the diterpene skeleton was determined as 4*R*, 5*R*, 6*R*, 9*S*, 11*S*, and 15*S*.

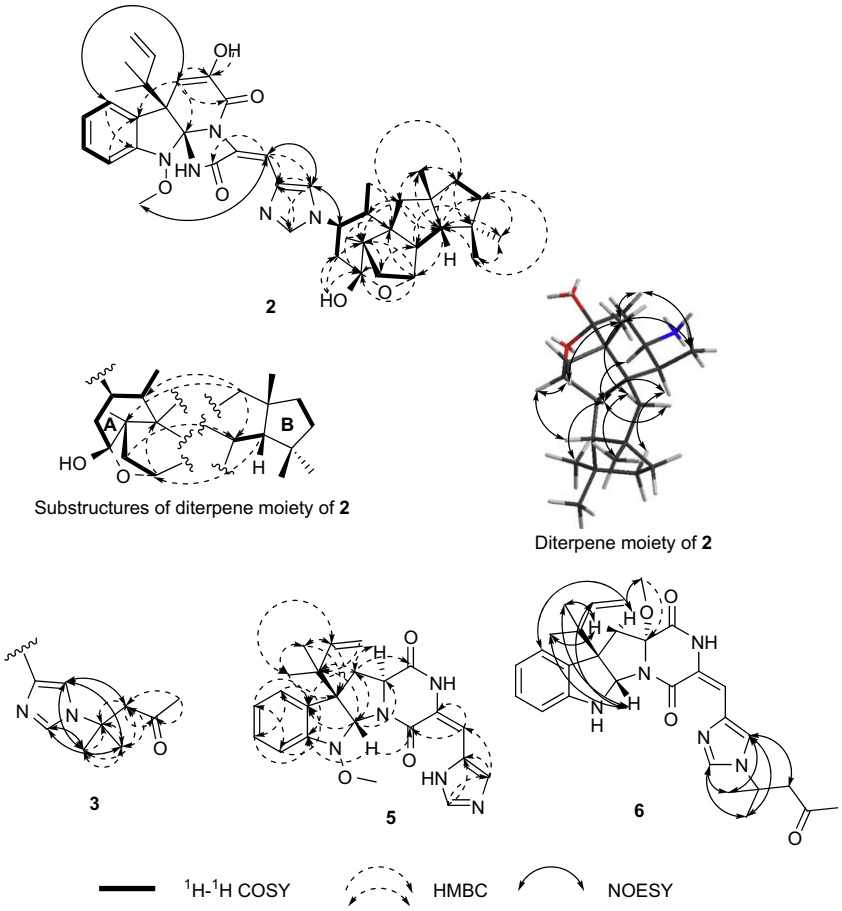


Figure 1. Selected ^{1}H–^{1}H COSY, HMBC, and NOESY data, correlations of compounds **2**, **3**, **5**, and **6**.

The biogenetic relationships of all the new alkaloids were postulated in a plausible route (Scheme 1). The biogenetic mechanisms of roquefortine C (**4**) and meleagrin (**1**) have been established and confirmed by isotopic labeling studies.[1f,5] A precursor, 4-methylpent-3-en-2-one (**a**), originated from mevalonic acid, could react with meleagrin (**1**) or the intermediate (**b**) to afford meleagrin C (**3**) or roquefortine G (**6**), respectively. Similar mechanism could be applied to meleagrin (**1**) and the diterpene precursor conidiogenone G (1**2**). A Michael addition reaction could lead to the formation of the intermediate (**c**), which could spontaneously undergo inner 1,2-addition reaction to afford meleagrin B (**2**). On biogenetic ground, the absolute configuration of the diterpene moiety in meleagrin B would be deduced as 1′*R*, 3′*R*, 4′*S*, 5′*R*, 6′*R*, 7′*R*, 9′*S*, 11′*S*, and 15′*S*.

The new compounds were evaluated for their cytotoxicity against the HL-60 and MOLT-4 cell lines by the MTT method,[10] and the A-549 and BEL-7402 cell lines by the SRB method.[11] The novel complex compound, meleagrin B (**2**), showed moderate cytotoxicity against all the four cell lines with the IC_{50} values as 6.7, 2.7, 1.8, and 2.9 μM. Conidiogenone C (**8**) showed potent cytotoxicity against HL-60 and BEL-7402 cell lines with the IC_{50} values as 0.038 and 0.97 μM, while it exhibited no cytotoxicity against A-549 and MOLT-4 cell lines at 50 μM. The antitumor activity of the conidiogenone diterpenes was discovered for the first time (Table 5).

3. Experimental

3.1. General

Optical rotations were obtained on a JASCO P-1020 digital polarimeter. UV spectra were recorded on Beckman DU® 640 spectrophotometer. IR spectra were taken on a NICOLET NEXUS 470 spectrophotometer in KBr discs. ^{1}H, ^{13}C NMR, DEPT spectra, and 2D NMR were recorded on a JEOL JNM-ECP 600 spectrometer using TMS as internal standard and chemical shifts were recorded as δ values. ESIMS was measured on a Q-TOF ULTIMA GLOBAL GAA076 LC mass spectrometer. Semiprepartive HPLC was performed using an ODS column [YMC-pak ODS-A, 10×250 mm, 5 μm, 4 mL/min].

3.2. Culture of *Penicillium* sp., strain F23-2, extraction and isolation of compounds 1–12

The fungus, strain F23-2, was obtained from a deep ocean sediment sample (depth 5080 m). It was identified as *Penicillium* sp. by Professor Xiang Xiao, The Third Institute of Oceanography, SOA, Xiamen, China, on the basis of its ribosomal internal transcribed spacers and the 5.8S ribosomal RNA gene (ITS1-5.8S-ITS2), which was deposited in Genbank (EU770318). Working stocks were prepared on Potato Dextrose agar slants stored at 4 °C.

The producing strain was cultured and the fermentation was carried out as follows. Spores growing on PDA slant were inoculated into 1000 mL Erlenmeyer flasks containing 200 mL seawater based culture medium (potato 200 g, glucose 20 g, mannitol 20 g, maltose 10 g, peptone 5 g, yeast extract 3 g, dissolved in 1 L seawater pH 6.0) and cultured at 28 °C for 45 days under static conditions. One hundred liters of the whole broth gave a crude ethyl acetate extract (45.0 g), which was subjected to silica gel column chromatography (petroleum ether/acetone, v/v, gradient). The active fraction Fr. 6 was recrystallized to afford meleagrin (**1**, 200 mg). The active fraction Fr. 9 was

Table 2
^{1}H and ^{13}C NMR data of compounds **5** and **6** (600 and 150 MHz, TMS, δ ppm)

No.	**5**[a]		**6**[a]	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		166.3		163.5
3		121.6		122.8
4		157.9		159.7
5a	5.89, s	85.5		79.2
6-OCH_3	4.06, s	64.2		
6a		151.4		149.0
7	7.09, d (7.8)	116.1	6.59, d (7.8)	108.8
8	7.28, t (7.8)	129.4	7.06, t (7.8)	128.2
9	7.11, t (7.8)	124.9	7.73, t (7.8)	118.4
10	7.28, d (7.8)	124.9	7.18, d (7.8)	124.6
10a		130.7		131.3
10b		61.4		60.0
11α	2.54, t (11.9)	38.7	2.74, d (14.2)	40.1
11β	2.44, dd (5.9, 12.3)		2.55, d (13.8)	
11a	4.04–4.07, m	58.1		91.4
11a-OCH_3			2.84, s	51.8
12	6.39, s	111.0	6.75, s	118.5
13		125.6		134.9
15	7.72, s	136.7	7.72, s	134.7
17	7.28, s	134.8	8.49, s	121.7
18		41.2		41.3
19	5.96, dd (10.4, 17.4)	143.0	5.94, dd (10.4, 17.4)	143.5
20a	5.18, d (10.4)	115.2	5.12, d (10.4)	114.8
20b	5.12, d (17.4)		5.10, d (17.4)	
21	0.99, s	22.8	0.99, s	22.8
22	1.12, s	23.8	1.14, s	22.2
2-NH	10.21, s		9.20, s	
6-NH			5.04, s	
14 or 16-NH	13.06, s			
1′			2.03, s	31.5
2′				204.8
3′a			2.97, s	54.7
3′b			2.96, s	
4′				56.7
5′			1.74, s	27.8
6′			1.75, s	28.2

[a] Measured in $CDCl_3$.

subjected to repeated Sephadex LH-20 column chromatography ($CHCl_3$/MeOH, v/v, 1:1), and further purification was carried out using HPLC on a ODS semi-preparative column (YMC-Park ODS-A, 10×250 mm, 5 μm, 4 mL/min) eluted with 70% of methanol/water to obtain meleagrin B (**2**, 8 mg), meleagrin C (**3**, 5 mg), roquefortine C (**4**, 30 mg), roquefortine F (**5**, 10 mg), and roquefortine G (**6**, 2 mg). The active fraction Fr. 2 was subjected to silica gel column chromatography (petroleum ether/acetone, 10/1) and further purified on HPLC (90%, methanol/water) to afford conidiogenone B (**7**, 11.2 mg). The active fraction Fr. 3 was subjected to repeated Sephadex LH-20 column chromatography ($CHCl_3$/MeOH, v/v, 1:1) and further purified on HPLC (65%, methanol/water) to afford conidiogenone C (**8**, 6.0 mg),

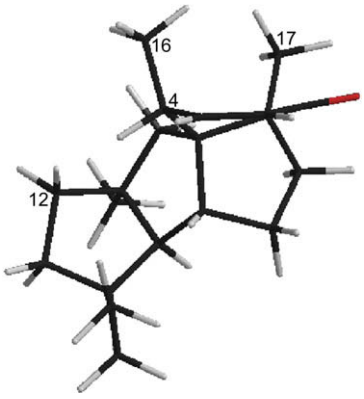


Figure 3. Relative stereochemistry for compound **7**.

conidiogenone D (**9**, 3.9 mg), conidiogenone E (**10**, 1.8 mg), conidiogenone F (**11**, 1.5 mg), and conidiogenone G (**12**, 1.2 mg).

3.3. Biological assays

Cytotoxic activity was evaluated using the HL-60 and MOLT-4 cell lines by the MTT method,[10] and the A-549 and BEL-7402 cell lines by the SRB method.[11] In the MTT assay, the cell line was grown in RPMI-1640 supplemented with 10% FBS under a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. Cell suspensions (200 μL) at a density of 5×10^4 cells/mL were plated in 96-well microtiter plates and incubated for 24 h. The test compound solutions (2 μL in MeOH) at different concentrations were added to each well and further incubated for 72 h under the same conditions. MTT solution (20 μL of a 5 mg/mL solution in IPMI-1640 medium) was added to each well and incubated for 4 h. An old medium (150 μL) containing MTT was then gently replaced by DMSO and pipetted to dissolve any formazan crystals formed. Absorbance was then determined on a SPECTRA MAX PLUS plate reader at 540 nm. In the SRB assay, cell suspensions (200 μL) were plated in 96-cell plates at a density of 2×10^5 cells/mL. Then the test compound solutions (2 μL in MeOH) at different concentrations were added to each well and further incubated for 24 h. Following drug exposure, the cells were fixed with 12% trichloroacetic acid and the cell layer was stained with 0.4% SRB. The absorbance of SRB solution was measured at 515 nm. Dose response curves were generated and the IC_{50} values were calculated from the linear portion of log dose response curves.

3.3.1. *Meleagrin B (**2**)*

Yellow solid (methanol); $[\alpha]_D^{20}$ −28.4 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 228 (4.22), 339 (4.15); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 197.1 (58.8), 225.1 (−26.6), 251.2 (12.1), 263.0 (10.6), 281.4 (10.7), 336.1 (−7.86); IR (KBr) ν_{max} 3295, 2938, 2869, 1715, 1656, 1589, 1460, 1388, 1335, 1213, 1121, 1048 cm^{-1}; ^{1}H NMR and ^{13}C NMR data

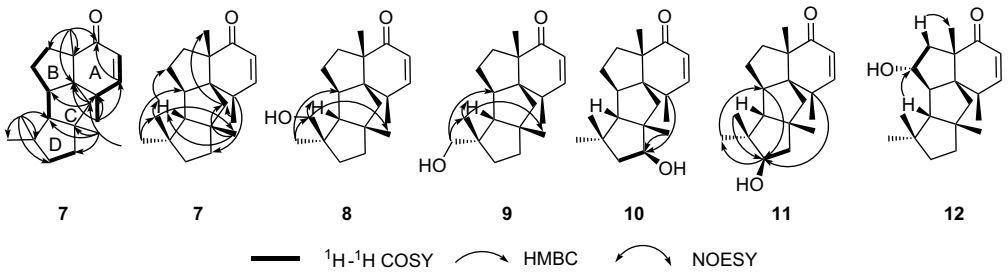


Figure 2. Selected ^{1}H–^{1}H COSY, HMBC, and NOESY correlations of compounds **7** to **12**.

Table 3
^{1}H NMR data of compounds **7–12** (600 MHz, TMS, δ ppm)

No.	**7**[a]	**8**[a]	**9**[a]	**10**[a]	**11**[a]	**12**[a]
2	5.97, dd, 9.9, 1.1	5.97, dd, 9.9, 1.1	5.97, dd, 9.9, 1.1	5.97, dd, 9.9, 1.1	5.97, d, 9.9	6.05, d, 10.4
3	6.93, dd, 9.9, 6.1	6.93, dd, 9.9, 6.1	6.93, dd, 9.9, 6.1	6.88, dd, 9.9, 5.5	6.93, dd, 9.9, 6.0	6.88, dd, 10.4, 6.0
4	2.66–2.70, m	2.70–2.75, m	2.65–2.69, m	2.66–2.69, m	2.62–2.67, m	2.69–2.74, m
6	2.25, dd, 9.4, 5.0	2.32, dd, 8.2, 4.9	2.29, dd, 7.6, 6.1	2.35, dd, 8.8, 5.5	2.24, dd, 7.6, 5.5	2.19, d, 4.3
7a	1.58–1.61, m	1.70–1.73, m	1.63–1.67, m	1.67–1.70, m	1.64–1.68, m	3.81, d, 4.9
7b	1.16–1.19, m	1.20–1.23, m	1.24–1.27, m	1.18–1.21, m	1.18–1.21, m	
8a	2.10, dd, 12.7, 6.0	2.13, ddd, 11.5, 5.5, 1.7	2.12, dd, 10.9, 4.9	2.11–2.14, m	2.07–2.11, m	2.24, d, 14.2,
8b	1.66–1.68, m	1.66–1.69, m	1.65–1.68, m	1.65–1.68, m	1.62–1.65, m	1.67, dd, 13.7, 4.9
10a	1.98, d, 14.2	2.01, d, 14.8	2.01, d, 14.8	2.11, d, 14.8	1.99, d, 14.8	2.02, d, 14.2
10b	1.64, d, 14.2	1.64, d, 14.8	1.64, d, 14.8	1.51, d, 14.8	1.64, d, 14.8	1.64, d, 14.2
12a	1.65–1.68, m	1.65–1.68, m	1.64–1.68, m	4.07, dd, 9.9, 7.1	2.07, dd, 13.8, 8.3	1.66–1.70, m
12b	1.60–1.63, m	1.60–1.63, m	1.60–1.63, m		1.60–1.63, m	1.61–1.65, m
13a	1.56–1.58, m	1.52–1.55, m	1.55–1.58, m	1.84, dd, 12.7, 7.1	3.96, dd, 10.0, 8.3	1.56–1.59, m
13b	1.48–1.51, m	1.43–1.47, m	1.46–1.50, m	1.64–1.67, m		1.52–1.55, m
15	1.39, d, 5.0	1.58, d, 4.9	1.54, d, 6.1	1.64, d, 5.5	1.52, d, 5.5	1.50, d, 4.3
16	1.20, d, 7.2	1.22, d, 7.1	1.20, d, 7.2	1.23, d, 7.7	1.21, d, 7.1	1.24, d, 7.7
17	1.18, s	1.19, s	1.18, s	1.23, s	1.17, s	1.18, s
18	1.28, s	1.21, s	1.30, s	1.14, s	1.34, s	1.27, s
19a	0.96, s	0.99, s	3.56, d, 10.9	0.97, s	0.99, s	1.02, s
19b			3.42, d, 10.9			
20a	1.06, s	3.41, d, 11.0	1.16, s	1.12, s	1.01, s	1.09, s
20b		3.38, d, 11.0				

[a] Measured in $CDCl_3$.

are provided in Table 1; HRESIMS $[M+H]^+$ m/z 736.4066 (calcd for $C_{43}H_{54}N_5O_6$ 736.4074).

3.3.2. *Meleagrin C* (***3***)

Yellow solid (methanol); $[\alpha]^{20}_D$ −54.1 (*c* 0.125, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.30), 342 (4.20); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 197.1 (52.8), 224.1 (−23.9), 248.2 (9.68), 258.0 (9.08), 274.4 (9.79), 342.1 (−6.74); IR (KBr) ν_{max} 3285, 2965, 1704, 1634, 1458, 1388, 1353, 1314, 1225, 1106, 1029 cm^{-1}; ^{1}H NMR and ^{13}C NMR data are provided in Table 1; HRESIMS $[M+H]^+$ m/z 532.2571 (calcd for $C_{29}H_{34}N_5O_5$ 532.2560).

Table 4
^{13}C NMR data of compounds **7–12** (150 MHz, TMS, δ ppm)

No.	**7**[a]	**8**[a]	**9**[a]	**10**[a]	**11**[a]	**12**[a]
1	206.0	205.7	205.7	205.3	205.6	204.6
2	127.4	127.3	127.5	127.6	127.6	127.8
3	154.5	154.2	154.2	153.9	154.0	152.3
4	37.5	37.8	37.7	38.7	37.4	36.6
5	58.9	59.9	59.1	60.8	58.7	58.7
6	56.0	54.7	54.8	54.5	56.1	66.0
7	34.3	34.2	34.4	34.5	34.5	81.4
8	39.0	38.7	39.1	39.0	39.0	48.0
9	57.4	57.5	57.3	57.5	57.5	55.3
10	47.9	46.5	47.3	42.7	48.8	47.4
11	52.3	52.7	52.5	56.1	46.7	52.4
12	38.9	38.4	38.3	78.5	47.5	38.6
13	40.7	36.6	36.2	49.7	80.5	41.0
14	42.7	48.1	47.9	36.0	44.7	42.8
15	73.6	68.2	71.3	72.5	72.4	70.1
16	18.8	18.5	18.8	18.6	18.9	18.8
17	21.0	21.1	20.9	21.1	21.0	21.6
18	32.5	31.1	32.3	22.3	33.3	32.7
19	27.1	22.5	70.7	28.9	24.3	27.4
20	31.0	71.9	26.1	34.5	23.2	31.2

[a] Measured in $CDCl_3$.

3.3.3. *Roquefortine F* (***5***)

Yellow solid (methanol); $[\alpha]^{20}_D$ −281.2 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 207 (4.05), 240 (4.08), 320 (4.24); IR (KBr) ν_{max} 3193, 2970, 1665, 1608, 1408, 1296, 1247, 1213, 1098, 1036 cm^{-1}; ^{1}H NMR and ^{13}C NMR data are provided in Table 2; HRESIMS $[M+H]^+$ m/z 420.2055 (calcd for $C_{23}H_{26}N_5O_3$ 420.2036).

3.3.4. *Roquefortine G* (***6***)

Yellow solid (methanol); $[\alpha]^{20}_D$ −244.1 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.24), 230 (4.17), 327 (4.20); IR (KBr) ν_{max} 3227, 2968, 1694, 1463, 1404, 1258, 1158, 1088 cm^{-1}; ^{1}H NMR and ^{13}C NMR data are provided in Table 2; HRESIMS $[M+H]^+$ m/z 518.2761 (calcd for $C_{29}H_{36}N_5O_4$ 518.2767).

3.3.5. *Conidiogenone B* (***7***)

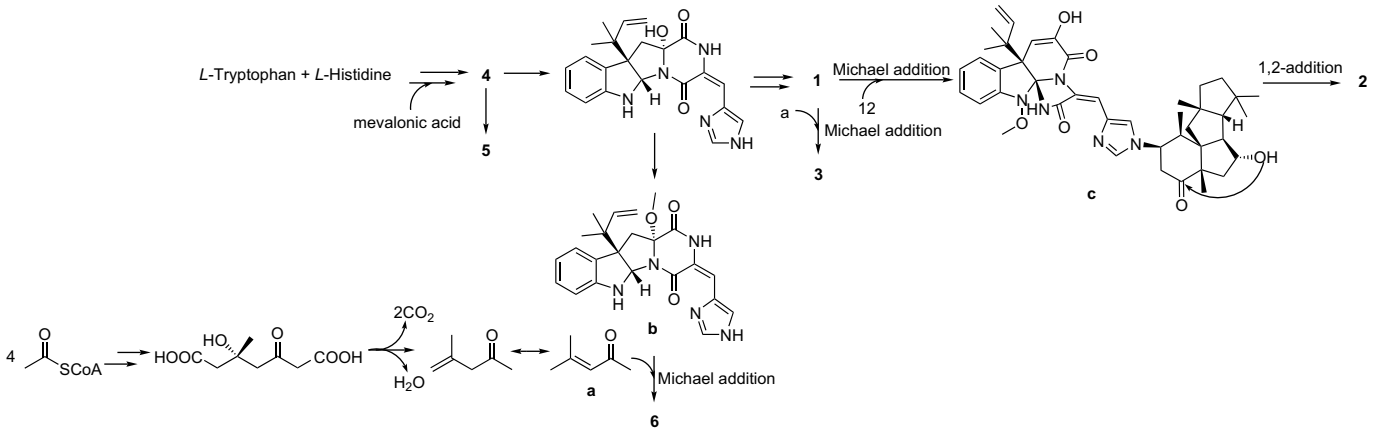
Colorless oil; $[\alpha]^{20}_D$ −6.0 (*c* 0.55, MeOH); UV (MeOH) λ_{max} (log ε) 231 (3.66); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 194.2 (−8.0), 234.1 (2.0), 305.3 (−0.20), 347.0 (0.55); ^{1}H NMR and ^{13}C NMR data are provided in Tables 3 and 4; HRESIMS m/z 287.2376 $[M+H]^+$ (calcd for $C_{20}H_{31}O$ 287.2375).

3.3.6. *Conidiogenone C* (***8***)

Colorless oil; $[\alpha]^{20}_D$ −11.9 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 231 (3.50); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 194.1 (−8.3), 234.2 (1.9), 304.3 (−0.88), 350.0 (1.1); ^{1}H NMR and ^{13}C NMR data are provided in Tables 3 and 4; HRESIMS m/z 303.2322 $[M+H]^+$ (calcd for $C_{20}H_{31}O_2$ 303.2324).

3.3.7. *Conidiogenone D* (***9***)

Colorless oil; $[\alpha]^{20}_D$ −8.6 (*c* 0.34, MeOH); UV (MeOH) λ_{max} (log ε) 231 (3.60); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 194.2 (−8.5), 238.4 (1.6), 303.3 (−0.50), 347.1 (0.59); ^{1}H NMR and ^{13}C NMR data are provided in Tables 3 and 4; HRESIMS m/z 303.2328 $[M+H]^+$ (calcd for $C_{20}H_{31}O_2$ 303.2324).



Scheme 1. Postulated biosynthetic pathway of **1**–**6**.

Table 5
Cytotoxicity data for compounds **2**, **3**, and **5**–**12** against established cancer cell lines

Compound	Cytotoxicity (IC_{50}, μM)			
	A-549	HL-60	BEL-7402	MOLT-4
2	2.7	6.7	1.8	2.9
3	9.9	>50	10.0	4.7
5	14.0	33.6	13.0	21.2
6	42.5	36.6	>50	>50
7	40.3	28.2	>50	>50
8	>50	0.038	0.97	>50
9	9.3	5.3	11.7	21.1
10	15.1	8.5	>50	25.8
11	42.2	17.8	17.1	>50
12	8.3	1.1	43.2	4.7

3.3.8. *Conidiogenone E* (***10***)

Colorless oil; $[\alpha]^{20}_D$ −26.2 (*c* 0.075, MeOH); UV (MeOH) λ_{max} (log ε) 231 (3.69); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 193.1 (−8.1), 245.1 (2.3), 305.7 (−0.32), 353.0 (0.43); ^{1}H NMR and ^{13}C NMR data are provided in Tables 3 and 4; HRESIMS *m*/*z* 303.2326 $[M+H]^+$ (calcd for $C_{20}H_{31}O_2$ 303.2324).

3.3.9. *Conidiogenone F* (***11***)

Colorless oil; $[\alpha]^{20}_D$ −13.7 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 231 (3.88); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 193.2 (−7.2), 237.1 (1.7), 305.7 (−0.47), 350.0 (0.44); ^{1}H and ^{13}C NMR data are provided in Tables 3 and 4; HRESIMS *m*/*z* 303.2334 $[M+H]^+$ (calcd for $C_{20}H_{31}O_2$ 303.2324).

3.3.10. *Conidiogenone G* (***12***)

Colorless oil; $[\alpha]^{20}_D$ +27.7 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 231 (3.88); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 193.2 (−7.6), 232.4 (6.4), 300.5 (−0.57), 330.1 (0.77); ^{1}H and ^{13}C NMR data are provided in Tables 3 and 4; HRESIMS *m*/*z* 325.2150 $[M+Na]^+$ (calcd for $C_{20}H_{30}O_2Na$ 325.2144).

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