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Penigrisacids A–D, Four New Sesquiterpenes from the Deep-Sea-Derived *Penicillium griseofulvum*

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Abstract: Four new (penigrisacids A–D, **1**–**4**) and one known (**5**) carotane sesquiterpenoids were isolated from the deep-sea-derived fungus *Penicillium griseofulvum*, along with four known compounds (**6**–**9**). The planar structures and relative configurations of the new compounds were determined by extensive analysis of the NMR and HRESIMS data. The absolute configurations were established by comparison of the experimental and calculated ECD (electronic circular dichroism) spectra or OR (optical rotation) value. Compound **9** exhibited potent anti-food allergic activity with IC_{50} value of 28.7 μM, while **4** showed weak cytotoxicity against ECA-109 tumor cells (IC_{50} = 28.7 μM).

Keywords: deep-sea-derived fungus; *Penicillium griseofulvum*; carotanes; sesquiterpenes; anti-food allergy

1. Introduction

Carotanes (also called daucanes) are bicyclic sesquiterpenes mainly isolated from the plants of the Umbelliferae family and the fungus *Aspergillus terreus* [1–4]. Some of these compounds showed significant biological activities, for example, aspterric acid was found to inhibit Arabidopsis pollen development at meiosis [5]. More recently, studies showed aspterric acid could be used as a herbicide [6]. In our current investigations on the bioactive secondary metabolites from the deep-sea-derived microorganisms [7–10], the crude extract of *Penicillium griseofulvum*, a fungus isolated from the Indian Ocean, showed potent in vitro anti-food allergic activity. Accordingly, a systematic isolation was conducted, which led to the isolation of five carotanes (**1**–**5**, Figure 1) and four other compounds (**5**–**9**). Herein, we report the isolation, structure elucidation, as well as anti-allergic and anti-tumor bioactivities of these compounds.

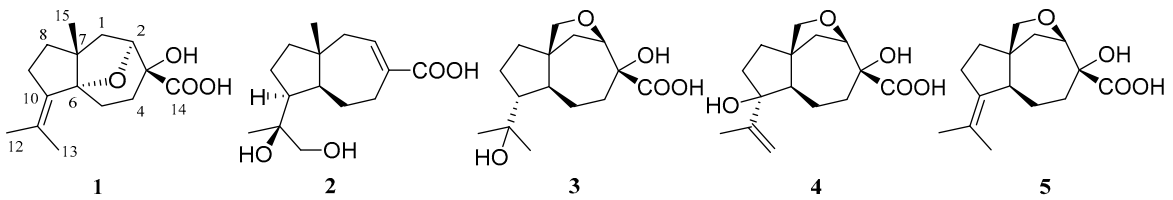

Figure 1. Chemical structures of **1**–**5**, isolated from *Penicillium griseofulvum*.

2. Results and Discussion

Compound **1** was obtained as a colorless oil. Its molecular formula was established as $C_{15}H_{22}O_4$ on the basis of the sodium adduct ion peak at *m*/*z* 289.1411 $[M + Na]^+$ in its HRESIMS spectrum, indicating

five degrees of unsaturation. The ^{1}H NMR spectrum (Table 1) showed the presence of three methyl singlets (δ_H 1.18, s, Me-15; 1.82, s, Me-12; 1.63, s, Me-13) and one oxymethine (δ_H 4.48, d, J = 7.5 Hz, H-2). The ^{13}C NMR spectrum, in association with the DEPT and HSQC spectra, indicated 15 carbon signals (Table 2), including three methyls (δ_C 20.1, 21.3, and 23.5, for Me-15, 12, and 13, respectively), five methylenes (δ_C 27.7, C-5; 28.5, C-4; 33.3, C-9; 40.4, C-1; 41.6, C-8), one oxygenated methine (δ_C 82.6, C-2), and six non-protonated carbons, including one carboxyl (δ_C 178.0, C-14), two olefinic (δ_C 135.6, C-10; δ_C 131.6, C-11), two oxygenated (δ_C 74.4, C-3; 93.9, C-6); and one aliphatic (δ_C 54.0, C-7) carbons. Since one carboxyl and two olefinic carbons accounted for two degrees of unsaturation, **1** was assumed to be a tricyclic molecule. The COSY correlations showed three isolated spin systems from H-2 (δ_H 4.48) to H_2-1 (δ_H 2.01, 1.90), H_2-4 (δ_H 2.18, 1.69) to H_2-5 (δ_H 2.74, 1.55), and H_2-8 (δ_H 1.63, 1.55) through H_2-9 (δ_H 2.38) to Me-12 and Me-13. The HMBC spectrum displayed correlations from Me-15 to C-1/C-6/C-7/C-8, H-2 to C-3/C-4/C-6/C-14, and H_2-5 to C-6/C-7/C-10. Taking together the COSY and HMBC correlations, the planar structure of **1** was established (Figure 2), which was very similar to aspterric acid except that the ether bond was between C-2 and C-6 in **1**, instead of C-2 and C-15 in aspterric acid. In the NOESY spectrum, strong correlations were observed from H-1a (δ_H 1.90) to Me-15, H-1b (δ_H 2.01) to H-2, Me-15 to H-5a (δ_H 1.55), suggesting the configurations of C-2 and C-6 at the epoxy moiety were opposite to that of Me-15. Accordingly, the relative structure of **1** was assigned as 10,11-dehydro-3α-hydroxy-2α,6α-epoxycarotan-14-oic acid.

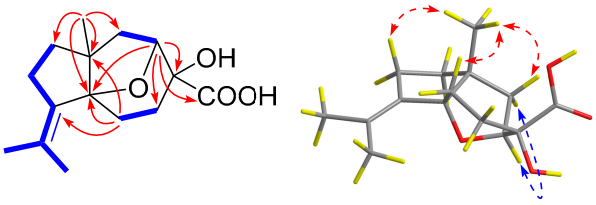

Figure 2. Key COSY (—), HMBC (⌒), and NOESY (⇢) correlations of **1**.

Table 1. ^{1}H (400 MHz) NMR spectroscopic data of **1**–**5** (δ in ppm, J in Hz within parentheses).

No.	1 [a]	2 [a]	3 [b]	4 [a]	5 [a]
1	2.01, dd (13.2, 7.6) 1.90, d (13.2)	2.41, dd (14.9, 9.1) 2.14, br d (14.2)	2.00, dd (12.7, 8.5) 1.85, d (12.7)	2.20, dd (13.0, 8.5) 2.00, d (13.0)	2.40, overlap 1.48, overlap
2	4.48, d (7.5)	7.07, dt (8.6, 3.1)	4.13, d (8.4)	4.28, d (8.4)	4.31, d (8.4)
4	2.18, dd (13.5, 6.4) 1.69, m	2.99, dd (15.8, 5.8) 1.93, overlap	2.18, dd (13.8, 7.6) 1.20, m	2.42, dd (13.7, 7.5) 1.33, m	2.28, m 2.03, overlap
5	2.74, dt (12.6, 6.2) 1.55, overlap	1.93, overlap 1.25, d (13.2)	1.93, dd (11.0, 8.0) 1.43, overlap	1.77, m 1.45, dd (13.6, 7.6)	2.16, dd (13.1, 8.4) 2.03, overlap
6	-	1.59, t (10.5)	1.42, overlap	1.72, m	2.32, m
8	1.63, m 1.55, overlap	1.41, m	1.56, m 1.26, dd (11.1, 7.6)	1.84, m 1.65, m	2.40, overlap 1.67, m
9	2.38, m	1.78, m 1.68, m	1.41, overlap	2.10, m 1.61, m	1.69, m 1.48, overlap
10	-	1.93, overlap	1.58, m	-	-
12	1.82, br s	1.19, s	1.03, s	1.76, s	1.73, br s
13	1.63, br s	3.45, br s	1.05, s	5.01, s 4.84, s	1.60, br s
15	1.18, s	0.79, s	3.64, d (8.0) 3.18, d (8.1)	4.62, d (8.0) 3.34, d (7.9)	3.70, d (8.0) 3.35, d (8.7)

[a] Recorded in CD_3OD. [b] Recorded in DMSO-d_6.

Table 2. ^{13}C (100 MHz) NMR spectroscopic data of **1**–**5**.

No.	1 [a]	2 [a]	3 [b]	4 [a]	5 [a]
1	40.4 CH_2	42.3 CH_2	35.8 CH_2	38.9 CH_2	35.2 CH_2
2	82.6 CH	142.8 CH	82.8 CH	84.0 CH	84.9 CH
3	74.4 C	136.5 C	77.6 C	79.6 C	79.5 C
4	28.5 CH_2	28.5 CH_2	34.3 CH_2	35.3 CH_2	33.0 CH_2
5	27.7 CH_2	27.0 CH_2	24.8 CH_2	19.8 CH_2	36.8 CH_2
6	93.9 C	55.8 CH	52.3 CH	58.4 CH	56.7 CH
7	54.0 C	43.5 C	52.7 C	52.7 C	54.2 C
8	41.6 CH_2	42.4 CH_2	33.6 CH_2	33.3 CH_2	24.9 CH_2
9	33.3 CH_2	24.6 CH_2	26.8 CH_2	40.0 CH_2	35.1 CH_2
10	135.6 C	50.1 CH	51.8 CH	86.0 C	136.7 C
11	131.6 C	76.3 C	71.5 C	150.7 C	125.2 C
12	21.3 CH_3	22.9 CH_3	26.6 CH_3	19.7 CH_3	21.0 CH_3
13	23.5 CH_3	69.5 CH_2	29.2 CH_3	110.3 CH_2	23.4 CH_3
14	178.0 C	172.0 C	175.8 C	177.9 C	177.9 C
15	20.1 CH_3	19.2 CH_3	74.5 CH_2	76.2 CH_2	76.5 CH_2

[a] Recorded in CD_3OD. [b] Recorded in DMSO-d_6.

To determine the absolute configuration, a theoretical calculation on its ECD spectrum was performed. The calculated ECD spectra of 2*S*,3*R*,6*S*,7*R*-**1** (**1a**) and 2*R*,3*S*,6*R*,7*S*-**1** (**1b**) were obtained by time-dependent density functional theory (TD-DFT) at the B3LYP/6-31+G(d,p) level in ACN (acetonitrile). As shown in Figure 3, the calculated ECD spectrum of **1a** fits well with the experimental one. From the above-mentioned evidences, the structure of **1** was then established as (2*S*,3*R*,6*S*,7*R*)-10,11-dehydro-2,6-epoxy-3-hydroxy-14-carotanoic acid, and was named penigrisacid A.

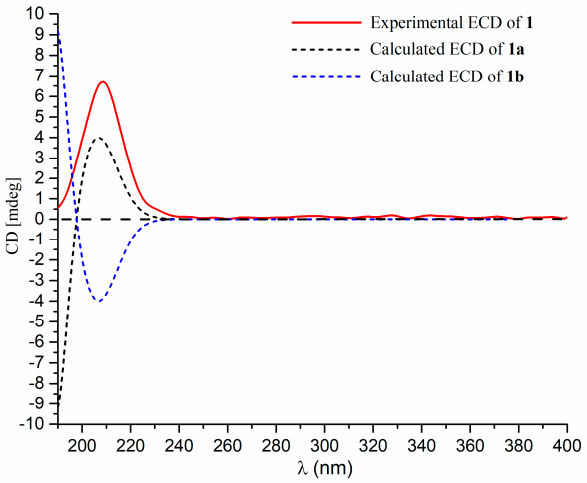

Figure 3. Experimental and calculated ECD spectra of **1**.

Compound **2** was also obtained as a colorless oil. The molecular formula $C_{15}H_{24}O_4$ was established according to its positive HRESIMS spectrum at *m*/*z* 291.1563 (calcd for $C_{15}H_{24}O_4Na$, 291.1572), indicating four degrees of unsaturation. The ^1H NMR spectrum exhibited two methyl singlets (δ_H 0.79, s, Me-12; 1.19, s, Me-15), one oxygenated methylene (δ_H 3.45, s, H_2-13), and one olefinic proton (δ_H 7.07, dt, J = 8.6, 3.1 Hz, H-2). The ^{13}C NMR spectrum displayed 15 carbons signals which, in combination with DEPT and HSQC spectra, could be categorized as two methyls (δ_C 19.2, Me-15; 22.9, Me-12), one oxymethylene (δ_C 69.5, C-13), five sp^3 methylene (δ_C 24.6, C-9; 27.0, C-5; 28.5, C-4; 42.3, C-1; 42.4, C-8), two methines (δ_C 50.1, C-10; 55.8, C-6), one protonated sp^2 (δ_C 142.8, C-2), and four non-protonated carbons, including one carboxyl (δ_C 172.0, C-14), one olefinic (δ_C 136.5, C-3) and one oxygenated (δ_C 76.3, C-11) carbons. Since one carboxyl and two olefinic carbons accounted for two degrees of unsaturation, **2** was deduced to be a bicyclic molecule. The COSY spectrum exhibited

correlations from H-2 (δ_H 7.07) to H_2-1 (δ_H 2.41, dd, J = 14.9, 9.1 Hz, H-1a; 2.14, br d, J = 14.2 Hz, H-1b) and the overlapped proton at δ_H 1.93; H-5a (δ_H 1.25, d, J = 13.2 Hz) to H-4a (δ_H 2.99, dd, J = 15.8, 5.8 Hz) and H-6 (δ_H 1.59, t, J = 10.5 Hz); H_2-9 (δ_H 1.78 m, 1.68 m) to H_2-8 (δ_H 1.41, m) and the overlapped proton at δ_H 1.93. The HMBC spectrum showed correlations from Me-12 to C-10, C-11, C-13, from Me-15 to C-1, C-6, C-7, C-8, from H_2-4 to C-2, C-3, C-14, and from H_2-9 to C-6, C-10, C-11. Taking together the molecular formula, the COSY, and HMBC spectra, the planar structure of **2** was then established. The NOESY spectrum showed correlations from Me-15 to H-1a (δ_H 2.41)/H-5a (δ_H 1.25)/H-9a (δ_H 1.78), from H-1b (δ_H 2.14) to H-6, and from H_2-13 to H-5b (δ_H 1.93)/H-6/H-9b (δ_H 1.68)/H-10 (Figure 4). Therefore, H-6, H-7, and H_2-13 were on the same face which was opposite to that of Me-15.

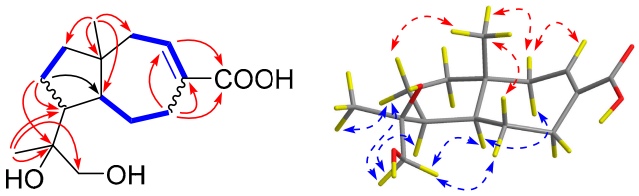

Figure 4. Key COSY (—), HMBC (⌒), and NOESY (⌒) correlations of **2**.

To determine the absolute configuration of the stereogenic carbons of **2**, a theoretical calculation of its ECD spectrum in ACN was performed. As shown in Figure 5, the calculated ECD spectrum of (6*S*,7*R*,10*S*,11*S*)-**2** (**2a**) fit well with that of the experimental one. Accordingly, **2** was elucidated as (6*S*,7*R*,10*S*,11*S*)-2,3-dehydro-11,13-dihydroxy-14-carotanoic acid, and was named penigrisacid B.

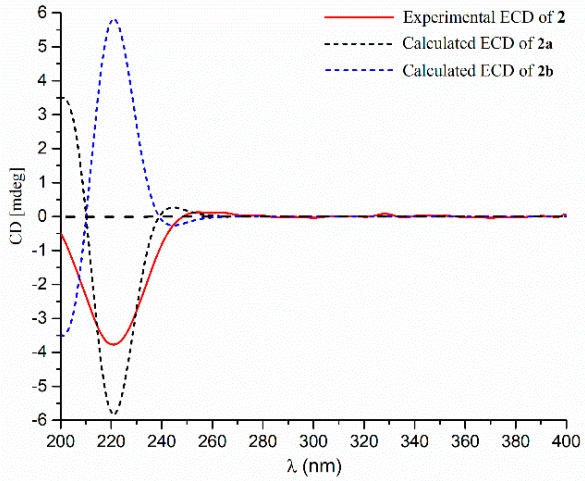

Figure 5. Experimental and calculated ECD spectra of **2**.

Compound **3** showed a molecular formula $C_{15}H_{24}O_5$, as established by its positive HRESIMS spectrum at *m*/*z* 307.1523 (calcd for $C_{15}H_{24}O_5Na$, 307.1521), indicating four degrees of unsaturation. The ^{13}C NMR spectrum showed 15 carbon signals which, according to DEPT and HSQC spectra, could be classified as two methyls (δ_C 26.6, C-12; 29.2, C-13), five methylenes (δ_C 24.8, C-5; 26.8, C-9; 33.6, C-8; 34.3, C-4; and 35.8, C-1), one oxymethylene (δ_C 74.5, C-15), two methines (δ_C 51.8, C-10; 52.3, C-6), one oxymethine (δ_C 82.8, C-2), and four non-protonated carbons including one carboxyl group (δ_C 175.8), two oxyquaternary carbons (δ_C 77.6 and 71.5). The general aspect of the ^{13}C NMR spectrum of **3** was similar to that of aspterric acid, except that the signals of the olefinic carbons (C-10/C-11) in aspterric acid were replaced by the signals of one methine (δ_H 1.58, m, H-10; δ_C 51.8, C-10) and one oxyquaternary carbon (δ_C 71.5, C-11) in **3**, respectively. This was confirmed by the HMBC correlations from Me-12 and Me-13 to C-10 and C-11. The NOESY spectrum showed a correlation from H-10 (δ_H 1.58, m) to H-15a (δ_H 3.64, d, J = 8.0 Hz), indicating H-10 is in the β position. Further by comparison

of the experimental OR ($[\alpha]_D^{25}$ −49.4) and the calculated one for (2*R*,3*R*,6*S*,7*S*,10*R*)-**3** ($[\alpha]_D^{25}$ −52.9), the full structure of **3** was then established as (10*R*)-10,11-dihydro-11-hydroxyaspterric acid, and was named penigrisacid C.

The molecular formula of **4** was assigned as $C_{15}H_{22}O_5$ on the basis of its positive HRESIMS spectrum at *m*/*z* 305.1365 (calcd for $C_{15}H_{22}O_5Na$, 305.1359), indicating five degrees of unsaturation. The ^{1}H NMR spectrum exhibited one methyl singlet (δ_H 1.76, Me-12), one sp^2 oxymethylene (δ_H 4.62, d, J = 8.0 Hz; 3.34, d, J = 7.9 Hz, H-15), one olefinic methylene (δ_H 5.01, s; 4.84, s, H_2-13), and one oxymethine (δ_H 4.28, d, J = 8.4, H-2). The ^{13}C NMR spectrum in association with the DEPT (distortionless enhancement by polarization transfer) and HSQC (heteronuclear single-quantum correlation) spectra indicated 15 carbon signals ascribed to one methyl (δ_C 19.7, Me-12); five aliphatic (δ_C 19.8, C-5; 33.3, C-8; 35.3, C-4; 38.9, C-1; 40.0, C-9), one oxygenated (δ_C 76.2, C-15), and one olefinic (δ_C 110.3, C-13) methylenes; one aliphatic (δ_C 58.4, C-6) and one oxygenated (δ_C 84.0, C-2) methines; one aliphatic (δ_C 52.7, C-7), two oxygenated (δ_C 79.6, C-3; 86.0, C-10), one olefinic (δ_C 150.7, C-11), and one carbonyl (δ_C 179.9, C-12) quaternary carbons (Table 2). These signals were very similar to those of **3**, except that the hydroxy moiety at the C-11 in **3** was located at the C-10 position in **4**, while the methyl group at the C-11 position was displaced by a terminal double bond. This was evidenced by the COSY correlations from H-2 (δ_H 4.28) to H_2-1a (δ_H 2.20), from H_2-5a (δ_H 1.45) to H_2-4a (δ_H 2.24) and H-6 (δ_H 1.72), from H_2-8a (δ_H 1.84) to H_2-9a (δ_H 2.10), and from Me-12 (δ_H 1.76) to H_2-13 (δ_H 5.01, 4.84); as well as the HMBC cross peaks of Me-12 (δ_H 1.76 s) to C-10 (δ_C 86.0 s), C-11 (δ_C 150.7 s), and C-13 (δ_C 110.3 t). Since H-13a (δ_H 5.01 s) was correlated to H-6 (δ_H 1.72 m) in the NOESY spectrum, the hydroxy group at the C-10 position was deduced to be β-orientation. Therefore, compound **4** was established as 10,11-dihydro-10β-hydroxy-11,13-dehydroaspterric acid, and was named penigrisacid D. All detailed data of theoretical calculations, NMR, and HRESIMS spectra of **1**–**4** can be found in the Supplementary Materials Figures S1–S30 Tables S1 and S2.

By comparison of the NMR and MS data with those in references, the structures of five known compounds were identified as aspterric acid (**5**) [11], aspermytin A (**6**) [12], 1-propanone,3-hydroxy-1-(1,2,4a,5,6,7,8,8a-octahydro-2,5-dihydroxy-1,2,6-trimethyl-1-naphthalenyl) (**7**) [13], craterellone D (**8**) [14], and 3,4-dihydro-3,4,8-trihydroxy-1(2H)-naphthalenone (**9**) [15].

All the nine isolates were tested for anti-food allergic activity. Compound **9** exhibited potent effect with an IC_{50} value of 28.7 μM, compared to 91.6 μM of the positive control, loratadine. While compound **6** showed moderate effect with an IC_{50} value of 97.3 μM. Therefore, **9** could be a promising lead compound for anti-food allergic agent. Moreover, **1**–**9** were also subjected to cytotoxicity assay against five different cancer cells, i.e., BIU-87, Bel-7402, ECA-109, Hela-S3, and PANC-1. However, only **4** showed weak effect on ECA-109 tumor cells with IC_{50} value of 28.7 μM.

3. Materials and Methods

3.1. General Experimental Procedures

Optical rotations were obtained from an MCP 100 polarimeter (Anton Paar Trading Co. Ltd., Shanghai, China). HRESIMS spectra were conducted on a Xevo G2 Q-TOF mass spectrometer (Waters Corporation, Milford, MA, USA). NMR spectra were recorded on a Bruker 400 MHz spectrometer (Bruker, Fällanden, Switzerland). UV data were recorded from a UV-8000 UV/Vis spectrophotometer (Shanghai Metash instrument Co., Ltd., Shanghai, China). ECD spectra were measured with a Chirascan spectropolarimeter (Applied Photophysic, Beverly, MA, USA). Materials for column chromatography involved silica gel, ODS (octadecylsilyl), and Sephadex LH-20.

3.2. Fermentation and Extraction

The fungus was isolated from the deep-sea-sediment (−1420 m) of the Indian Ocean in 2005 by Prof. De-Zan Ye of the Third Institute of Oceanography. It was purchased from the Marine Culture Collection of China with the accession number of MCCC 3A00225. The fungus was cultured on a PDA

plate at 25 °C for 3 days. The fresh mycelia and spores were inoculated to 10 × 250 mL Erlenmeyer flasks containing 120 mL of the ISP medium **2** (1.0 L contains 4.0 g yeast extract, 10.0 g malt extract, 4.0 g dextrose, and 20.0 g agar), which were incubated in a 180 rpm rotary shaker at 28 °C for 5 days. Then the spore cultures were used to inoculate 100 × 1 L Erlenmeyer flasks containing corn medium (100 g corn and 120 mL tap water for each flask) to perform the large-scale fermentation. After 62 days, the fermentation broth was extracted with EtOAc three times to provide a crude extract (55.4 g).

3.3. Isolation and Purification

The extract was fractionated into six fractions (Fr.1−Fr.6) by column chromatography (CC) on silica gel using a gradient CH_2Cl_2-MeOH (0→100%). Fr.4 (4.9 g) was subjected to CC (26 mm × 310 mm) over ODS using H_2O-MeOH (10→100%) and Sephadex LH-20 (MeOH), followed by purification using preparative TLC (CH_2Cl_2-acetone, 2:1) to provide **1** (7.8 mg) and **6** (25.9 mg). Fr.5 (40.0 g) was chromatographed over ODS (H_2O-MeOH, 5→80%, 49 mm × 460 mm) to get 15 subfractions (Fr.5.1–Fr.5.15), which were further chromatographed on a Sephadex LH-20 (MeOH). Compounds **8** (4.5 mg) and **9** (9.0 mg) were obtained from fractions Fr.5.3 and Fr.5.2 by prep. TLC using EtOAc-MeOH (20:1) and CH_2Cl_2-MeOH (10:1), respectively. Fraction Fr.5.4 was subjected to CC over Sephadex LH-20 (CH_2Cl_2-MeOH, 1:1), subsequent purification by prep. TLC (PE-EtOAc, 1:2) provided **3** (29.5 mg) and **7** (19.5 mg). Fr.5.5 was subjected to HPLC (MeOH-H_2O, 20→40%), followed by prep. TLC (CH_2Cl_2-MeOH, 10:1) to give **4** (21.4 mg). By prep. TLC using CH_2Cl_2-MeOH (10:1), **2** (6.0 mg) and **5** (9.3 mg) were obtained from fractions Fr.5.11 and Fr.5.10, respectively.

Penigrisacid A (**1**): colorless oil; $[\alpha]_D^{25}$ +66.2 (c 0.14, EtOH); UV (MeOH) λ_{max} (log ε) 211 (3.29) nm; ECD (ACN) $\Delta\varepsilon_{209}$ +0.32; ^{1}H and ^{13}C NMR data, see Table 1; Table 2; HRESIMS *m*/*z* 289.1411 $[M + Na]^+$ (calcd. for $C_{15}H_{22}O_4Na$, 289.1410).

Penigrisacid B (**2**): colorless oil; $[\alpha]_D^{25}$ −29.4 (c 0.11, EtOH); UV (MeOH) λ_{max} (log ε) 209 (3.94) nm; ECD (ACN) $\Delta\varepsilon_{221}$ −0.24; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 291.1563 $[M + Na]^+$ (calcd. for $C_{15}H_{24}O_4Na$, 291.1572).

Penigrisacid C (**3**): colorless oil; $[\alpha]_D^{25}$ −49.4 (c 0.16, EtOH); UV (MeOH) λ_{max} (log ε) 201 (2.98) nm; ECD (ACN) $\Delta\varepsilon_{207}$ −0.68; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 307.1523 $[M+Na]^+$ (calcd. for $C_{15}H_{24}O_5Na$, 307.1521).

Penigrisacid D (**4**): colorless oil; $[\alpha]_D^{25}$ −30.9 (c 0.41, EtOH); UV (MeOH) λ_{max} (log ε) 209 (3.51) nm; ECD (ACN) $\Delta\varepsilon_{198}$ +0.44; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 305.1365 $[M + Na]^+$ (calcd. for $C_{15}H_{22}O_5Na$, 305.1359).

3.4. Theoretical Calculations

As reported previously [7], the preliminary conformational analyses were carried out using RDKit Toolkit [16] by Genetic algorithm at MMFF94 force field. Subsequently, the dominating conformers were re-optimized using density functional theory (DFT) at the B3LYP/6-31+G(d) level. Further calculation in the same level with PCM were conducted for ECD in ACN and OR in MeOH. The ECD spectra of different conformers were simulated by the overlapping Gaussian function [17]. The final spectrum was averaged according to the Boltzmann distribution theory and their relative Gibbs free energy (ΔG).

3.5. Anti-Allergic Experiment

As reported previously [18], anti-allergic bioassay was conducted on RBL-2H3 cells. In brief, RBL-2H3 cells were seeded into 96-well cell culture plates to incubate with dinitrophenol specific IgE overnight. IgE-sensitized RBL-2H3 cells were pre-treated with tested compounds for 1 h and stimulated with dinitrophenyl-bovine serum albumin. Phosphate-buffered saline (PBS) buffer and loratadine were used as negative and positive controls, respectively. The bioactivity was quantified by measuring the fluorescence intensity of the hydrolyzed substrate in a fluorometer.

3.6. Cytotoxicity Assay

The in vitro antiproliferative assay was performed using MTT method according to the previously reported protocol [19]. Five different cancer cell lines (BIU-87, Bel-7402, ECA-109, Hela-S3, and PANC-1) were seeded into 96-well cell culture plates. After 24 h, different concentrations of tested compounds were added, and the incubation was continued for another 48 h. Then 20 μL MTT solution were added and cell viability was evaluated by measuring the absorbance at 570 nm.

4. Conclusions

From the deep-sea-derived fungus *Penicillium griseofulvum*, four new (penigrisacids A–D) and five known compounds (aspterric acid, aspermytin A, 1-propanone,3-hydroxy-1-(1,2,4a,5,6,7,8,8a-octahydro-2,5-dihydroxy-1,2,6-trimethyl-1-naphthalenyl), craterellone D, 3,4-dihydro-3,4,8-trihydroxy-1(2H)-naphthalenone) were obtained. 3,4-Dihydro-3,4,8-trihydroxy-1(2H)-naphthalenone, showed potent anti-food allergic activity (IC_{50} = 28.7 μM), whereas penigrisacid D showed weak cytotoxicity against esophageal cancer cell line ECA-109 tumor cells (IC_{50} = 28.7 μM).

Supplementary Materials: The following are available online at http://www.mdpi.com/1660-3397/17/9/507/s1, Figures S1–S30 and Tables S1–S2: The theoretical calculation data for **1**–**3,** 1D and 2D NMR as well as HRESIMS spectra of **1**−**4**.

Author Contributions: X.-W.Y. designed and coordinated the project; C.-P.X. conducted fungal fermentation as well as compound isolation and characterization. C.-L.X. assisted C.-P.X.'s experiments. J.-M.X. contributed to the structure identification of isolated compounds. Q.-M.L. and G.-M.L. conducted anti-food allergic experiments. W.-X.L. contributed to the extraction and isolation. D.-Z.Y. isolated the fungus and deposited it to MCCC. C.-P.X. and X.-W.Y wrote the paper, while critical revision of the publication was performed by all authors.

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