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PENICITRINOLS F–I, NEW CITRININ DERIVATIVES FROM THE MARINE-DERIVED FUNGUS *PENICILLIUM CITRINUM*

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Abstract – Four new citrinin derivatives, namely, penicitrinols F, G, H, and I (**1**–**4**), along with four known compounds, namely, 4,6-dihydroxy-2,3-dimethyl-benzaldehyde (**5**), pennicitrinone A (**6**), dicitrinone B (**7**), and dicitrinone C (**8**), were isolated from the marine-derived fungus *Penicillium citrinum*. Their structures were established using spectroscopic methods.

Penicillium citrinum is a rich source of various citrinin derivatives.[1,2] Our previous chemical investigation of *P. citrinum* resulted in the isolation of three new citrinin derivatives, namely, penicitrinols C–E,[3] Our continuing search for bioactive compounds from this organism has further resulted in the isolation of another four new derivatives, namely, penicitrinols F–I (**1**–**4**), along with four known analogues (**5**–**8**).[2,4] In this paper, we report the isolation and structural elucidation of these metabolites.

Bioactive ethyl acetate extract of *P. citrinum* was seperated by chromatography on Si gels and Sephadex LH-20 columns and purified by reversed-phase HPLC to yield the eight compounds (**1**–**8**).

Compound **1** was established to have the molecular formula $C_{15}H_{20}O_4$ by its HRESIMS. The ^{1}H NMR spectrum of **1** indicated the presence of an exchangeable proton (δ 9.13), an aromatic proton (δ 6.18), three protons attached to oxygenated carbons (δ 5.31, 4.51, and 3.98), and four methyl groups (δ 3.41, 1.95, 1.19, and 1.10). The ^{13}C NMR and DEPT data indicated the presence of the following: six aromatic carbons, two of which were oxygenated (δ 155.1 and 148.7) and one was protonated (δ 100.8); three oxymethine carbons (δ 100.2, 73.5, and 60.3); one methylene carbon (δ 36.3); one methine carbon (δ 33.7); and four methyl carbons (δ 55.1, 21.5, 17.7, and 9.9). The connectivities of these groups and

carbons were deduced from the COSY and HMBC spectra (Figure 2). The NOESY correlations between H-3/H-12, H-4/H-11, H-1/H-11, and H-1/H-15 observed in **1** indicated that H-1, H-4, H-15, and CH_3-11 were on the same side, whereas CH_3-12 and OCH_3 were on the reverse side. Therefore, the structure of **1** was elucidated as shown (Figure 1).

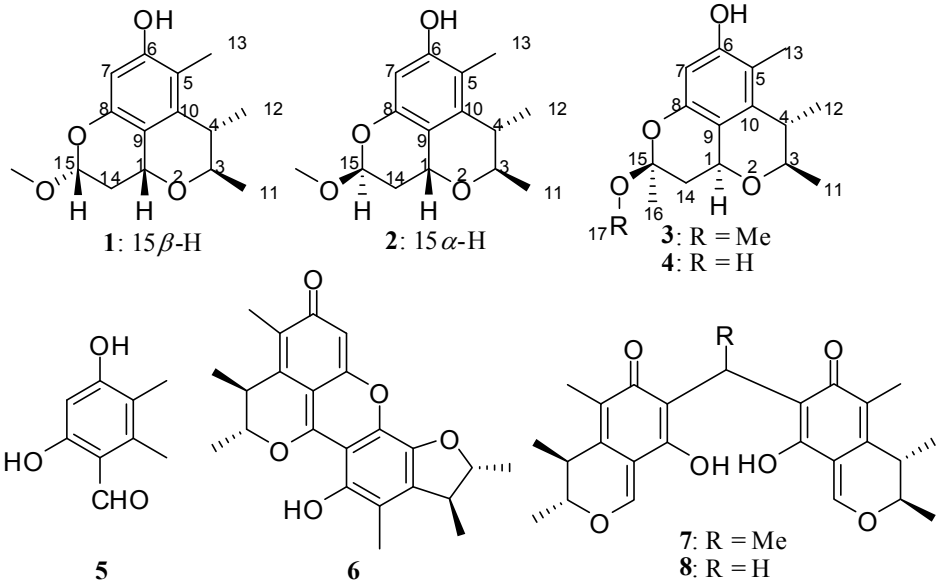

Figure 1. Structures of compounds **1**–**8**

Compound **2** has the same molecular formula ($C_{15}H_{20}O_4$) as **1**, based on HRESIMS data (*m/z*: 263.1266 $[M - H]^-$). The NMR (1D NMR, COSY, HMQC, and HMBC) data revealed that **2** and **1** possessed the same planar structure. However, the NOESY correlations of H-1 with H-11 and OCH_3, as well as H-4 with H-11 suggested that **2** and **1** were stereoisomers with different C-15 configurations.

Compound **3** was obtained as a yellow gum. Its molecular formula, $C_{16}H_{22}O_4$, was established using HRESIMS (*m/z* 277.1451 $[M - H]^-$). The analysis and comparison of the 1D NMR of **3** with those of **1** and **2** (Tables 1 and 2) suggested that the planar structure of **3** had one methyl group (δ_H 1.52, 3H; δ_C 23.3 q) more than **1** or **2**, which was supported by the MS data. The COSY and HMBC spectra suggested that this methyl group was connected to C-15. The NOESY correlations between H-3/H-12, H-4/H-11, H-1/H-3, and H-1/H-16 observed in **3** indicated that H-4, CH_3-11, and OCH_3 were on the same side. On the other hand, H-1, H-3, CH_3-12 and CH_3-16 were located on the reverse side.

Compound **4** was obtained as a yellow gum. Its molecular formula, $C_{15}H_{20}O_4$, was established using negative HRESIMS (*m/z* 263.1275, $[M - H]^-$, calcd for $C_{15}H_{19}O_4$, 263.1283). Comparing the 1D and 2D NMR data of **4** with those of **3** (Tables 1 and 2), an exchangeable proton (δ_H 6.44) in **4** replaced the *O*-methyl group (δ_H 3.28, 3H; δ_C 49.0 q) in **3**. The similar NOESY correlations indicated that the

configuration of **4** was the same as that of **3**.

Table 1. ^{1}H NMR data (400 MHz, *J* in Hz and δ in ppm) of compounds **1**–**4**

No	1[a]	2[a]	3[b]	4[a]
1	4.51 (H, dd, 12.1, 4.8)	4.62 (H, dd, 12.4, 4.8)	4.65 (H, dd, 12.0, 6.0)	4.49 (H, dd, 11.8, 6.0)
3	3.98 (H, q, 6.8)	3.98 (H, q, 6.8)	3.70 (H, dq, 6.8, 6.0)	3.56 (H, dq, 6.8, 6.2)
4	2.57 (H, q, 6.9)	2.60 (H, q, 6.9)	2.80 (H, dq, 6.8, 6.8)	2.67 (H, dq, 6.8, 6.8)
7	6.18 (H, s)	6.16 (H, s)	6.15 (H, s)	6.07 (H, s)
11	1.19 (3H, d, 6.8)	1.24 (3H, d, 6.8)	1.36 (3H, d, 6.0)	1.24 (3H, d, 6.2)
12	1.10 (3H, d, 6.9)	1.08 (3H, d, 6.9)	1.21 (3H, d, 6.8)	1.11 (3H, d, 6.8)
13	1.95 (3H, s)	1.95 (3H, s)	2.10 (3H, s)	1.96 (3H, s)
14	1.52 (H, m) 2.43 (H, m)	1.70 (H, m) 2.09 (H, m)	1.74 (H, t, 12.0) 2.39 (H, dd, 12.0, 6.0)	1.45 (H, t, 11.8) 2.15 (H, dd, 11.8, 6.0)
15	5.31 (H, dd, 8.2, 5.2)	5.21 (H, dd, 3.2, 1.5)		
16			1.52 (3H, s)	1.48 (3H, s)
OCH_3	3.41 (3H, s)	3.41 (3H, s)	3.28 (3H, s)	
OH-15				6.44 (H, brs)
OH-6	9.13 (H, brs)	9.13(H, brs)	5.58 (H, brs)	9.06 (H, brs)

[a]DMSO-d_6 as solvent. [b]$CDCl_3$ as solvent.

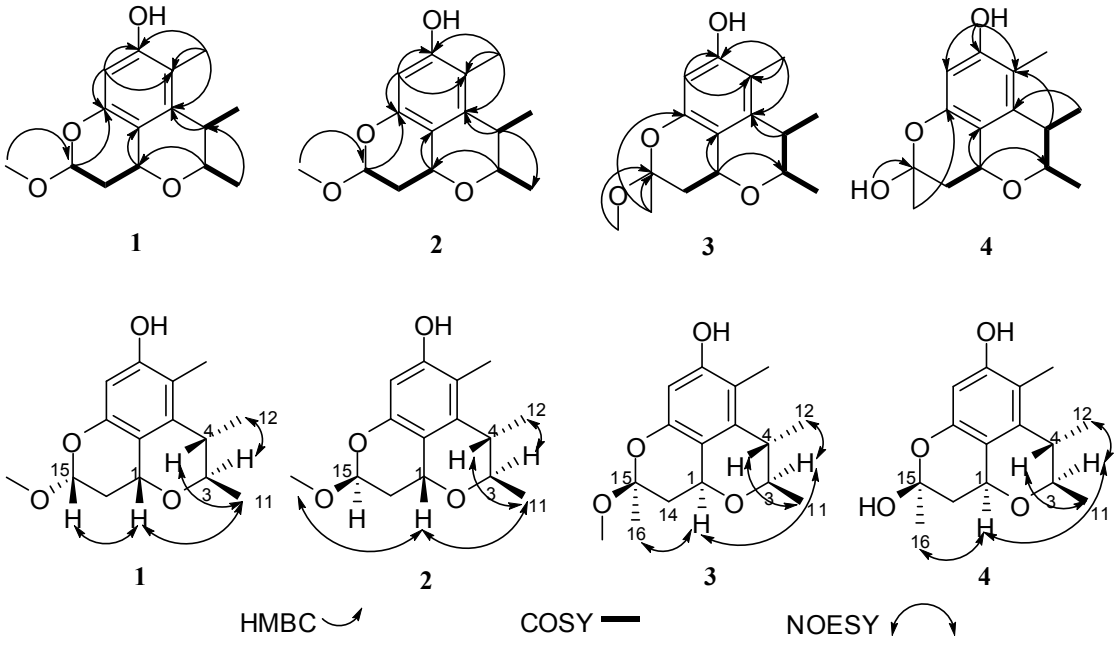

Figure 2. Key COSY, HMBC, and NOESY correlations of compounds **1**–**4**

Table 2. ^{13}C NMR data (100 MHz, δ in ppm) of compounds **1**–**4**

No	1[a]	2[a]	3[b]	4[a]
1	60.3 d	57.4 d	67.3 d	67.0 d
3	73.5 d	73.5 d	79.0 d	78.2 d
4	33.7 d	34.1 d	38.0 d	37.4 d
5	114.4 s	114.1 s	114.7 s	113.4 s
6	155.1 s	155.1 s	154.4 s	155.4 s
7	100.8 d	99.6 d	100.3 d	99.5 d
8	148.7 s	148.1 s	148.6 s	148.7 s
9	112.8 s	110.4 s	113.8 s	112.2 s
10	135.4 s	136.1 s	138.3 s	137.7 s
11	17.7 q	18.1 q	21.5 q	21.5 q
12	21.5 q	22.2 q	19.6 q	19.5 q
13	9.9 q	10.0 q	11.1 q	11.1 q
14	36.3 t	32.9 t	38.2 t	38.4 t
15	100.2 d	98.7 d	100.0 s	97.0 s
16			23.3 q	29.0 q
OCH_3	55.1 q	55.4 q	49.0 q	

[a]DMSO-d_6 as solvent. [b]$CDCl_3$ as solvent.

The cytotoxic effects of compounds **1**–**4** were evaluated using the MTT method on A-549, HL-60, hela, and K562 cancer cell lines. Unfortunately, the results showed that all of their IC_{50} values were larger than 100 μM.

EXPERIMENTAL

General Experimental Procedures. Optical rotations were obtained from a Shenguang SGW-1 digital polarimeter. UV spectra were recorded on a Shimadzu UV-2450 spectrophotometer. ^{1}H NMR, ^{13}C NMR, DEPT spectra and 2D NMR were recorded on a BRUKER BIOSPIN AVANCE III spectrometer using TMS as the internal standard. ESI-MS were obtained by an AGILENT 1200/Q-TOF 6510 LC mass spectrometer. Semipreparative HPLC was performed using an ODS column (ODS-A, 10×250 mm, 5 μm) at 5 mL/min.

Fungal Material. The fungus *P. citrinum* was isolated from marine sediments collected from Langqi Island, Fujian, China. It was identified according to its morphological characteristics and ITS by Beijing Sunbiotech Co. Ltd, and preserved in our laboratory at −80 ˚C. The producing strain was prepared on

Martin medium and stored at 4 ˚C.

Fermentation and Extraction. The fungus was cultured under static conditions at 28 ˚C for 30 days in 1000-mL conical flasks containing the liquid medium (400 mL/flask) composed of glucose (10 g/L), maltose (20 g/L), mannitol (20 g/L), monosodium glutamate (10 g/L), KH_2PO_4 (0.5 g/L), $MgSO_4 \cdot 7H_2O$ (0.3 g/L), yeast extract (3 g/L), and seawater. The fermented whole broth (60 L) was filtered through cheese cloth to separate supernatant from mycelia. The former was extracted two times with EtOAc to yield an EtOAc solution that was concentrated under reduced pressure to give a crude extract (32.0 g).

Purification. The crude extract (32.0 g) was separated into 1L fractions on a Si gel column using a step gradient elution of petroleum ether, CH_2Cl_2, and MeOH. Fraction 5 (2.1 g) eluted with petroleum ether/CH_2Cl_2 (1:3) was further purified on a Si gel column using a step gradient elution. Subfraction 5-4 (437 mg) eluted with petroleum ether/EtOAc (2:1) was purified on a Sephadex LH-20 (CH_2Cl_2/MeOH, 1:1) to give compound **8** (52.3 mg). Subfraction 5-6 (326 mg) eluted with petroleum ether/EtOAc (1:1) was purified on a Sephadex LH-20 (CH_2Cl_2/MeOH, 1:1) and a reversed-phase column (MeOH/H_2O, 2:1) to give compound **7** (48.6 mg). Fraction 6 (1.5 g) eluted with CH_2Cl_2 was further purified on a Sephadex LH-20 (CH_2Cl_2/MeOH, 1:1) and a reversed-phase column (MeOH/H_2O, 3:2) to give compound **6** (15.8 mg). Fraction 9 (5.6 g) eluted with CH_2Cl_2/MeOH (50:1) was further separated on a Sephadex LH-20 (CH_2Cl_2/MeOH, 1:1). Subfraction 9-8 (650 mg) was purified by a reversed-phase column (MeOH/H_2O, 3:2) and semipreparative HPLC (45% MeCN), yielding compounds **1** (2.8 mg), **2** (4.2 mg), **3** (36.5 mg), **4** (40.2 mg), and **5** (5.8 mg).

Penicitrinol F (1): white solid; $[\alpha]^{25}_D$ −9.7° (*c* 0.4, MeCN); UV λ_{max} (MeCN) nm (log ε): 282 (2.71); ^{1}H and ^{13}C NMR (see Tables 1 and 2); HRESIMS *m/z* 263.1297 $[M - H]^-$ (Calcd for $C_{15}H_{19}O_4$: 263.1283).

Penicitrinol G (2): white solid; $[\alpha]^{25}_D$ +16.9° (*c* 0.6, MeCN); UV λ_{max} (MeCN) nm (log ε): 285 (2.90); ^{1}H and ^{13}C NMR (see Tables 1 and 2); HRESIMS *m/z* 263.1266 $[M - H]^-$ (Calcd for $C_{15}H_{19}O_4$: 263.1283).

Penicitrinol H (3): yellow gum; $[\alpha]^{25}_D$ –69.4° (*c* 0.6, MeCN); UV λ_{max} (MeCN) nm (log ε): 284 (3.03); ^{1}H and ^{13}C NMR (see Tables 1 and 2); HRESIMS *m/z* 277.1451 $[M - H]^-$ (Calcd for $C_{16}H_{21}O_4$: 277.1440).

Penicitrinol I (4): yellow gum; $[\alpha]^{25}_D$ –79.5° (*c* 1.0, MeCN); UV λ_{max} (MeCN) nm (log ε): 287 (2.87); ^{1}H and ^{13}C NMR (see Tables 1 and 2); HRESIMS *m/z* 263.1275 $[M - H]^-$ (Calcd for $C_{15}H_{19}O_4$: 263.1283).

Biological assay. Cytotoxic activity was evaluated by the MTT method using A-549, HL-60, hela, and K562 cell lines.[3] The cell lines were grown in RPMI-1640 supplemented with 10% FBS under a humidified atmosphere of 5% CO_2 and 95% air at 37 ˚C. Those cell suspensions (200 μL) at a density of 5×10^4 cell mL^{-1} were plated in 96-well microtiter plates and incubated for 24 h at the above condition. The test compound solution (2 μL in DMSO) at different concentrations was added to each well and further incubated for 72 h in the same condition. Then 20 μL of the MTT solution (5 mg/ml in

RPMI-1640 medium) was added to each well and incubated for 4 h. The old medium containing MTT (150 μL) 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.

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REFERENCES

1. a) M. Sasaki, M. Tsuda, M. Sekiguchi, Y. Mikami, and J. Kobayashi, *Org. Lett.*, 2005, **7**, 4261; b) H.-C. Liu, L. Du, T.-J. Zhu, D.-H. Li, M.-Y. Geng, and Q.-Q. Gu, *Helv. Chim. Acta*, 2010, **93**, 2224; c) B. R. Clark, R. J. Capon, E. Lacey, S. Tennant, and J. H. Gill, *Org. Biomol. Chem.*, 2006, **4**, 1520; d) Z.-Y. Lu, Z.-J. Lin, W.-L. Wang, L. Du, T.-J. Zhu, Y.-C. Fang, Q.-Q. Gu, and W.-M. Zhu, *J. Nat. Prod.*, 2008, **71**, 543; e) D. Wakana, T. Hosoe, T. Itabashi, K. Okada, G. M. C. Takaki, T. Yaguchi, K. Fukushima, and K. Kawai, *J. Nat. Med.*, 2006, **60**, 279.
2. L. Du, D. Li, G. Zhang, T. Zhu, J. Ai, and Q. Gu, *Tetrahedron*, 2010, **66**, 9286.
3. L. Chen, W. Liu, X. Hu, K. Huang, J.-L. Wu, and Q.-Q. Zhang, *Chem. Pharm. Bull.*, 2011, **59**, 515.
4. a) N. Shangguan, S. Kiren, and L. J. Williams, *Org. Lett.*, 2007, **9**, 1093; b) Z.-H. Xin, W.-L. Wang, Y.-P. Zhang, H. Xie, Q.-Q. Gu, and W.-M. Zhu, *J. Antibiot.*, 2009, **62**, 225.