

HETEROCYCLES, Vol. 83, No. 8, 2011, pp. 1853 - 1858. © The Japan Institute of Heterocyclic Chemistry
Received, 12th April, 2011, Accepted, 13th May, 2011, Published online, 20th May, 2011
DOI: 10.3987/COM-11-12234

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

Li Chen,^{a,*} Wei Liu,^a Kai Huang,^a Xiao Hu,^a Zhe-Xiang Fang,^a Jiu-Lin Wu,^a
and Qi-Qing Zhang^{a,b,*}

^aInstitute of Biomedical and Pharmaceutical Technology & College of Chemistry and Chemical Engineering, Fuzhou University, Fuzhou, 350002, P. R. China

^bInstitute of Biomedical Engineering, Chinese Academy of Medical Science, Peking Union Medical College, Tianjin, 300192, P. R. China. E-mail: ibptcl@fzu.edu.cn; zhangqiq@126.com

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-dimethylbenzaldehyde (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 separated 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 C₁₅H₂₀O₄ by its HRESIMS. The ¹H 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 ¹³C 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 CH₃-11 were on the same side, whereas CH₃-12 and OCH₃ were on the reverse side. Therefore, the structure of **1** was elucidated as shown (Figure 1).

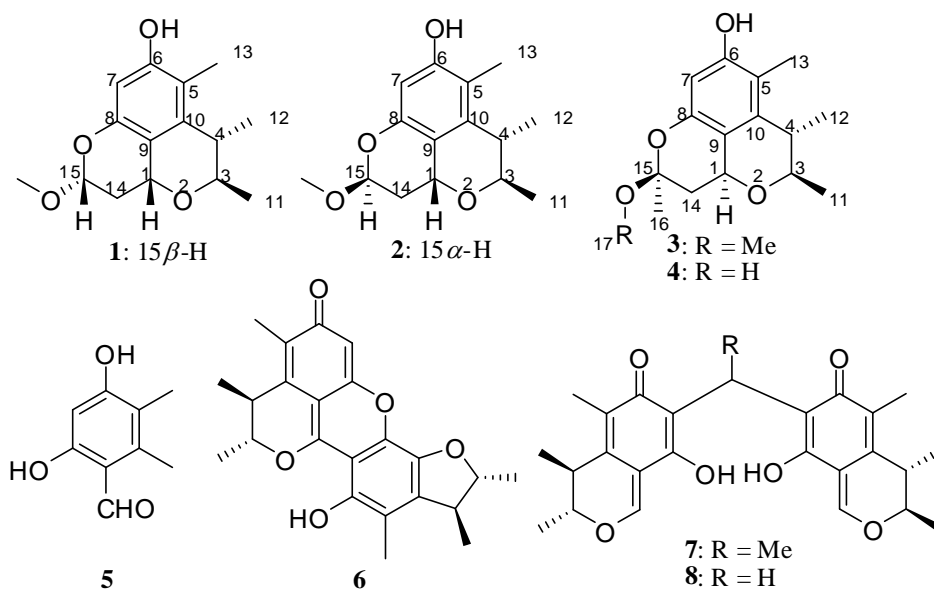


Figure 1. Structures of compounds **1**–**8**

Compound **2** has the same molecular formula (C₁₅H₂₀O₄) 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 OCH₃, 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, C₁₆H₂₂O₄, 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, CH₃-11, and OCH₃ were on the same side. On the other hand, H-1, H-3, CH₃-12 and CH₃-16 were located on the reverse side.

Compound **4** was obtained as a yellow gum. Its molecular formula, C₁₅H₂₀O₄, was established using negative HRESIMS (m/z 263.1275, [M – H][–], calcd for C₁₅H₁₉O₄, 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. ^1H 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)	1.70 (H, m)	1.74 (H, t, 12.0)	1.45 (H, t, 11.8)
	2.43 (H, m)	2.09 (H, m)	2.39 (H, dd, 12.0, 6.0)	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)

^aDMSO-*d*₆ as solvent. ^bCDCl₃ as solvent.

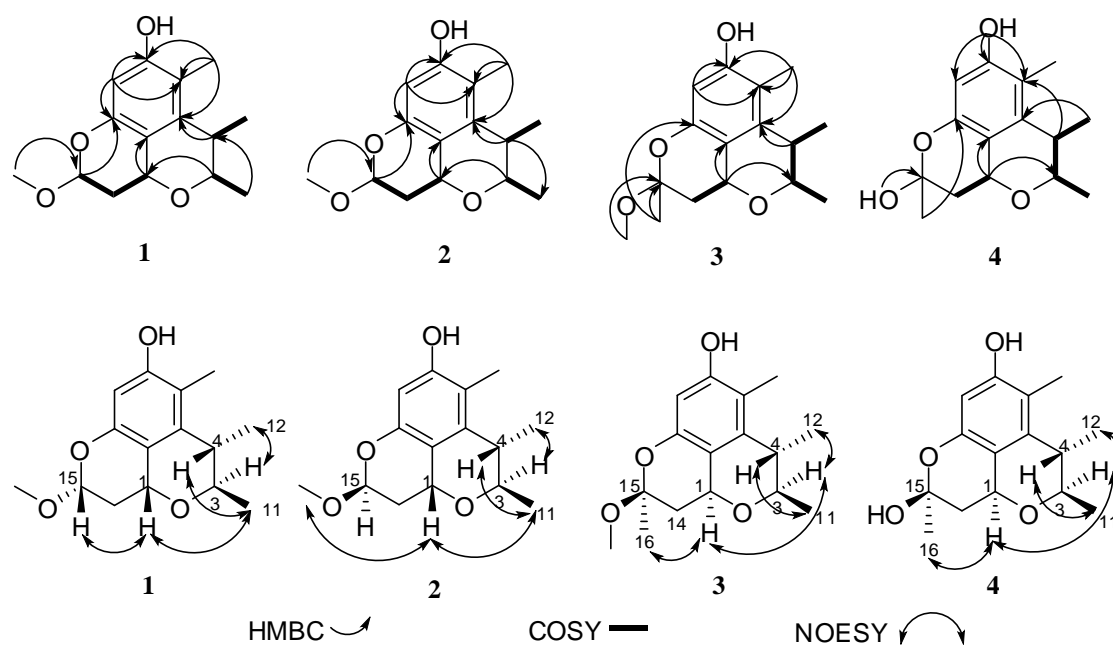


Figure 2. Key COSY, HMBC, and NOESY correlations of compounds **1–4**

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	

^aDMSO-*d*₆ as solvent. ^bCDCl₃ 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 IC₅₀ 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. ^1H 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 \times 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 $-80\text{ }^\circ\text{C}$. 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), KH₂PO₄ (0.5 g/L), MgSO₄·7H₂O (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, CH₂Cl₂, and MeOH. Fraction 5 (2.1 g) eluted with petroleum ether/CH₂Cl₂ (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 (CH₂Cl₂/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 (CH₂Cl₂/MeOH, 1:1) and a reversed-phase column (MeOH/H₂O, 2:1) to give compound **7** (48.6 mg). Fraction 6 (1.5 g) eluted with CH₂Cl₂ was further purified on a Sephadex LH-20 (CH₂Cl₂/MeOH, 1:1) and a reversed-phase column (MeOH/H₂O, 3:2) to give compound **6** (15.8 mg). Fraction 9 (5.6 g) eluted with CH₂Cl₂/MeOH (50:1) was further separated on a Sephadex LH-20 (CH₂Cl₂/MeOH, 1:1). Subfraction 9-8 (650 mg) was purified by a reversed-phase column (MeOH/H₂O, 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; $[\alpha]_D^{25} -9.7^\circ$ (*c* 0.4, MeCN); UV λ_{\max} (MeCN) nm (log ϵ): 282 (2.71); ¹H and ¹³C NMR (see Tables 1 and 2); HRESIMS *m/z* 263.1297 [M – H][–] (Calcd for C₁₅H₁₉O₄: 263.1283).

Penicitrinol G (2): white solid; $[\alpha]_D^{25} +16.9^\circ$ (*c* 0.6, MeCN); UV λ_{\max} (MeCN) nm (log ϵ): 285 (2.90); ¹H and ¹³C NMR (see Tables 1 and 2); HRESIMS *m/z* 263.1266 [M – H][–] (Calcd for C₁₅H₁₉O₄: 263.1283).

Penicitrinol H (3): yellow gum; $[\alpha]_D^{25} -69.4^\circ$ (*c* 0.6, MeCN); UV λ_{\max} (MeCN) nm (log ϵ): 284 (3.03); ¹H and ¹³C NMR (see Tables 1 and 2); HRESIMS *m/z* 277.1451 [M – H][–] (Calcd for C₁₆H₂₁O₄: 277.1440).

Penicitrinol I (4): yellow gum; $[\alpha]_D^{25} -79.5^\circ$ (*c* 1.0, MeCN); UV λ_{\max} (MeCN) nm (log ϵ): 287 (2.87); ¹H and ¹³C NMR (see Tables 1 and 2); HRESIMS *m/z* 263.1275 [M – H][–] (Calcd for C₁₅H₁₉O₄: 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% CO₂ and 95% air at 37 °C. Those cell suspensions (200 μ L) at a density of 5×10⁴ cell mL^{–1} were plated in 96-well microtiter plates and incubated for 24 h at the above condition. The test compound solution (2 μ L in DMSO) at different concentrations was added to each well and further incubated for 72 h in the same condition. Then 20 μ L 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 μ L) 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.

ACKNOWLEDGEMENTS

This research was supported by the Natural Science Foundation of Fujian Province (2009J05075), the Scientific Research Foundation in Fuzhou University (2009-XY-16, 022229), Scientific Key Research Project of Fujian Province (2010N0015), Key Laboratory of Biomedical Material of Tianjin and Scientific Major Research Project of Fujian Province (2010NZ0001-1).

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. Shanguan, 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.