有机化学 DOI: 10.6023/cjoc201812008 研究简报
Chinese Journal of Organic Chemistry NOTE

一株红树来源真菌 *Penicillium citrinum* HL-5126 中两个新异香豆素化合物

梅荣清 黄国雷 王 斌 白 猛 罗由萍 陈光英 郑彩娟*

(热带药用植物化学教育部重点实验室 海南省热带药用植物化学重点实验室 海南师范大学化学与化工学院 海口 571158)

摘要 综合运用多种现代色谱学分离方法对红树来源真菌 *Penicillium citrinum* HL-5126 中的次级代谢产物进行了研究, 从中分离获得 2 个新的异香豆素类化合物, 通过 1D NMR, 2D NMR 和 HR-ESI-MS 等波谱鉴定技术确定结构分别为 penicimarin J (**1**)和 penicimarin K (**2**). 通过测定圆二色谱并与文献数据对照, 确定了化合物 **1** 和 **2** 的绝对构型. 化合物 **1** 和 **2** 对 *α*-Glucosidase 显示一定的抑制活性, IC_{50} 值分别为 18.37 和 25.86 μg/mL.

关键词 *Penicillium citrinum*; 异香豆素; 抗菌活性; *α*-Glucosidase 抑制活性

Two New Isocoumarins Isolated from a Mangrove-Derived Fungus *Penicillium citrinum* HL-5126

Mei, Rongqing Huang, Guolei Wang, Bin Bai, Meng Luo, Youping Chen, Guangying Zheng, Caijuan*

(*Key Laboratory of Tropical Medicinal Plant Chemistry of Ministry of Education*, *Key Laboratory of Tropical Medicinal Plant Chemistry of Hainan Province*, *College of Chemistry and Chemical Engineering*, *Hainan Normal University*, *Haikou 571158*)

Abstract Two new isocoumarins derivatives, penicimarins J (**1**) and K (**2**), were isolated from the mangrove-derived fungus *Penicillium citrinum* HL-5126. Their structures were elucidated through extensive 1D NMR, 2D NMR and HR-ESI-MS spectroscopic analyses. The absolute configurations of **1** and **2** were determined by comparison of their circular dichroism (CD) spectra with the literature. Compounds **1** and **2** showed inhibitory activities against *α*-glucosidase with the IC_{50} values of 18.37 and 25.86 μg/mL, respectively.

Keywords *Penicillium citrinum*; isocoumarin; antibacterial activity; *α*-glucosidase inhibitory activity

1 Introduction

Marine-derived fungus had recently come into the focus of research as one of the richest sources of novel and bioactive secondary metabolites in the mangrove environment.[1] In particular, secondary metabolites isolated from the marine-derived fungi in the genus *Penicillium* can produce various bioactive metabolites, including cytotoxic chromones,[2] anticancer steroids,[3] antimigratory diketopiperazine alkaloids,[4] antibacterial benzopyran derivatives,[5] and anti-inflammatory polyketides.[6] In our research for new bioactive products from mangrove-derived fungi,[7~13] three new isocoumarins penicimarins G-I were isolated from liquid fermentation broth of the fungus *Penicillium citrinum* HL-5126.[14] In order to search for new bioactive products from the fungus *P. citrinum* HL-5126, two new isocoumari penicimarins J (**1**) and K (**2**) were isolated from the rice solid medium fermention of *P. citrinum* HL-5126. Herein, details of the isolation, structure elucidation, and the *α*-glucosidase inhibitory and antibacterial activities of these compounds are described.

* Corresponding author. E-mail: caijuan2002@163.com
Received December 5, 2018; revised December 29, 2018; published online January 18, 2019.
Project supported by the National Natural Science Foundation of China (Nos. 21462015, 31760093), the National Natural Science Foundation of Hainan Province (No. 218MS045), the Program for Innovative Research Team in University (No. IRT-16R19).
国家自然科学基金(Nos. 21462015, 31760093)、海南省自然科学基金(No. 218MS045)、教育部创新团队发展计划(No. IRT-16R19)资助项目.

2 Results and discussion

2.1 Structure identification of compound 1

Compound **1** (Figure 1) was obtained as a colorless oil, with the molecular formula of $C_{12}H_{12}O_5$ (seven degrees of unsaturation) determined by HR-ESI-MS. The ^{1}H NMR spectrum (Table 1) displayed a chelated phenolic hydroxy at δ_H 10.92 (s), a set of meta-coupled aromatic protons at δ_H 7.41 (dd, J=8.4, 8.0 Hz), 6.90 (d, J=8.4 Hz), and 6.70 (d, J=8.0 Hz), one oxygenated methane proton signal at δ_H 4.64～4.69 (m), and three methylene groups at δ_H 2.95～2.98 (m), 2.67～2.70 (m), and 2.10～2.16 (m). The ^{13}C NMR and DEPT NMR data revealed the presences of three methylenes, one methine, six aromatic carbons and two lactone carbonyl carbons at δ_C 176.8 (C) and 169.7 (C). The above ^{1}H NMR and ^{13}C NMR data suggested a close structural relationship to penicimarin I.[14] The obvious differences in ^{1}H NMR spectra were the absence of one methoxy signal at δ_H 3.82 (s) in **1**. The ^{1}H-^{1}H COSY, HSQC, and HMBC spectra allowed the complete assignment for **1** (Figure 2). The absolute configuration of C-3 was also determined by CD spectroscopy. The negative circular dichroism at 259 nm suggested the *R* configuration at C-3, by comparison with data for dihydroisocoumarins described in the literature (Figure 3).[15] Thus, compound **1** was a new natural compound, and named as penicimarin J.

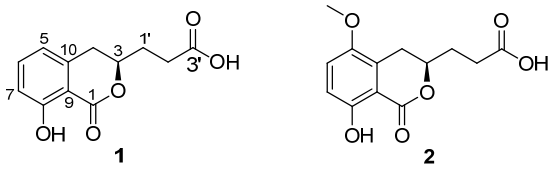

Figure 1 Structures of compounds **1**～**2**

2.2 Structure identification of compound 2

Compound **2** (Figure 1) was also isolated as colorless gum. Its molecular formula was determined to be $C_{13}H_{14}O_6$, on the basis of its HR-ESI-MS, requiring seven degrees of unsaturation. In the ^{1}H NMR spectrum (Table 1), two aromatic protons at δ_H 7.03 (d, J=8.0 Hz) and 6.65 (d, J=8.0 Hz), one oxygenated methine proton signal at δ_H 4.61～4.68 (m), one methoxyl signal at δ_H 3.90 (s), and three methylene groups at δ_H 2.90～2.91(m), 2.64～2.68 (m) and 2.08～2.16 (m) were observed. The ^{13}C NMR spectrum displayed three methylenes, one methines, one methoxy, and six aromatic carbons and two lactone carbonyl carbons. Detailed analyses of its 1D NMR spectroscopic features implied that it was closely related structurally to **1**, except for the absence of an aromatic proton signal, and the presence of a methoxyl signal at δ_H 3.90 (s) in **2**. The aromatic protons at δ_H 7.03 (d, J=8.0 Hz) and 6.65 (d, J = 8.0 Hz) indicated the presence of a 1,2,3,4-tetrasubstituted benzene system in **2**, instead of the 1,2,3-trisubstituted benzene in **1**. In the ^{13}C NMR spectrum, an aromatic methine carbon (δ_C 118.1, C-5) in **1** was replaced by a downfield aromatic quaternary carbon (δ_C 147.6, C-5) in **2**. These results were confirmed by the HMBC correlations of H-4 and H-7 to C-5. The methoxyl signal attached at C-5 was determined by the HMBC correlation of 5-OMe to C-5 (Figure 2). The structure of **2** was further confirmed by the 2D NMR spectra. The absolute configuration of C-3 was also determined to be *R* by CD spectra (Figure 3), and **2** was named as penicimarin K.

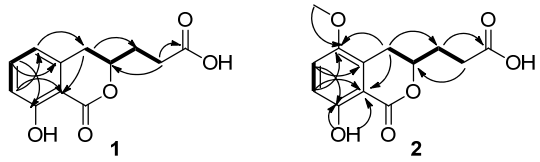

Figure 2 Key ^{1}H-^{1}H-COSY (▬) and HMBC correlations (H→C) of compounds **1** and **2**

2.3 Biological activity of compounds 1 and 2

Compounds **1** and **2** were tested for their inhibitory activities against α-glucosidase. Both compounds showed

Table 1 ^{1}H NMR and ^{13}C NMR spectroscopic data (400/100 MHz, $CDCl_3$) for compounds **1** and **2**

Position	1		2	
	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	169.7, C		170.0, C	
2				
3	78.4, CH	4.64～4.69 (m)	79.0, CH	4.61～4.68 (m)
4	33.1, CH_2	2.95～2.98 (m)	32.6, CH_2	2.90～2.91 (m)
5	118.1, CH	6.70 (d, 8.0)	147.6, C	
6	136.5, CH	7.41 (dd, 8.4, 8.0)	117.7, CH	7.03 (d, 8.0)
7	116.6, CH	6.90 (d, 8.4)	117.2, CH	6.65 (d, 8.0)
8	162.4, C		152.7, C	
9	108.5, C		108.5, C	
10	139.1, C		129.7, C	
1'	29.7, CH_2	2.10～2.16 (m)	29.7, CH_2	2.08～2.16 (m)
2'	29.0, CH_2	2.67～2.70 (m)	29.0, CH_2	2.64～2.68 (m)
3'	176.8, C		176.6, C	
5-OMe			56.5, CH_3	3.90 (s)
8-OH		10.92 (s)		11.13 (s)

Table 2 Antibacterial activity of compounds **1** and **2**

Compound	Minimum inhibitory concentration (MIC)/(μg•mL^{-1})					
	S. aureus	*S. albus*	*E. coli*	*P. aeruginosa*	*V. parahaemolyticus*	*V. alginolyticus*
1	50	>50	>50	>50	>50	>50
2	50	>50	>50	>50	>50	>50
Ciprofloxacin[a]	0.31	0.62	0.62	1.25	0.31	1.25

[a] Ciprofloxacin was used as a positive control.

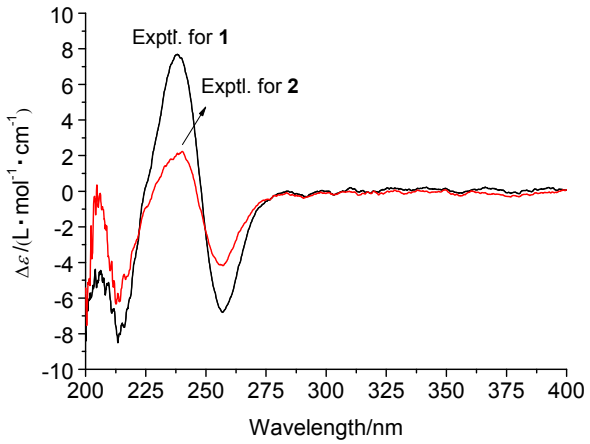

Figure 3 Experimental ECD spectra of compounds **1** and **2** in MeOH

inhibitory activities with the IC_{50} values of 18.37 and 25.86 μg/mL, respectively. Acarbose was used as a positive control with the IC_{50} value of 1.98 μg/mL. They were also evaluated for their antibacterial activities against four terrestrial pathogenic bacteria and two marine pathogenic bacteria. Compounds **1** and **2** exhibited antibacterial activities against *Staphylococcus aureus* with the same minimum inhibitory concentration (MIC) values of 50 μg/mL (Table 2).

3 Conclusions

In summary, two isocourmarins were isolated from the mangrove-derived endophytic fungus *P. citrinum* HL-5126. The absolute configurations of **1** and **2** were determined by comparison of their CD spectra with literature. Compounds **1** and **2** showed inhibitory activities against *α*-Glucosidase with the IC_{50} values of 18.37 and 25.86 μg/mL, respectively, and they exhibited antibacterial activities against *S. aureus* with the same MIC values of 50 μg/mL.

4 Experimental section

4.1 Instruments and reagents

HR-ESI-MS spectra were obtained on a Bruker Daltonics Apex-Ultra 7.0 T (Bruker Corporation, Billerica, MA, USA) and a Q-TOF Ultima Global GAA076 LC mass spectrometer. IR spectra were recorded on a Thermo Nicolet 6700 (using KBr disks) spectrophotometer (Thermo Scientific, Madison, WI, USA). CD spectra were recorded on a MOS-450 spectrometer. Prep. HPLC were used for Agilent 1260 prep-HPLC system with an Agilent C18 analytical HPLC column. Both 1D and 2D NMR spectra were measured on a Bruker AV-400 (Bruker Corporation, Switzerland) instrument with TMS as the internal standard (400 MHz for ^{1}H and 100 MHz for ^{13}C). Sephadex LH-20 (Pharmacia Co. Ltd, Sandwich, UK) and Silica gel (200～300 mesh, 300～400 mesh Qingdao Marine Chemical Factory, Qingdao, China) were used for column chromatography (CC). Precoated silica gel GF254 plates (Yantai Zifu Chemical Group) was applied to thin layer chromatography (TLC). All solvents were purchased from Xilong Chemical Reagent Factory (Guangzhou, China).

4.2 Fungal materials

The fungal strain *P. citrinum* HL-5126 was isolated from the mangrove *Bruguiera sexangula var. rhynchopetala*, collected from the South China Sea. The strain *P. citrinum* HL-5126 was deposited in the Key Laboratory of Tropical Medicinal Plant Chemistry of Ministry of Education, Hainan Normal University, Hainan with an accession number KJ466981.[14] The fungal strain was cultivated in solid medium, 80 g of rice with 120 mL seawater (33 g of sea salt in 1L distilled water), in 1 L Erlenmeyer flasks (total of 24 bottles). Each flask was inoculated with 5 mL of the culture medium and incubated at room temperature for 35 days.

4.3 Extraction, isolation and purification

The fermented soild medium was extracted repeatedly with EtOAc three times, and the combined EtOAc layers were evaporated to dryness under vacuum to afford the EtOAc extract (10.0 g). The EtOAc extract was chromatographed on a silica gel (200～300 mesh) column eluted with a step gradient of petroleum ether (PE)-EtOAc and EtOAc-MeOH to give 10 fractions (Fr.1～Fr.10). Fr.6 was chromatographed on a silica gel (300～400 mesh) column eluted with PE-EtOAc (gradient V∶V=100∶10～10∶100) and further purified on HPLC with 50% MeOH-H_2O to afford **1** (5.4 mg). Fr.7 was separated by ODS column chromatography (CC) using a gradient of increasing methanol in water (30%, 50%, 60%, 80%, 100%) to give five subfractions Fr.7.1～Fr.7.5, and then purified on HPLC with 60% MeOH-H_2O to afford **2** (7.3 mg).

Penicimarin J (**1**): colorless oil; ^{1}H NMR and ^{13}C NMR data see Table 1; CD (5.0×10^{-4} mol/L, MeOH) λ_{max} [$\Delta\varepsilon$/(L•mol^{-1}•cm^{-1})]: 235 (+7.50), 260 (−7.00), 280 (−0.75) nm; IR (KBr) ν_{max}: 3354, 1710, 1690, 1272, 1250, 1230, 1112, 1082, 1064, 802 cm^{-1}; HR-ESI-MS calcd for $C_{12}H_{13}O_5$ $[M+H]^+$ 237.0763, found 237.0759.

Penicimarin K (**2**): colorless gum; ^{1}H NMR and ^{13}C

NMR data see Table 1; CD (4.2×10^{-4} mol/L, MeOH) λ_{max} [$\Delta\varepsilon$/(L•mol^{-1}•cm^{-1})]: 239 (+2.00), 257 (−4.00), 280 (−0.75) nm; IR (KBr) ν_{max}: 3450, 1708, 1683, 1272, 1255, 1230, 1110, 1088, 1069, 803 cm^{-1}. HR-ESI-MS calcd for $C_{13}H_{15}O_6$ $[M+H]^+$ 267.0869, found 267.0864.

4.4 Biological activity

The activity of α-glucosidase inhibitory was determined in 96-well plates,[16] and the absorbance was determined at 405 nm measured with a ELISA Microplate Reader (Bio Tek ELX800). Acarbose was utilized as the positive control. Antibacterial activity was evaluated by the conventional broth dilution assay,[17] six bacterial strains, including four terrestrial pathogenic bacteria, *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus albus* (ATCC 8799), and two marine pathogenic bacteria, *Vibrio parahaemolyticus* (ATCC 17802) and *Vibrio alginolyticus* (ATCC 17749). Ciprofloxacin was used as a positive control.

Supporting Information ^{1}D NMR, 2D NMR spectra and CD spectra of compounds **1** and **2**. These materials can be available free of charge via the internet at http://sioc-journal.cn/.

References

[1] Blunt, J. W.; Carroll, A. R.; Copp, B. R.; Davis, R. A.; Keyzer, R. A.; Prinsep, M. R. *Nat. Prod. Rep.* **2018**, *35*(1), 8.

[2] Sun, Y. L.; Bao, J.; Liu, K. S.; Zhang, X. Y.; He, F.; Nong, X. H.; Qi, S. H. *Planta Med.* **2013**, *79*(15), 1474.

[3] Qi, J.; Shao, C. L.; Liu, M.; Qi, X.; Wang, C. Y. *Chem. Nat. Compd.* **2014**, *50*(3), 568.

[4] Asiri, I. A. M.; Badr, J. M.; Youssef, D. T. A. *Phytochem. Lett.* **2015**, *13*, 53.

[5] Zheng, C. J.; Huang, G. L.; Xu, Y. Song, X. M.; Yao, J. Liu, H. Wang, R. P.; Song X. P. *Nat. Prod. Res.* **2016**, *30*, 821.

[6] Ha, T. M.; Ko, W.; Lee, S. J.; Kim, Y. C.; Son, J. Y.; Sohn, J. H.; Yim, J. H.; Oh, H. *Mar. Drugs* **2017**, *15*(9), 282.

[7] Zhou, X. M.; Zheng, C. J.; Chen, G. Y.; Song, X. P.; Han, C. R.; Li, G. N.; Fu, Y. H.; Chen, W. H.; Niu, Z. G. *J. Nat. Prod.* **2014**, 77, 2021.

[8] Liao, H. X.; Sun, D. W.; Zheng, C. J.; Wang, C. Y. *Nat. Prod. Res.* **2017**, *31*(14), 1640.

[9] He, K. Y.; Zhang, C.; Duan, Y. R.; Huang, G. L.; Yang, C. Y.; Lu, X. R.; Zheng, C. J; Chen, G. Y. *J. Antibiot.* **2017**, *70*, 823.

[10] Zheng, C. J; Wu, L. Y.; Li, X. B.; Song, X. M. Niu, Z. G.; Song, X. P.; Chen, G. Y.; Wang, C. Y. *Helv. Chim. Acta* **2015**, *98*, 368.

[11] Zheng, C. J.; Huang, G. L.; Xu, Y.; Song, X. M.; Yao, J. Liu, H.; Wang, R. P.; Song, X. P. *Nat. Prod. Res.* **2016**, *30*, 821.

[12] Zheng, C. J.; Bai, M. Zhou, X. M.; Huang, G. L.; Shao, T. M.; Luo, Y. P.; Niu, Z. G.; Niu, Y. Y.; Chen, G. Y.; Han, C. R. *J. Nat. Prod.* **2018**, *81*(4), 1045.

[13] Zheng, C. J.; Liao, H. X.; Mei, R. Q.; Huang, G. L.; Yang, L. J.; Zhou, X. M.; Shao, T. M.; Chen, G. Y.; Wang, C. Y. *Nat. Prod. Res.* **2019**, *33*, 1127.

[14] Huang, G. L.; Zhou, X. M.; Bai, M.; Liu, Y. X.; Zhao, Y. L.; Luo, Y. P.; Niu, Y. Y.; Zheng, C. J.; Chen, G. Y. *Mar. Drugs* **2016**, *14*(10), 177.

[15] Choukchou-Braham, N.; Asakawa, Y.; Lepoittevin, J. P. *Tetrahedron Lett.* **1994**, 35, 3949.

[16] Rivera-Chávez, J.; Figueroa, M.; González, M. D. C.; Glenn, A. E.; Mata, R. *J. Nat. Prod.* **2015**, *78*, 730.

[17] Pierce, C. G.; Uppuluri, P.; Teistan, A. R.; Wormley, J. F. L.; Mowat, E.; Ramage, G.; Lopez-ribot, J. L. *Nat. Protoc.* **2008**, *3*, 1494.

(Li, L.; Fan, Y.)

