Bioorganic & Medicinal Chemistry Letters 20 (2010) 3284–3286

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Penicinoline, a new pyrrolyl 4-quinolinone alkaloid with an unprecedented ring system from an endophytic fungus *Penicillium* sp.

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ARTICLE INFO

Article history:
Received 26 February 2010
Revised 4 April 2010
Accepted 12 April 2010
Available online 14 April 2010

Keywords:
Penicillium sp.
Penicinoline
Penicinotam
X-ray analysis
Biological activity

ABSTRACT

A new pyrrolyl 4-quinolinone alkaloid with an unprecedented ring system, named penicinoline (**1**) was isolated from a mangrove endophytic fungus. The structure of **1** was elucidated by spectroscopic methods and comparison with its derivative, penicinotam (**1a**), an unexpected lactam that was obtained from **1** by intramolecular dehydration. The structure of **1a** was unambiguously confirmed by single-crystal X-ray analysis. Penicinoline (**1**) showed potent in vitro cytotoxicity toward 95-D and HepG2 cell lines with IC_{50} values of 0.57 and 6.5 μg/mL, respectively.

Endophytic fungi have proved to be a productive source of structurally diverse and biologically active secondary metabolites.[1,2] In our ongoing investigation on new bioactive compounds from marine microorganisms in the South China Sea,[3–5] the methanolic extract of the mycelium of a mangrove endophytic fungus *Penicillium* sp.[6,7] exhibited significant cytotoxicity against HepG2 cell line. Bioassay-guided fractionation of the bioactive extract resulted in the isolation of a new and unusual pyrrolyl 4-quinolinone alkaloid, penicinoline (**1**) (Fig. 1). Herein we report the isolation, structure elucidation, and biological activity of the new compound.

Penicinoline (**1**)[8] was obtained as a yellow amorphous powder. The HREIMS results (*m/z* 254.0681, calcd 254.0691) together with its ^{13}C NMR spectrum indicated that it has a molecular formula of $C_{14}H_{10}N_2O_3$ with 11 degrees of unsaturation. A characteristic $[M-44]^+$ peak in the EIMS spectrum suggested that the compound has a carboxyl group. In the ^1H NMR spectrum (Table 1), the proton signals and the coupling constants at δ_H 7.86 (dd, J = 8.0, 1.5 Hz), 7.81 (ddd, J = 8.0, 8.0, 1.5 Hz), 7.51 (ddd, J = 8.0, 8.0, 1.5 Hz) and 8.23 (dd, J = 8.0, 1.5 Hz) indicated the presence of an *ortho*-disubstituted benzene system. Three proton signals with multiple peaks at δ_H 7.14, 6.82 and 6.28 and an exchangeable NH proton signal at δ_H 11.80 were assigned to the mono-substituted pyrrole ring. The distinctive $^2J_{NH}$ and $^3J_{NH}$ couplings of pyrrole were observed in the ^1H–^1H COSY spectrum. The remaining two exchangeable proton signals observed in the low-field at δ_H 12.33 and δ_H 16.13 were assigned to NH on the quinolinone ring and OH of the carboxyl group, respectively. The ^{13}C NMR spectrum revealed 14 sp^2-carbons which were attributed to seven methines and seven quaternary carbons, including one α,β-unsaturated ketone carbon at δ_C 178.4 and one carboxyl carbon at δ_C 166.2 (Table 1). Taking into account the above data, it was clear that **1** was a 4-quinolinone alkaloid with one carboxyl group and one pyrrole ring. However, the positions of the carboxyl group and the pyrrole ring at which of the two positions C-3 and C-2 could not be confirmed because of lacking HMBC correlation between H-3′ and C-3 or C-2. Many attempts were made to grow single crystals of **1** in various organic solvents without success.

In order to improve its crystallization property, compound **1** was methylated with methyl iodide and K_2CO_3.[9] An unexpected dehydrated lactamic product, penicinotam (**1a**),[10] was obtained rather than the expected methyl ester. Methylation with dimethyl sulfate instead of methyl iodide produced the same product **1a**. The HREIMS results (*m/z* 250.0733, calcd 250.0737) of **1a** implied it has a molecular formula of $C_{15}H_{10}N_2O_2$. The ^1H NMR and ^{13}C

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doi:10.1016/j.bmcl.2010.04.043

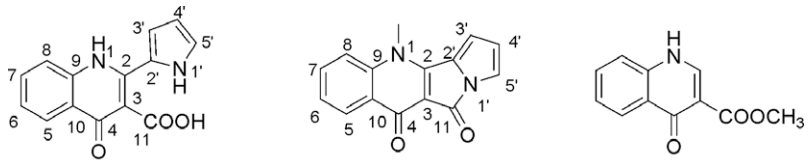


Figure 1. Structures of compounds **1**, **1a** and **2**.

Table 1
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of **1** and **1a** in DMSO-d_6

Position	1		1a	
	δ_H[a]	δ_C	δ_H[a]	δ_C
2		147.6, –C		153.8, –C
3		106.3, –C		104.3, –C
4		178.4, –C		169.9, –C
5	8.23, dd, 8.0, 1.5	124.9, CH	8.20, dd, 8.0, 1.6	125.9, CH
6	7.51, ddd, 8.0, 8.0, 1.5	125.3, CH	7.51, ddd, 8.0, 8.0, 1.6	125.6, CH
7	7.81, ddd, 8.0, 8.0, 1.5	133.7, CH	7.78, ddd, 8.0, 8.0, 1.6	132.7, CH
8	7.86, dd, 8.0, 1.5	119.1, CH	7.89, dd, 8.0, 1.6	117.7, CH
9		138.8, –C		140.4, –C
10		122.8, –C		128.7, –C
11		166.2, –C		159.5, –C
2′		123.6, –C		125.6, –C
3′	7.14, m	123.0, CH	7.09, dd, 3.5, 1.0	116.6, CH
4′	6.28, m	108.8, CH	6.43, dd, 3.5, 3.0	111.6, CH
5′	6.82, m	113.7, CH	7.47, dd, 3.0, 1.0	120.2, CH
NCH_3			3.99, s	36.6, CH_3
OH	16.13, s			
NH	12.33, s			
NH	11.80, s			

[a] Mult, *J* in hertz.

NMR spectra of **1a** were similar to those of **1** except that three exchangeable protons disappeared and one *N*-methyl group (δ_H 3.99; δ_C 36.6) emerged. However, the linkages of the carboxyl group and the pyrrole ring with the rest of the molecule remained undetermined due to the insufficient long range coupling. Fortunately, by slow crystallization from DMF, single crystals of **1a** suitable for X-ray diffraction analysis were obtained, allowing the structure of **1a** to be unambiguously established. It is a structurally unique 4-quinolinone conjugating with a bicyclic ring consisting of a γ-lactam ring and a pyrrole ring and all the atoms are almost coplanar in the molecule (Fig. 2). Thus, the structure of **1** was deduced as 3-carboxylic acid-2-pyrrole-4-quinolinone (**1**) on the basis of the structure of **1a**.

Quinolinone alkaloids, a group of secondary metabolites, are found more commonly in terrestrial materials than in marine organisms.[11,12] A structurally related 4-quinolinone alkaloid has been reported from chestnut honey, with a carboxyl group at C-2 and a pyrrolidinyl substituent at C-3.[13] To the best of our knowledge, naturally-occurring 4-quinolinone alkaloids containing a pyrrole ring have not previously been reported, so compound **1** is the first example. Taking into account of the structure of penicinoline (**1**), two amino acids ornithine and tryptophan might be the biosynthetic precursors. A plausible biogenetic pathway for penicinoline (**1**) is proposed in Scheme 1. This deduction was partially confirmed by the co-isolation of the possibly intermediate alkaloid, methyl 4-oxo-1,4-dihydroquinoline-3-carboxylate (**2**), from the same fungus.

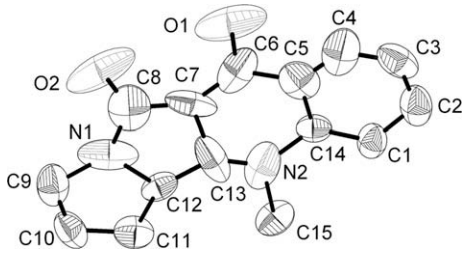
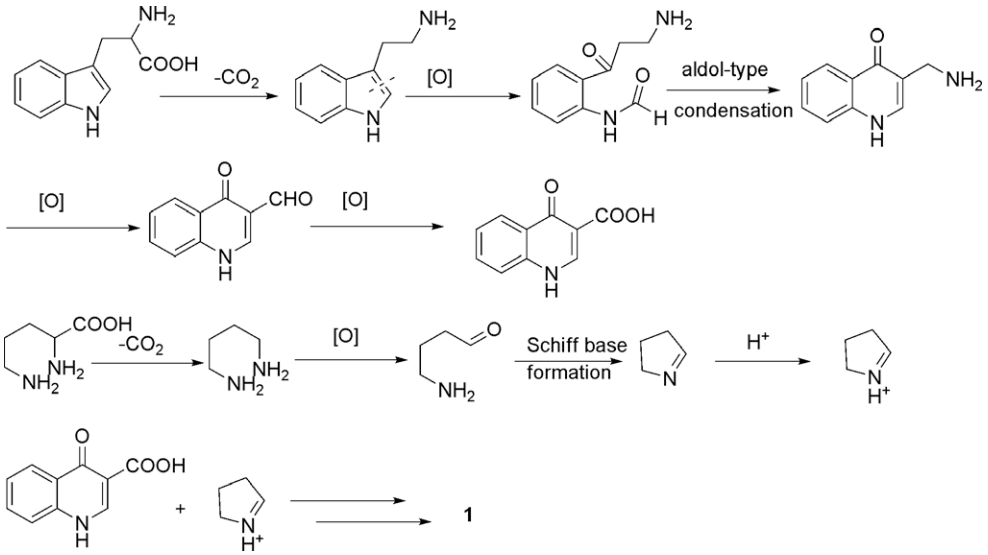


Figure 2. ORTEP drawing for penicinotam (**1a**).

Penicinoline (**1**) showed potent in vitro cytotoxicity toward 95-D and HepG2 cell lines with IC_{50} values of 0.57 and 6.5 μg/mL, respectively, while compound **1** was inactive against Hela, KB, KBv200, and Hep2 cell lines at the concentration of 100 μg/mL (Table 2).[14]

Compounds **1** and **1a** were also tested for their insecticidal activity against *Aphis gossypii*, *Plutella xylostella*, *Heliothis virescens*, *Septoria tritici*, and *Uromyces fabae* using assays based on insect mortality and the area of leaf eaten by the methods described previously.[15] Compound **1** exhibited strong activity against *A. gossypii* with 100% mortality on a mixed population at 1000 ppm, a very impressive result for a natural product. The semi-synthetic compound **1a** not only retained this strong activity on the sucking pest *A. gossypii*, but also showed total control of larvae of the chewing pest *P. xylostella* at 500 ppm and some activity on *H. virescens* at 1000 ppm (Table 3). Unfortunately, limited amounts of compounds **1** and **1a** prevented further structural modification as well as SAR studies. Further studies on penicinoline and penicinotam, including the synthesis of analogues, the biogenetic pathway, and structure–activity relationships, are in progress.



Scheme 1. Possible biogenetic pathway to penicinoline (**1**).

Table 2
Cytotoxic activity of **1**

Cell lines	IC_{50} (μg/mL) **1**
95-D	0.57
HepG2	6.5
Hela	>100
KB	>100
KBv200	>100
Hep2	>100

Table 3
Insecticidal activity of **1** and **1a**

Insect types	100% mortality (ppm)	
	1	**1a**
Aphis gossypii	1000	1000
Plutella xylostella	>1000	500
Heliothis virescens	>1000	>1000
Septoria tritici	>1000	>1000
Uromyces fabae	>1000	>1000

Acknowledgments

We thank Syngenta for the fellowship to C.L.S. and the agrochemical assays. This work was supported by the National Natural Science Foundation of China (Nos. 40976077, 30901879, 40776073), the Research Fund for the Doctoral Program of Higher Education, Ministry of Education of China (No. 20090132110002), the Basic Research Program of Science and Technology, Ministry of Science and Technology of China (No. 2007FY210500), and the Open Research Fund Program of Key Laboratory of Marine Drugs (Ocean University of China), the Ministry of Education (No. KLMD (OUC) 200801).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.04.043.

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6. *Fungal material:* The fungus strain *Penicillium* sp. was isolated from the bark of mangrove *Acanthus ilicifolius* Linn. (endophyte) collected from the South China Sea in September, 2005. The strain was deposited in the School of Life Science and the School of Chemistry and Chemical Engineering, Sun Yat-sen (Zhongshan) University, Guangzhou, PR China with the access code ZJ-2005032. Ninety liters of the fungal strain were cultivated in liquid medium (10.0 g of glucose, 2.0 g of yeast extract, 2.0 g of peptone in 1 L of seawater, in a 1.0 L Erlenmeyer flasks each containing 400 mL of culture broth) at 27 °C without shaking for five weeks.
7. *Extraction and separation of metabolites:* The mycelium (450.0 g, dry weight) was crushed and extracted successively with 3 L of MeOH at room temperature for three times. The solution was concentrated in vacuum and gave a yellow residue (12.5 g). The crude extract was partitioned between EtOAc and H_2O. The EtOAc solution was evaporated to give a dark yellow residue (4.6 g). The extract was subjected to a silica gel CC (200–300 mesh, 500 g) eluted with a gradient of petroleum ether and EtOAc and yielded **1** (15.5 mg) from the EtOAc/petroleum ether (100:0) fractions.
8. *Penicinoline* (**1**): A yellow amorphous powder; mp 350–352 °C; UV (CH_3OH) λ_{max} (log ε) 214.5 (3.00), 312.5 (1.82), 353.0 (1.80) nm; IR (KBr) ν_{max} 3292, 3113, 1626, 1569, 1524, 1500, 1471, 1442, 1396, 1353, 1132, 867, 777, 754 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz), see Table 1; EIMS (pos.) *m/z* 254 $[M]^+$, 236, 210, 179, 154, 127; HREIMS *m/z* 254.0681 (calcd for $C_{14}H_{10}N_2O_3$, 254.0691).
9. *Preparation of* **1a**: To a solution of compound **1** (10.0 mg) in acetone (10.0 mL), 50.0 mg of K_2CO_3 and 0.5 mL CH_3I or $(CH_3)_2SO_4$ were added. The solution was stirred at room temperature for 12 h. The reaction mixture was filtered through Celite, and the residue was washed with acetone (20.0 mL). The combined filtrates were evaporated to dryness under reduced pressure and the product **1a** was purified by a silica gel CC using 80:20 EtOAc/petroleum ether (v/v).
10. *Penicinotam* (**1a**): A yellow needle crystal; mp 323–325 °C; UV (CH_3OH) λ_{max} (log ε) 191.1 (5.4), 213.4 (4.20), 287.6 (3.30), 339.8 (1.90) nm; IR (KBr) ν_{max} 2930, 2872, 1737, 1674, 1608, 1544, 1496, 1458, 1403, 1377, 1232, 1094, 763 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz), see Table 1; EIMS (pos.) *m/z* 250 $[M]^+$, 222, 194, 166, 140, 80; HREIMS *m/z* 250.0733 (calcd for $C_{15}H_{10}N_2O_2$, 250.0737). Crystallizes in monoclinic, space group *P*2 (1)/*n* with $a = 7.3216(16)$ Å, $b = 22.498(5)$ Å, $c = 14.536(3)$ Å, $\alpha = 90°$, $\beta = 103.416(3)°$, $\gamma = 90°$, $C_{15}H_{10}N_2O_2$, $M_r = 250.07$, $V = 2329.1$ (9) $Å^3$, $Z = 4$, $D_c = 1.476$ g/cm^3, $F(0\,0\,0) = 1076$, $\mu = 0.103$ mm^{-1}, the final $R = 0.1380$ and $wR = 0.3376$ for 4332 observed reflections ($I > 2\sigma(I)$). The paragraph crystallographic data for **1a** have been deposited at the Cambridge Crystallographic Data Centre (CCDC No.734744).
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