Tetrahedron 72 (2016) 5679–5683

Contents lists available at ScienceDirect

Tetrahedron

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

Campyridones A–D, pyridone alkaloids from a mangrove endophytic fungus *Campylocarpon* sp. HDN13-307

Meilin Zhu, Xiaomin Zhang, Huimin Feng, Qian Che, Tianjiao Zhu, Qianqun Gu, Dehai Li *

Key Laboratory of Marine Drugs, Chinese Ministry of Education; School of Medicine and Pharmacy, Ocean University of China, 5 Yushan Road, Qingdao, Shandong, 266003, PR China

ARTICLE INFO

Article history:
Received 1 June 2016
Received in revised form 20 July 2016
Accepted 30 July 2016
Available online 2 August 2016

Keywords:
Pyridone alkaloids
Endophytic fungus
Mangrove plant
Campylocarpon sp.
Cytotoxicity

ABSTRACT

Two pairs of epimers, campyridones A and B (**1** and **2**) and campyridones C and D (**3** and **4**), were isolated from the mangrove endophytic fungus *Campylocarpon* sp. HDN13-307, as well as the biogenetically related known ilicicolin H (**5**). Their structures including absolute configurations were elucidated on the basis of the spectroscopic analysis and ECD calculations. The new compounds **1**–**4** possess unprecedented ring systems featured by the appearance of an additional spiro-furanone (**1**/**2**) or γ-pyrone (**3**/**4**) moieties (C ring), which represents a new family of 4-hydroxy-2-pyridone alkaloids. Compounds **4** and **5** were cytotoxic against Hela cell with IC_{50} values of 8.8 μM and 4.7 μM, respectively. A plausible biosynthetic pathway for **1**–**4** is also elucidated.

1. Introduction

4-Hydroxy-2-pyridone alkaloids are a family of natural products distributing in both terrestrial and marine organisms and the subtype featured with a decalin moiety linked via a carboxide bridge is attractive for the diverse structure and broad bioactivity.[1] Since the discovery of ilicicolin H from *Cylindrocladium ilicicola* in 1971,[2,11] other compounds have been reported including fischerin,[3] apiosporamide,[4] *N*-hydroxyapiosporamide,[5] YM-215343,[6] didymellamides A–D,[7] and arthpyrones A–C[8] (Fig. S1 in Supplementary data). They showed a wide range of biological activities, such as antifungal (ilicicolin H and didymellamides A-B),[2,7] antitumor (arthpyrones A–B and *N*-hydroxyapiosporamide)[5,8] and AchE inhibitory activity (arthpyrone C).[8] They were considered to be biosynthesized via a tetramic acids intermediate formed by hybriding a polyketide unit to a tyrosine moiety.[9–11]

Mangrove endophytic fungi appear to be abundant resource for bioactive secondary metabolites.[12–14] During our exploration for novel antitumor agents from marine derived fungi, an endophytic fungus *Campylocarpon* sp. HDN13-307, isolated from the root of mangrove plant *Sonneratia caseolaris*, was selected for the significant cytotoxic activity against P388 cells (inhibitory rate 81.5% for the EtOAc extract at the concentration 100 μg/mL). Chemical investigation led to the isolation of four new 4-hydroxy-2-pyridone alkaloids, named as campyridones A–D (**1**–**4**) (Fig. 1), as well as the biogenetically related ilicicolin H (**5**). Campyridones A–D (**1**–**4**) existed as two pairs of diastereoisomers, and featured with an additional C ring between the decalin and pyridone units which represented as new ring systems for this family of alkaloids. Herein, the isolation, structure elucidation and activities are described.

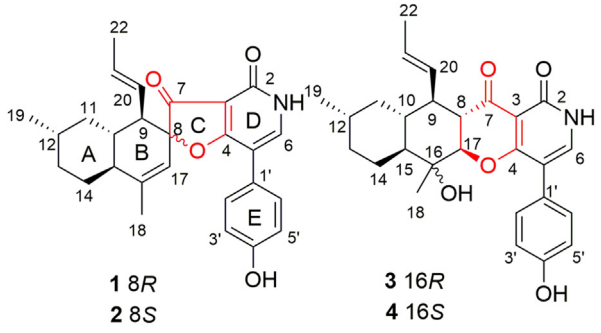

Fig. 1. The structures of compounds **1**–**4**.

* Corresponding author. Fax: +86 532 82033054; e-mail address: dehaili@ouc.edu.cn (D. Li).

http://dx.doi.org/10.1016/j.tet.2016.07.080
0040-4020/

2. Results and discussion

Campyridone A (**1**) was obtained as a yellow oil. The molecular formula was determined as $C_{27}H_{29}NO_4$ by HRESIMS peak at *m*/*z* 432.2175 $[M+H]^+$, which indicated 14 degrees of unsaturation. The 1D NMR data showed the existence of three methyls, three methylenes, twelve methines and nine non-protonated carbons including two carbonyls, as well as two exchangeable protons (δ_H 9.59 and 11.70) (Table 1). The NMR data of **1** were similar to those of ilicicolin H (**5**), which was also isolated from this strain. The major differences were the replacement of an sp^3 methine signal (δ_H 4.95) in **5**[2,15] by a downfield non-protonated carbon signal (δ_C 92.0) in **1**. Considering the molecular formula and the downfield shift of C-4 (δ_C 176.0 in **5** vs 180.8 in **1**), a hydrogenated furan ring (ring C) with ether linkage between C-4 and C-8 was suggested, which was confirmed by the chemical shift of C-8 (δ_C 92.0). The structure of **1** was further supported by the COSY and HMBC correlations (Fig. 2).

Campyridone B (**2**) was obtained with the same molecular formula to **1**. Although the 1D and 2D NMR spectroscopic data (Table 1 and Fig. S2) indicated that they share the same planar structure, the chemical shifts for C-7, C-8, C-9, and C-17 were different (δ_C 197.7, 92.0, 49.5, 117.1 in **1** vs δ_C 196.1, 93.5, 51.1, 118.5 in **2**, respectively), which suggested that they are C-8 epimers.

The relative configurations of **1** and **2** were partially determined through NOESY spectrum (Fig. 3). The correlations from H-9 to H-15, H-15 to Me-19, and H-10 to H-12 indicated that H-9, H-15, and Me-19 were on the same side of the decalin while H-10 and H-12 were on the other side. However, the configurations of C-8 cannot be deduced by NMR because of lacking any available signals. Given that **1** and **2** were C-8 epimers and the biogenetic consideration, the ECD spectra of all the possible relative configurations were calculated based on time-dependent density-functional theory (TDDFT), and compared with the experimental ECD curves. The MMFF (molecular mechanics force field) conformational searches of the arbitrarily chosen (8*S*, 9*R*, 10*S*, 12*S*, 15*S*)-**1** and (8*R*, 9*R*, 10*S*, 12*S*, 15*S*)-**2** were carried out. And then the DFT reoptimization of the two epimers were performed at the B3LYP/6-31G(d) level with a PCM solvent model for MeOH, which afforded one major lowest-energy conformer for both **1** and **2** with 98.6% and 99.7% population (Fig. S3, supplementary data). The ECD spectra were calculated at the same basis set and the results showed well agreement with the experiment ones (Fig. 4), suggesting the absolute configurations of **1** and **2** to be (8*S*, 9*R*, 10*S*, 12*S*, 15*S*) and (8*R*, 9*R*, 10*S*, 12*S*, 15*S*), respectively.

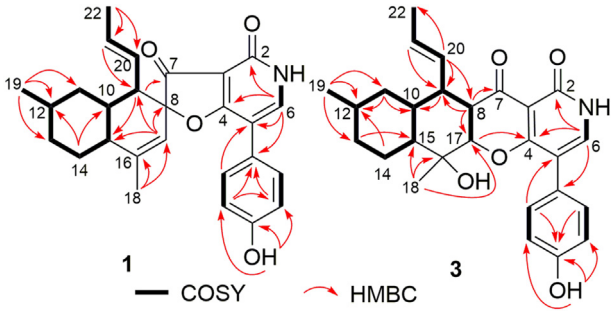

Fig. 2. Key COSY and HMBC correlations of **1** and **3**.

Campyridones C (**3**) and D (**4**) were obtained as pale yellow power. Their molecular formulas were all deduced as $C_{27}H_{31}NO_5$ on the basis of HRESIMS protonated peaks at *m*/*z* 450.2283 and

Table 1
^1H and ^{13}C NMR data for **1**–**4** (500, 125 MHz, DMSO-d_6, TMS, δ, ppm)

No.	**1**		**2**		**3**		**4**	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1-NH		11.70, br s		11.70, br s		11.52, br s		11.46, br s
2	157.5		157.4		159.1		158.9	
3	106.5		106.7		107.0		107.1	
4	180.8		180.9		169.6		169.1	
5	108.7		108.6		111.7		111.8	
6	143.2	7.74, s	142.9	7.73, s	139.2	7.45, s	138.9	7.41, s
7	197.7		196.1		190.3		190.4	
8	92.0		93.5		48.8	2.65, dd (13.8, 10.9)	47.7	2.83, dd (13.6, 10.8)
9	49.5	2.33, dd (11.2, 9.9)	51.1	2.41, dd (11.2, 9.9)	40.9	1.85, m	42.5	1.83, m
10	39.7	1.40, m	38.9	2.04, m	40.4	0.95, m	38.7	1.28, m
11	38.8	0.54, m	39.0	0.52, m	39.9	0.39, m	40.9	0.35, m
		1.68, m		1.56, m		1.85, m		1.84, m
12	32.4	1.37, m	32.4	1.37, m	32.2	1.21, m	32.1	1.16, m
13	35.1	0.93, m	35.3	0.93, m	34.7	0.75, dt (11.2, 3.3)	34.9	0.76, dt (11.2, 3.3)
		1.70, m		1.73, m		1.67, d (11.2);		1.65, d (11.2);
14	29.0	0.98, m	29.2	0.98, m	24.7	0.94, m	24.7	1.16, m
		1.99, m		1.99, m		1.91, m		1.68, m
15	44.3	1.66, m	44.5	1.69, m	42.9	0.90, m	46.7	0.80, m
16	145.5		145.3		72.7		70.9	
16-OH						4.35, s		4.44, s
17	117.1	5.10, s	118.5	5.06, s	86.9	3.98, d (13.8)	85.5	4.07, d (13.6)
18	20.9	1.67, s	20.8	1.66, s	15.5	1.04, s	22.0	1.08, s
19	22.9	0.83, d (6.6)	23.0	0.84, d (6.6)	23.0	0.81, d (6.5)	23.1	0.79, d (6.4)
20	126.5	5.04, dd (14.9, 9.8)	127.1	4.91, dd (14.9, 9.8)	134.3	5.26, dd (15.3, 6.4)	134.7	5.25, dd (15.5, 7.8)
21	129.9	5.32, m	130.0	5.47, m	125.3	5.41, m	124.9	5.38, m
22	18.2	1.45, d (6.0)	18.2	1.45, d (6.0)	18.5	1.60, d (6.3)	18.5	1.59, d (6.5)
1′	122.6		122.5		124.1		124.3	
2′	129.4	7.31, d (8.4)	129.3	7.31, d (8.4)	130.4	7.31, d (8.7)	130.7	7.29, d (8.7, 2.0)
3′	116.0	6.82, d (8.4)	115.9	6.80, d (8.4)	115.4	6.74, d (8.7)	115.3	6.73, d (8.7, 2.0)
4′	157.5		157.4		156.9		157.0	
4′-OH		9.59, s		9.55, s		9.46, s		9.43, s
5′	116.0	6.82, d (8.4)	115.9	6.80, d (8.4)	115.4	6.74, d (8.7)	115.3	6.73, d (8.7)
6′	129.4	7.31, d (8.4)	129.3	7.31, d (8.4)	130.4	7.31, d (8.7)	130.7	7.29, d (8.7)