



## A novel antifouling alkaloid from halotolerant fungus *Penicillium* sp. OUCMDZ-776

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### ABSTRACT

Penispirolloid A (**1**), a novel alkaloid possessing a unique spiro imidazolidinyl skeleton, was isolated from a halotolerant fungal strain, *Penicillium* sp. OUCMDZ-776. Its structure was elucidated on the basis of spectroscopic methods and quantum chemical CD calculation. Compound **1** showed significant antifouling activity toward *Bugula neritina* larvae with EC<sub>50</sub> of 2.40 μg/ml. Plausible biogenesis of compound **1** was also proposed.

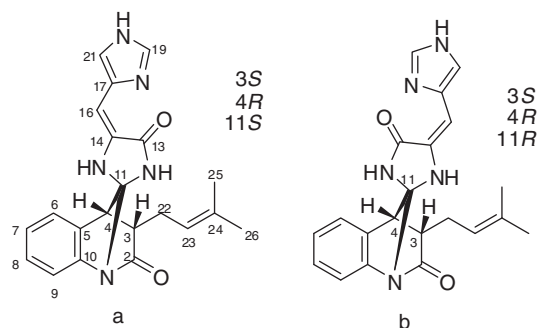
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### Introduction

Biofouling, the colonization of submerged surfaces by living organism is becoming a widespread global phenomenon and causes serious problems and huge economic losses every year.<sup>1–3</sup> Marine microorganisms have proved to be an important source of antifouling metabolites.<sup>4</sup> In our preliminary experiment, we found the EtOAc extract of a culture broth of the halotolerant fungal strain *Penicillium* sp. OUCMDZ-776 exhibited significant antifouling activity toward laboratory-reared *Bugula neritina* larvae. Bioassay-guided fractionation of the extract led to the isolation of a new alkaloid penispirolloid A (**1**) possessing a unique spiro imidazolidinyl skeleton. On the basis of extensive spectroscopic methods and quantum chemical CD calculation, the planar structure and partial absolute configuration of **1** were determined. In this Letter, the isolation, structure elucidation, antifouling activity and plausible biogenesis of **1** are described.

### Results and discussion

The EtOAc extract of a 10 L culture broth of *Penicillium* sp. OUCMDZ-776 was subjected to RP-C 18 column and Sephadex



Proposed structure of **1**

LH-20 column chromatography, followed by further purification with preparative-reversed HPLC, eluting with MeOH/H<sub>2</sub>O (55:45) to give compound **1**.

The molecular formula of penispirolloid A (**1**) was inferred as C<sub>21</sub>H<sub>21</sub>N<sub>5</sub>O<sub>2</sub> by the analysis of its HRESIMS (*m/z* 376.1768 [M+H]<sup>+</sup>), which indicated 14 degrees of unsaturation.<sup>5</sup> The UV spectrum showed absorption maxima at 238 and 322 nm, suggesting the presence of aromatic ring. The IR spectrum showed absorption bands at 3428, 1722, 1641, 1613 cm<sup>-1</sup>, which were assigned to

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**Table 1**  
NMR spectral data for penispinoloid A (**1**) in CD<sub>3</sub>OD<sup>a</sup>

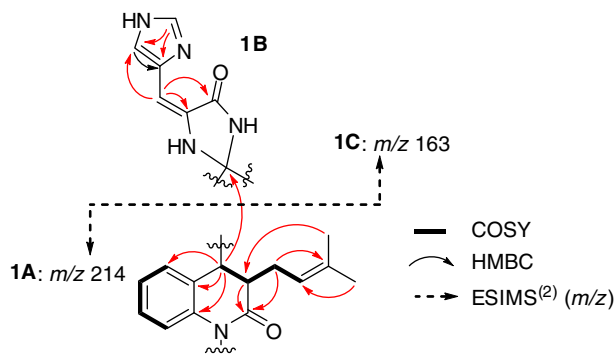
Position	Penispinoloid A			
	$\delta_{\text{H}}$ , mult (J Hz)	$\delta_{\text{C}}$ , mult	<sup>1</sup> H, <sup>1</sup> H COSY	HMBC
2		176.2 (C)		
3	2.84, ddd (4.5, 8.0, 8.5)	51.6 (CH)	H-4, 22	C-5, 23
4	3.62, d (8.5)	53.7 (CH)	H-3	C-3, 5, 6 <sup>*</sup> , 11, 22
5		126.7 (C)		
6	7.09, d (8.0) <sup>b</sup>	124.1 (CH)	H-7	C-5, 8, 10
7	6.75, dd (8.0, 8.0)	119.0 (CH)	H-6, 8	C-5, 6 <sup>*</sup> , 8 <sup>*</sup> , 9
8	7.08, dd (8.0, 8.0) <sup>b</sup>	128.6 (CH)	H-7, 9	C-6, 10
9	6.61, d (8.0)	108.7 (CH)	H-8	C-5, 7
10		150.0 (C)		
11		97.9 (C)		
13		165.8 (C)		
14		122.1 (C)		
16	7.26, s	110.8 (CH)		C-11, 13, 14, 17, 19
17		125.9 (C)		
19	7.67, s	136.4 (CH)		C-17, 21
21	7.26, s	133.1 (CH)		C-19
22a	2.64, ddd (5.5, 6.5, 14.0)	27.9 (CH <sub>2</sub> )		
22b	2.45, ddd (8.0, 7.0, 14.5)		H-3, 23	C-2, 23, 24
23	5.15, dd (7.0, 7.0)	119.9 (CH)		C-25, 26
24		134.9 (C)		
25	1.77, s	25.6 (CH <sub>3</sub> )		C-23, 24, 26
26	1.71, s	17.7 (CH <sub>3</sub> )		C-23, 24, 25

<sup>a</sup> 3–5 Drops of CDCl<sub>3</sub> added in CD<sub>3</sub>OD.

<sup>b</sup> Overlapped signals.

<sup>\*</sup> Weak signals.

NH, acylamino, and aromatic rings, respectively. The <sup>13</sup>C and DEPT NMR spectra (Table 1) displayed 21 carbon signals, including 2 methyls, 1 methylene, 10 methines, and 8 quaternary carbons. The <sup>1</sup>H NMR spectrum showed four characteristic aromatic protons at  $\delta_{\text{H}}$  6.61 (d,  $J = 8.5$  Hz, H-9), 6.75 (dd,  $J = 8.5, 8.5$  Hz, H-7), 7.08 (dd,  $J = 8.5, 8.5$  Hz, H-8), and 7.09 (d,  $J = 8.5$  Hz, H-6). The <sup>1</sup>H–<sup>1</sup>H COSY spectrum showing correlations of H-6 with H-7, H-7 with H-8, H-8 with H-9, H-3 ( $\delta_{\text{H}}$  2.84, ddd,  $J = 4.5, 8.0, 8.5$  Hz) with H-4 ( $\delta_{\text{H}}$  3.62, d,  $J = 8.5$  Hz), and H-22 ( $\delta_{\text{H}}$  2.64, 2.45) with H-3/H-23 ( $\delta_{\text{H}}$  5.14, t,  $J = 7.0$  Hz), and HMBC spectrum showing correlations of H-4 with C-5 ( $\delta_{\text{C}}$  126.7)/C-6 ( $\delta_{\text{C}}$  124.1)/C-10 ( $\delta_{\text{C}}$  150.0), H-3 with C-2 ( $\delta_{\text{C}}$  176.2), H-22 with C-2/C-24 ( $\delta_{\text{C}}$  134.9), and Me-25 ( $\delta_{\text{H}}$  1.77, s, 3H)/Me-26 ( $\delta_{\text{H}}$  1.71, s, 3H) with C-23 ( $\delta_{\text{C}}$  119.9), suggested the presence of a 3,4-disubstituted 2-quinolone moiety with an isopentenyl group at C-3 (partial structure **1A** in Fig. 1). The suggestion was proved by the comparison of the <sup>1</sup>H and <sup>13</sup>C NMR data

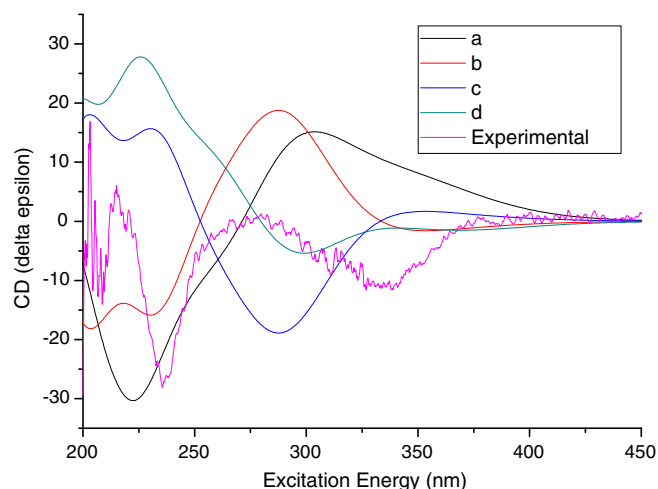


**Figure 1.** Key <sup>1</sup>H–<sup>1</sup>H COSY, HMBC correlations, and ESIMS<sup>(2)</sup> fragmentation of **1**.

of **1** with those of 2-quinolone derivatives.<sup>6,7</sup> The large coupling constant ( $^3J_{\text{H-3/H-4}} = 8.5$  Hz) suggested the cis-fused correlation of H-3 and H-4.

The <sup>1</sup>H NMR spectrum also exhibited two aromatic protons of an imidazole nucleus at  $\delta_{\text{H}}$  7.26 (s, H-21), 7.67 (s, H-19). In the HMBC spectrum, correlations of H-19 with C-17 ( $\delta_{\text{C}}$  125.9)/C-21 ( $\delta_{\text{C}}$  133.1), H-16 ( $\delta_{\text{H}}$  7.26, s) with C-13 ( $\delta_{\text{C}}$  165.8)/C-14 ( $\delta_{\text{C}}$  122.1)/C-17/C-21, suggested the presence of a 2-substituted urocanic acid amide moiety (partial structure **1B** in Fig. 1). Compared with the <sup>13</sup>C NMR data of roquefortine,<sup>8,9</sup> the chemical shifts for C-14, C-16 ( $\delta_{\text{C}}$  110.8), and C-21 in **1** indicated the *E* geometry of the double bond between C-14 and C-16. Specifically, pseudo ion peaks at  $m/z$  214 [ $\text{M}_{1\text{A}}^+\text{H}$ ]<sup>+</sup> and 163 [ $\text{M}_{1\text{C}}^+\text{H}$ ]<sup>+</sup>, caused by the fission of C11–C4 and C11–N1 bonds, were detected in ESIMS<sup>(2)</sup>. Key HMBC correlation of H-4 with C-11 ( $\delta_{\text{C}}$  97.9, s), combined with the molecular formula, degrees of unsaturation, and ESIMS<sup>(2)</sup> of **1**, suggested the linkage of **1A** with **1B** through a spiro-carbon C-11 as shown in Figure 1.

The relative configuration of **1** was established by the coupling  $^3J_{\text{H-3/H-4}}$  and ROESY spectrum showing the NOE correlations of H-4 with H-6/H-22/H-23. Considering the existence of three chiral centers in the molecular and the cis relative configuration between C-3 and C-4, there were four possible configurations for **1**: (a) 3*S*/4*R*/11*S*, (b) 3*S*/4*R*/11*R*, (c) 3*R*/4*S*/11*S*, and (d) 3*R*/4*S*/11*R* (see Supplementary data). The quantum chemical CD calculation method was used to further establish the absolute configurations of **1**.<sup>10</sup> The preliminary conformational distribution search was performed by SYBYL 8.0 software package using the TRIPOS force field. The corresponding minimum geometries were then optimized by using density functional calculations at the B3LYP/6-31G(d) level in GAUSSIAN 09.<sup>11</sup> The stable conformers obtained were submitted to CD calculation by TDDFT [B3LYP/6-31G(d)] method. The overall predicted CD spectra of **1** (a, b, c, and d) were subsequently compared with the experimental ones (Fig. 2). It is evident that the calculated ECD spectra of structures a and b match well with the experimental data in the region of 200–450 nm, indicating that the configuration of **1** should be the structure a or b, whereas the calculated CD spectra of the isomers c and d were opposite, suggesting that the isomers c and d can be ruled out. As there was no sufficient evidence to further differentiate the structures a and b, the absolute configuration of C-11 in **1** was undetermined finally.



**Figure 2.** Calculated CD spectra of four proposed stereoisomers of **1** and experimental CD spectrum of **1**.

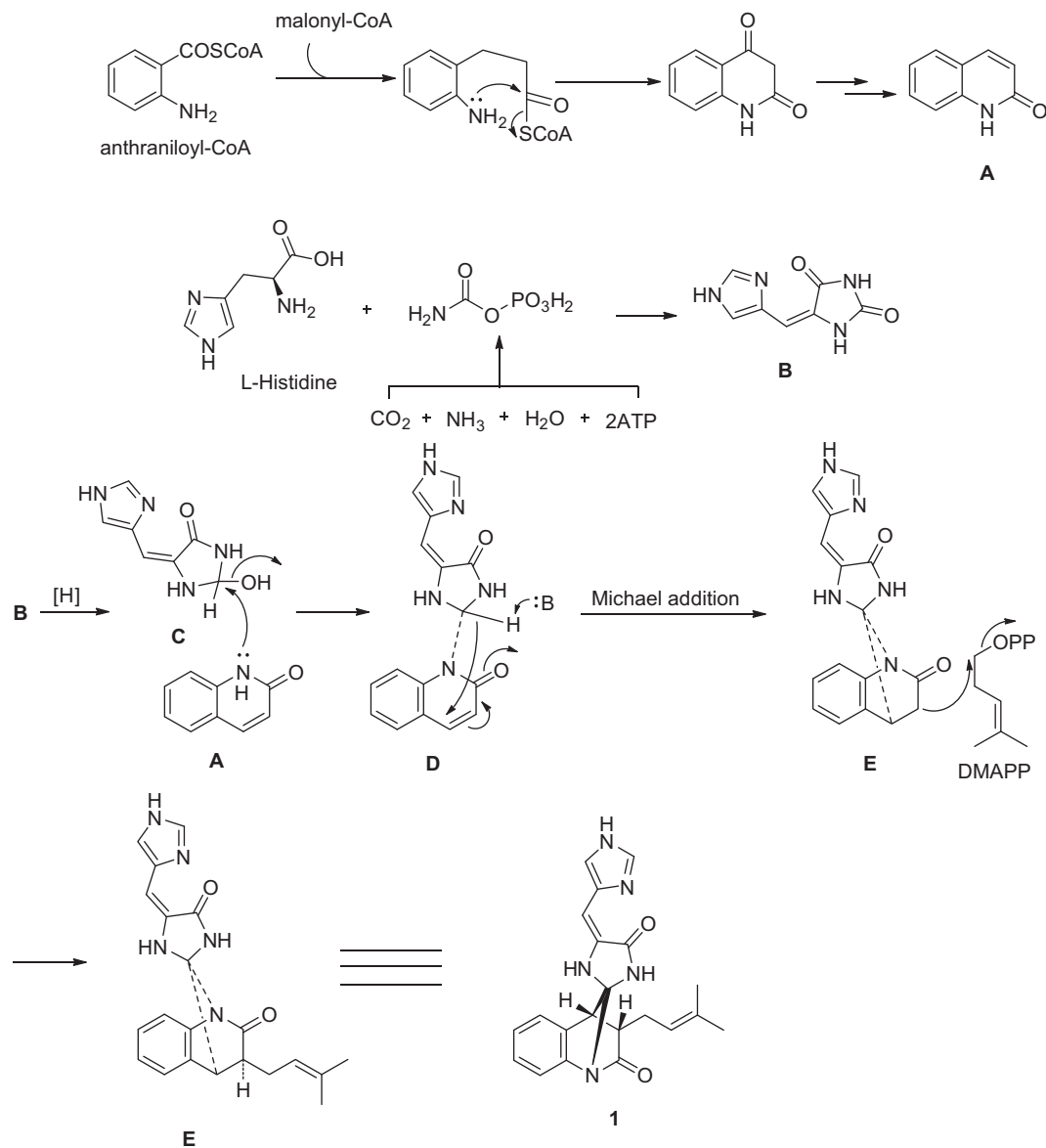


Figure 3. Proposed biogenetic pathway of **1**.

Compound **1** is a new alkaloid possessing a unique spiro imidazolidinyl skeleton. From the viewpoint of biosynthesis, **1** might be originated from anthraniloyl-CoA<sup>12</sup> and histidine, after the selective reduction of **B** to give **C**, nucleophilic substitution of **C** with **A** to give **D**, and subsequent Michael cycloaddition to form the spiro cyclic intermediate **E**. Because a bicyclic lactam of **E** possessing a bridgehead nitrogen, should have the property of an amide ketone rather than a lactam, such as 7-oxo-haemanthamine.<sup>13</sup> Finally the nucleophilic substitution between **E** and DMAPP to form compound **1** as shown in Figure 3. Compound **1** was tested for its antifouling activity against larval settlement of *B. neritina* larvae with  $EC_{50}$  of 2.40  $\mu\text{g/ml}$ .

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#### Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2012.02.063.

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