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Speramides A–B, two new prenylated indole alkaloids from the freshwater-derived fungus *Aspergillus ochraceus* KM007

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ABSTRACT

Two new prenylated indole alkaloids, named Speramides A (**1**) and B (**2**) were isolated from the extraction of the freshwater-derived fungus *Aspergillus ochraceus* KM007. Their structures were elucidated by means of spectroscopic analysis. Compound **1** is the first prenylated brevianamide derivative. These compounds isolated were evaluated for their antimicrobial activity, among which, compound **1** showed moderate activity against *Pseudomonas aeruginosa* with a MIC value of 0.8 μM.

A family of prenylated indole alkaloids including brevianamides, paraherquamides, stephacidins, asperparalines, marcfortines, sclerotiamides, notoamides, et al. which all contain a diketopiperazine or a core bicyclo-[2,2,2]diazaoctane ring, have been isolated from various fungi of the genera *Aspergillus* and *Penicillium*.[1–8] Biosynthetically, these alkaloids are derived from L-tryptophan, a second cyclic amino acid residue, and one or two isoprene units. Due to their intriguing structural features and remarkable diverse bioactivities, including insecticidal, antitumor, anthelmintic, calmodulin inhibitory, and antibacterial properties, this family has become a fascinating target for chemical synthesis, biosynthesis, and bioactivity studies.[9–16]

In our continuing search for novel compounds with bioactivity,[17–20] we obtained a fungal strain named *Aspergillus ochraceus* KM007 from the freshwater of Fuxian Lake. Chemical investigation of this strain led to the isolation of two new prenylated alkaloids (**1**–**2**). Their structures were characterized by physical data (Fig. 1).

Speramide A (**1**) was obtained as a white amorphous powder with $[\alpha]_D^{26}$ −106.6 (*c* 0.11, MeOH).[21] Its molecular formula $C_{26}H_{29}N_3O_4$ was determined by HRESIMS at *m*/*z* 447.2167 ($[M+H]^+$, calcd 447.2158), which represented 14 degrees of unsaturation. The IR spectrum of **1** showed absorption bands at 1753 and 1630 cm^{-1}, suggesting the existence of carbonyl moiety. Examination of the ^1H NMR resonances revealed the presence of one 1,2,3,4-tetrasubstituted benzene unit (7.16, d, J = 7.9 Hz; 6.57, d, J = 7.9 Hz), one cis 1,2-disubstituted double bond (δ_H 5.86, d, J = 9.8 Hz; 6.83, d, J = 9.8 Hz;), four methyls (δ_H 1.17, s; 1.21, s; 1.39, s), two NH singlets (δ_H 8.78, s; 7.11, s). The ^{13}C NMR spectrum (Table 1) displayed 26 resonances, which were classified by DEPT and HSQC experiments as one ketone group (δ_C 190.1), two amide (δ_C 172.1 and 171.2), eight olefinic or aromatic carbons, four methyls, five sp^3 methylenes, one methines, and five sp^3 quaternary carbons (one oxygenated and two nitrogenated). These data accounted for 7 out of 14 degrees of unsaturation, indicating the presence of a heptacyclic skeleton of **1**.

Comprehensive analysis of 1D- and 2D-NMR spectra, confirmed the fusion of a pyrrolidine ring to bicyclo-[2,2,2]diazaoctane subunit (Fig. 2A).[22] Interpretation of HMBC indicated the presence of a 5,6-disubstituted 2,2-dimethyl-2*H*-chromene moiety in which the benzene ring and the double bond are incorporated. Furthermore, the HMBC correlations from H-21 to C-10, C-22, C-23, and C-24, and from H_2-10 to C-2 led to the identification of the partial structural fragment of 2,2-disubstituted cyclopentane, which was fused to the right segment via C-11 and C-21. The signal for NH-1 showed HMBC correlations to C-2, C-9, and C-10, indicating that

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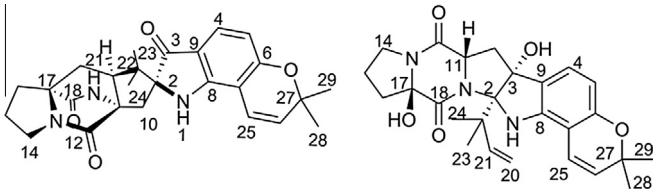


Figure 1. Chemical structures of compounds **1** and **2**.

C-2 and C-8 were connected with a nitrogen atom. In addition, the BV HMBC correlations from H-4, H_3-23 (J^4), and H_3-24 (J^4) to the ketone carbonyl group showed that the ketone carbon was connected to C-2, being assignable to C-3. The connection between C-3 and C-9 was deduced by their corresponding chemical shifts. The structure of **1** was accordingly revealed to possess an aza-spiro structure with a bicyclo[2.2.2]diazaoctane fused 2,2-dimethyl-2*H*-furan moiety attached to a 3-ox-indole fragment.

The relative configuration assigned for **1** was deduced by the analysis of ROESY data (Fig. 2B). Examination of a Dreiding molecular model of **1**, suggested that **1** adopts a conformation in which the central five-membered ring is orthogonal to the plane of the indoline subunit. ROESY correlations of H-10α/NH-1/H_3-23 indicated that H-10α and H_3-23 are both on the face of the cyclopentanoid ring, thus, orienting them toward NH-1, and fixing the relative configuration at C-2 as shown. ROESY interactions of H-21 with H_3-24 and H-10β, and H-10β with NH-19 placed the corresponding substituents together on the opposite face of the cyclopentanoid ring. The remaining stereocenter is C-17, which must have the relative configuration depicted to permit connection of the amide bridge to C-11.

The absolute configuration of compound **1** was established by a CD exciton chirality method (Fig. 3). Williams et al. reported the

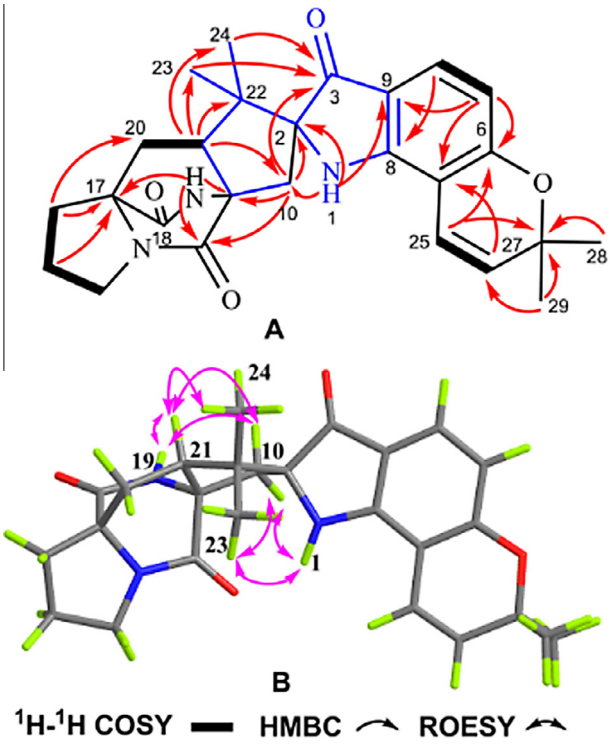


Figure 2. Key HMBC and ^{1}H–^{1}H COSY correlations (A) and ROESY correlations (B) of compound **1**.

Cotton effect at 200–250 nm is diagnostic for the bicyclo[2.2.2]diazaoctane diketopiperazine core, which arises from an n–π* transi-

Table 1
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of Speramides A (**1**) and B (**2**) in DMSO-d_6 (δ in ppm, *J* in Hz)

No.	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1	7.11 (m)		6.72 (s)	
2		80.3		91.3
3		190.1		87.4
4	7.16 (d, 7.9)	122	7.00 (d, 8.1)	124.0
5	6.57 (d, 7.9)	112.7	6.13 (d, 8.1)	106.3
6		153.3		153.7
7		113.7		104.6
8		147.6		145.8
9		134.7		122.9
10	3.03 (d, 14.8)	40.1	α 1.60 (dd, 7.6, 12.5) β 2.49 (overlapped)	34.9
11		60.4	4.01 (dd, 11.4, 7.8)	57.2
12		171.2		167.1
14	3.48 (m)	44.2	3.42 (m), 3.31 (m)	44.3
15	1.86 (m)	24.1	1.95 (m), 1.79 (m)	20.6
16	1.89 (m), 2.51 (m)	28.4	2.00 (m), 1.95 (m)	36.7
17		66.7		89.8
18		172.1		170.3
19	8.78 (s)			
20	2.00 (dd, 13.5, 6.5) 2.03 (dd, 13.5, 6.5)	30.4 137.3	4.82 (dd, 17.0, 2.4) 4.81 (dd, 11.0, 2.4)	110.8
21	2.12 (dd, 9.6, 6.5)	54.8	6.30 (dd, 17.0, 11.0)	145.6
22		40.2		44.8
23	1.17 (s)	17.2	1.17 (s)	24.7
24	1.21 (s)	26.2	1.21 (s)	24.0
25	6.83 (d, 9.8)	117.2	6.54 (d, 9.8)	117.5
26	5.86 (d, 9.8)	131.9	5.61 (d, 9.8)	128.2
27		76.1		75.6
28	1.39 (s)	27.6	1.33 (s)	27.5
29	1.39 (s)	27.5	1.36 (s)	28.0
3-OH			5.60 (s)	
17-OH			6.51 (s)	

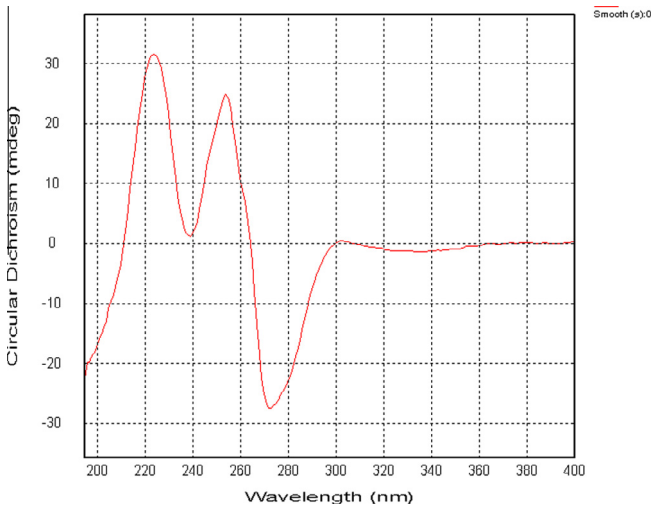
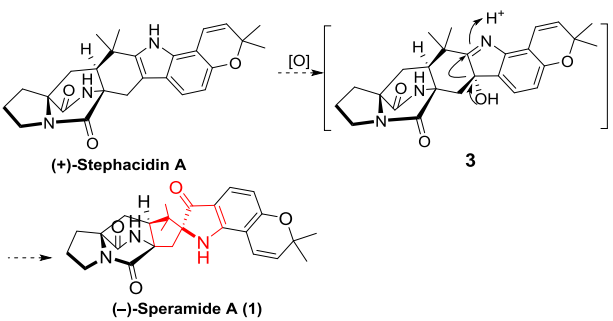


Figure 3. CD spectrum of Speramide A (**1**) in MeOH.

tion of the diketopiperazine amide bonds.[22] The CD spectrum of **1** correlated to the relevant regions in that of (−)-Notamide B and revealed that the absolute stereochemistry of **1** should be 2S,11S,17S,21S.[23]

A proposed biogenetic pathway for the assembly of **1** is shown in Scheme 1. (+)-Stephacidin A seems to be a plausible precursor, which is converted to **3** via oxidation.[24] The subsequent rearrangement of **3** would afford alkaloid **1**.

Speramide B (**2**) was isolated as a yellow oil and had a molecular formula of $C_{26}H_{31}N_3O_5$ on the basis of HRFABMS (*m/z* 466.2339 $[M+H]^+$, calcd 466.2342).[25] Its ^1H and ^{13}C spectrum data were similar to those of the known compound, Notoamide D, with exception of the loss of an α-nitrogen methine (δ_C 62.2 in acetone-d_6) in the latter and the addition of an amino ketal quaternary carbon (δ_C 89.8 in DMSO-d_6) in the former, supporting the fact that compound **2** has the same skeleton as Notoamide D but with the additional hydroxyl group at C-17. Data from the ^1H–^1H COSY, HMQC, and HMBC spectra further justified the structure of **2**, as shown in Figure 3. In the ROESY spectrum, the correlation of 17-OH with H-11 indicated that the H-11 and 17-OH were located at the same side, and taken α-axial orientation. In addition, the relative configuration at the remaining chiral centers of **2** was analogous to that of Notoamide D on the basis of ^{13}C NMR shifts and NOE data. The specific rotation value of **2** ($[\alpha]_D^{22}$ −95.1) matched up to the computated value ($[\alpha]_D^{22}$ −148.2), [26] which was also in accordance to the literature value of Notoamide K ($[\alpha]_D^{21}$ −128). The absolute configuration of the latter has been determined by an analysis of its acid hydrolysate.[27] Therefore, on the basis of biogenetic considerations, the absolute configuration of **2** is tentatively assigned as 2S,3R,11S,17R (Fig. 4).



Scheme 1. Possible biogenetic pathway of **1**.

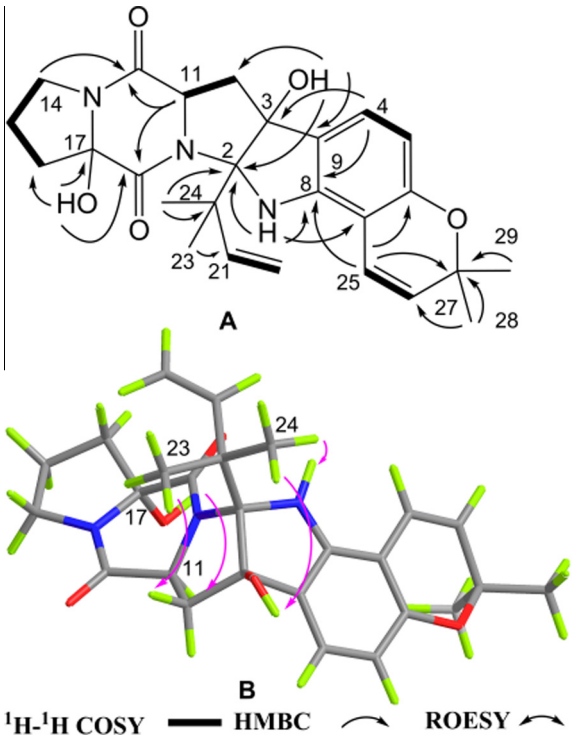


Figure 4. Key HMBC and ^1H–^1H COSY correlations (A) and ROESY correlations (B) of compound **2**.

Cytotoxicity of compounds **1** and **2** were tested on PC3, DU145 and Lncap cell lines, however, none exhibited significant activities (IC_{50} >40 μM). We also evaluated their antimicrobial activities against four microorganisms, MRSA 92#, MRSA 98#, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Finally, only compound **1** demonstrated moderate antimicrobial activities against *Pseudomonas aeruginosa* with a MIC value of 0.8 μM in the 2-fold dilution method.[28]

Acknowledgments

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Supplementary data

Supplementary data (1D and 2D NMR spectra, and CD spectrum of Speramides A–B) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.09.071.

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25. *Speramide B* (***2***): colorless oil; $[\alpha]_D^{22}$ −95.1 (*c* 0.09, MeOH); UV (MeOH): λ_{max} 292, 273, 320 nm; IR (KBr): ν_m 3423, 2928, 1700, 1056, 1030 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS: 466.2339 $[M+H]^+$ (calcd for $C_{26}H_{31}N_3O_5$ 466.2342).
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