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Isolation of a new indoxyl alkaloid, Amoenamide B, from *Aspergillus amoenus* NRRL 35600: biosynthetic implications and correction of the structure of Speramide B

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ABSTRACT

A new prenylated indoxyl alkaloid, Amoenamide B (**1**), was isolated from *Aspergillus amoenus* NRRL 35600 along with Asperochramide A (**2**). Although many prenylated oxyindole alkaloids, containing bicyclo[2.2.2]diazaoctane cores, have been isolated from the fungus of the genera *Aspergillus* and *Penicillium* to date, **1** is the fourth compound with the indoxyl unit containing the cores. During the structure elucidation of **1**, we found that the planar structure matched to that of Speramide A (**3**), isolated from *A. ochraceus* KM007, but the reported structure of **3** was incorrect and turned out to be that of Taichunamide H (**4**), recently isolated from *A. versicolor* HDN11-84.

Introduction

In 2007, we reported the isolation of four new prenylated indole alkaloids, Notoamides A–D, from *A. protuberus* MF297-2.[1] During our studies on the biosynthesis of the family of Notoamides in *A. protuberus*,[2] the opposite enantiomers of Stephacidin A (**5**) and Notoamide B (**6**) (Figure 1) were isolated from *A. amoenus* (formerly *A. versicolor*) NRRL 35600.[3] Compounds **5** and **6** contain bicyclo[2.2.2]diazaoctane cores, which may be formed through an intramolecular hetero Diels–Alder reaction from an isoprene unit and dioxopiperazine core. We are currently studying the mechanism of this fascinating construction. Recently, we isolated a new biosynthetically-interesting congener, Amoenamide A (**7**) (Figure 1), from *A. amoenus* along with five new enantiomers as minor metabolites.[4] Herein, we report the isolation of a new congener, Amoenamide B (**1**), along with Asperochramide A (**2**), which was isolated from *Aspergillus ochraceus* (cgmcc 3.6281), recently.[5]

Results and Discussion

Aspergillus amoenus NRRL 35600 was cultured on rice and the metabolites were purified by column chromatography and HPLC to afford **1** and **2**.[6] The molecular formula of **1** was determined to be $C_{26}H_{29}N_3O_4$ by HRESIMS. The ^{1}H NMR spectrum in DMSO-d_6 (Table 1) showed four doublet olefinic and aromatic protons (δ_H 5.66 (d, J = 10.0 Hz, H-26), 6.77 (d, J = 10.0 Hz, H-25), 6.11 (d, J = 8.4 Hz, H-5), and 7.18 (d, J = 8.4 Hz,

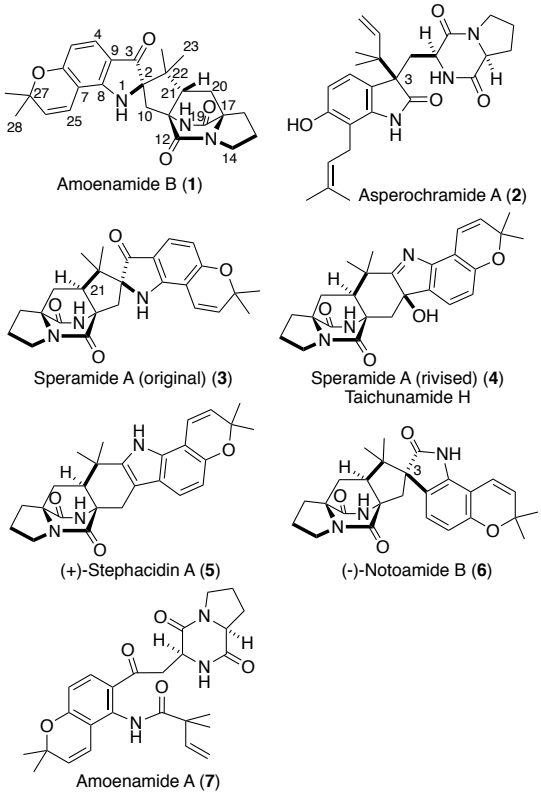

Fig. 1. Structures of **1**–**7**.

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Table 1
^{1}H and ^{13}C NMR data for **1** in DMSO-d_6.

No.	δ_H, mult (J in Hz)	δ_C, type	HMBC
1	7.73, s		2, 3, 8, 9
2		79.7, C	
3		197.0, C	
4	7.18, d (8.4)	124.9, CH	3, 6, 8
5	6.11, d (8.4)	107.6, CH	6, 7, 9
6		159.9, C	
7		102.7, C	
8		157.0, C	
9		113.7, C	
10	2.39, d (15.5)	35.6, CH_2	2, 3, 11, 12
	2.44, d (15.5)		2, 11, 22
11		66.6, C	
12		169.7, C	
14	3.25, m	43.4, CH_2	
	3.30, m		
15	1.78, m	24.7, CH_2	17
	1.95, m		17
16	1.80, m	28.5, CH_2	15, 18
	2.47, m		15, 18
17		68.7, C	
18		172.3, C	
19	8.67, s		11, 17
20	1.66, dd (13.4, 5.8)	27.3, CH_2	
	1.84, dd (13.4, 9.2)		17
21	2.71, dd (9.2, 5.8)	52.6, CH	12, 20, 22
22		48.3, C	
23	0.67, s	21.5, CH_3	2, 21, 22, 24
24	0.95, s	18.1, CH_3	2, 21, 22, 23
25	6.77, d (10.0)	116.4, CH	6, 8, 27
26	5.66, d (10.0)	127.3, CH	7, 27
27		77.2, C	
28	1.36, s	27.9, CH_3	26, 27, 29
29	1.40, s	27.3, CH_3	26, 27, 28

H-4)), two exchangeable protons (δ_H 7.73 (s, H-1) and 8.67 (s, H-19)), and four singlet methyl groups (δ_H 0.67 (3H, s, H_3-23), 0.95 (3H, s, H_3-24), 1.36 (3H, s, H_3-28), and 1.40 (3H, s, H_3-29)). The analysis of ^{13}C and 2D NMR spectra readily indicated that the structure of **1** was similar to that of (–)-Notoamide B (**6**),[1] a *spiro*-oxindole derivative. However, the HMBCs from H-4 to C-3 (δ_C 197.0) and from H_3-23/H_3-24 to C-2 (δ_C 79.7) (Figure 2a) clearly showed an indoxyl structure with a quaternary center at C-2 for **1**. The detailed analysis of 2D NMR spectra are consistent with the planar structure of **1** corresponding to that of (+)-Brevianamides A (**8**) and B[7] (**9**) (Figure 3), isolated from *P. brevicompactum*, but containing a 2,2-dimethylpyran ring fused to the indoxyl aromatic ring. NOE correlations, H-21 (δ_H 2.71, dd, J = 9.2, 5.8 Hz)/H-1, H-21/H_3-23, and H-19/H_3-24 (Figure 2b), indicated the relative configurations at C-2 and C-21, which corresponds to **8**, but not **9**. The CD spectrum of **1** showed the negative Cotton effects around 225 and 320 nm (Figure 2c), which were diagnostic for the dioxopiperazine amide bonds and the stereogenic center of the indoxyl core, respectively,[8] and was superimposable on that of (+)-**8**.[8] The absolute configuration of **1** appears to be consistent with that of (+)-**8**. HPLC analysis with a chiral-phase column (CHIRAL CELL OJ-H (4.6 x 250 mm), 93% *n*-hexane-2-PrOH) of **1** showed that **1** was optically pure. In addition, due to the presence of the indoxyl chromophore, **1** appears as a brilliant yellow color, characteristic of the indoxyl chromophore and both Brevianamides A (**8**) and B (**9**) exhibit this color.

During the structure elucidation of **1**, we noticed that the planar structure of **1** was identical to that of Speramide A (**3**) (Fig. 1), which was isolated from *A. ochraceus* KM007 by Hao.[9] However, the chemical shifts (δ_C 113.7 (C-7), 134.7 (C-9), and 147.6 (C-8)) of the indoxyl moiety in **3** exhibited significant differences from those of **1** (δ_C 102.7 (C-7), 113.7 (C-9), and 157.0 (C-8)). Further, two four-bond HMBC correlations from H_3-23 and H_3-24 to C-3 reported by Hao[9] are apparently inappropriate for the proposed structure of **3**. Although **3** is an indoxyl derivative, Hao compared the CD spectrum with that of a oxindole derivative (–)-**6**,[1] and these spectra were significantly different. These data clearly indicate that the proposed structure

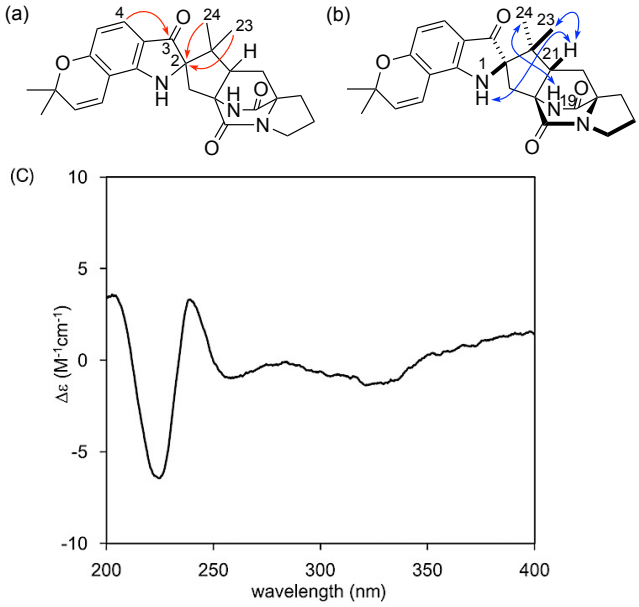


Fig. 2. Key HMBCs (a), NOEs (b), and ECD spectrum (c) of **1**.

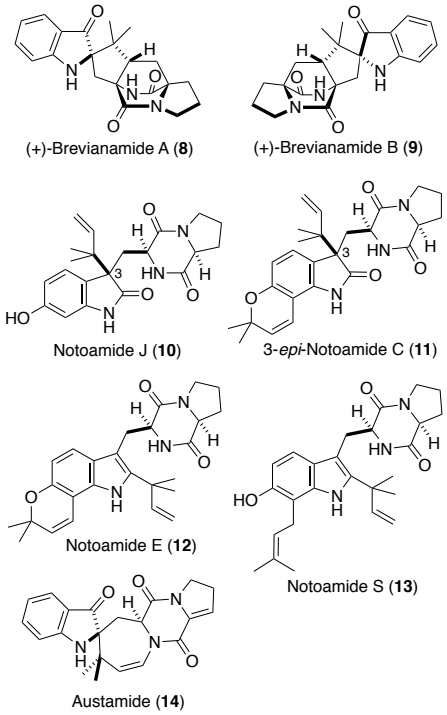
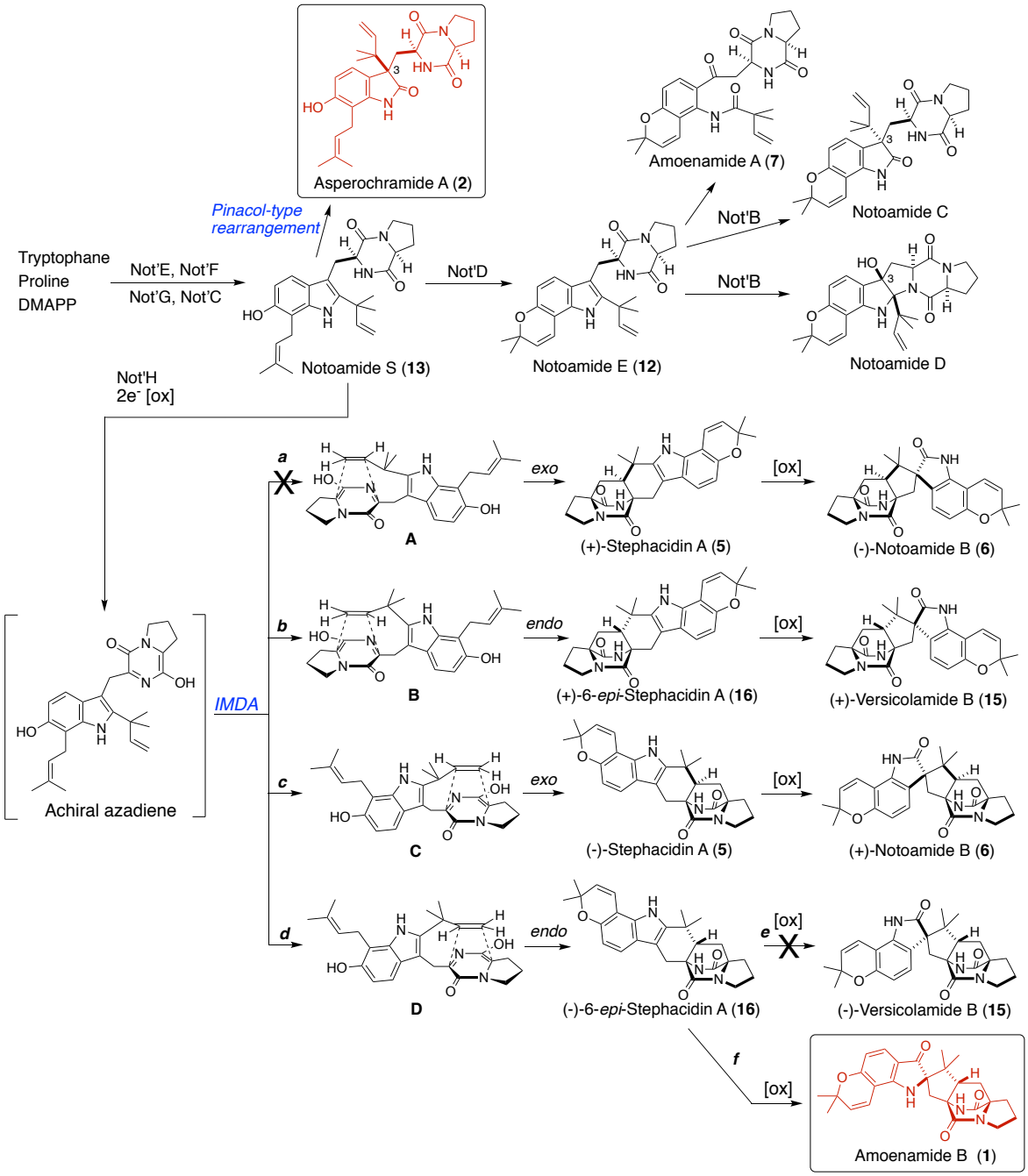


Fig. 3. Structures of **8**–**14**.

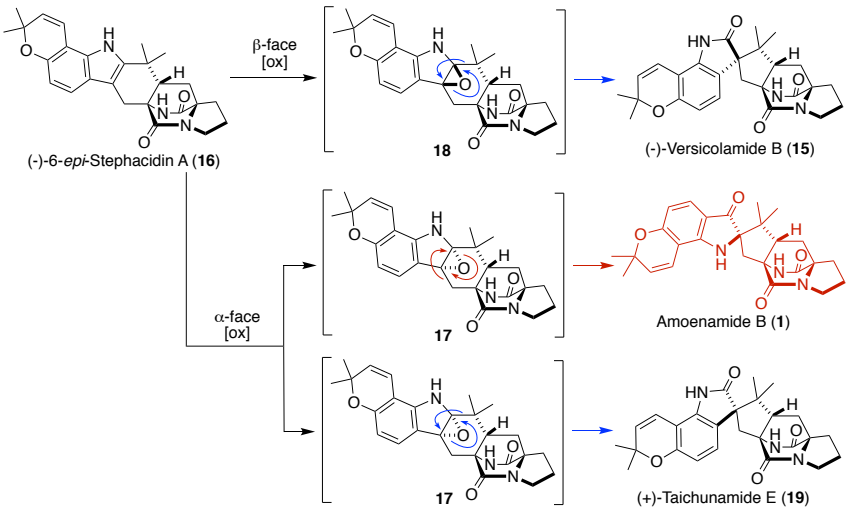


Scheme 1. Proposed formation of **1** and **2** in *A. amoenus*.

of **3** was incorrect. We analyzed the reported spectra in the literature[9] and found that the correct structure should be **4**, which is identical to that of Taichunamide H, recently isolated from *A. versicolor* HDN11-84.[10]

Asperochramide A (**2**) has the 3*R*-configuration (Fig. 1), although most prenylated indole alkaloids produced by fungi of the genera *Aspergillus* and *Penicillium* have the 3*S*-configuration (e.g. Notoamide C (Scheme 1)). Compound **2** is the third example with the 3*R*-configuration which includes Notoamide J (**10**),[11] isolated from *A. protuberus* MF297-2, and 3-*epi*-Notoamide C (**11**),[2a] obtained by the feeding experiment of Notoamide E (**12**) (Figure 3) in the strain.

Amoenamide B (**1**) was discovered as the fourth compound containing an indoxyl structure with a quaternary center at C-2 among the family of prenylated indole alkaloids after the isolation of (+)-**8**, (+)-**9**, and austamide (**14**)[12] (Figure 3) from *A. ustus*. In *A. amoenus*, (+)-Versicolamide B (**15**) and (+)-**6** (Scheme 1) are the major and minor metabolites[3] and are likely formed from Notoamide S (**13**) by the intra-molecular Diels–Alder (IMDA) reaction through intermediates **B** and **C**, respectively. Recently, we reported the isolation of an enantiomeric mixture of **16** enriched with the (–)-isomer together with (+)-**15** from *A. amoenus*.[2j] This result strongly suggests that the fungus possesses the indole oxidase, which converts (+)-**16** into (+)-**15**, but not for (–)-**15**. These experimental observations require further insight into the substrate specificity of the indole oxidases present in these fungi. We previously speculated that (–)-**16** is a minor shunt metabolite in *A. amoenus*, but the isolation of **1** in this study clearly suggests that this fungus may possess the indoxyl oxidase (pathway ***f***), which converts (–)-**16** to **1**, instead of the indole oxidase (pathway ***e***) for (–)-**15**. Possibly, **1** is converted from (–)-**16** through the oxidized intermediate **17** produced by α-face oxidation (Scheme 2). On the other hand, (–



Scheme 2. Possible pathways of **1** and (+)-**19** through **17** and (-)-**15** through **18** from **16**.

)-**15** could be formed through intermediate **18** by β-face oxidation. Interestingly, the product afforded by a distinct pinacol rearrangement through **17** corresponds to (+)-Taichunamide E (**19**), whose (–)-antipode was isolated from *A. taichungensis* IBT 19404.[13]

Biosynthetically, **2** may be formed from Notoamide S (**13**) by the Pinacol-type rearrangement (Scheme 1). IMDA reaction of the achiral azadiene derived from the oxidation of **13** affords the observed natural metabolites through intermediates **A**–**D**. Although we isolated both enantiomers of **5**, **16**, and **6** along with a single enantiomer of (+)-**15** from three fungi, *A. protuberus* MF297-2,[1] *A. amoenus* NRRL 35600,[2j] and *A. taichungensis* IBT 19404,[13] only (–)-**15** has yet to be isolated from any fungi. Biochemical investigations to address these subtle stereochemical anomalies are under intensive study in our laboratories.

Acknowledgments

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6. The fungus, *A. amoaenus* NRRL 35600, which was obtained from the basidioma of *Ganoderma australe* collected in a Hawaiian forest. The fungus was cultured on rice media (100 g × 200) at 25 °C for a month. The culture was extracted with *n*-BuOH and the concentrated aqueous solution was extracted with *n*-BuOH. The *n*-BuOH extract was partitioned between *n*-hexane and 90% MeOH/H_2O. The 90% MeOH/H_2O fraction (202 g) was subjected to SiO_2 chromatography with 5% MeOH/CH_2Cl_2 to yield a fraction (6.9 g) containing the prenylated indole alkaloids. The fraction was further purified by ODS chromatography with 75% MeOH/H_2O and then SiO_2 chromatography with EtOAc followed by HPLC (Develosil C30-UG-5, 45% CH_3CN/H_2O) to afford **1** (0.76 mg) and **2** (0.63 mg).
   Amoenamide B (**1**): $[\alpha]_D^{21}$ +64° (*c* 0.64, MeOH); UV (MeOH) λ_{max} (log ε) 228 (5.14), 264 (5.03), 320 (4.39), 334 (4.32) nm; ECD (200 μM, MeOH) λ_{max} (Δε) 323 (-1.4), 257 (-0.96), 240 (3.2), 225 (-6.4), 205 (3.4) nm; IR (film) ν_{max} 3320, 2925, 2854, 1670, 1601, 1439, 1401, 1312, 1114 cm^{-1}; ^1H and ^{13}C NMR data (DMSO-d_6), see Table 1.; HRESIMS *m/z* 448.2231 $[M + H]^+$ (calcd for $C_{26}H_{30}N_3O_4$, 448.2231).
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