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Isolation of amoenamide A and five antipodal prenylated alkaloids from *Aspergillus amoenus* NRRL 35600

Kayo Sugimoto[a], Yusaku Sadahiro[a] , Ippei Kagiyama[a], Hikaru Kato[a], David H. Sherman[b], Robert M. Williams[c,d], Sachiko Tsukamoto[a,*]

[a] *Graduate School of Pharmaceutical Sciences, Kumamoto University, Oe-honmachi 5-1, Kumamoto 862-0973, Kumamoto, Japan*
[b] *Life Sciences Institute and Departments of Medicinal Chemistry, Chemistry, Microbiology & Immunology, The University of Michigan, 210 Washtenaw Avenue, Ann Arbor, Michigan 48109-2216, United States*
[c] *Department of Chemistry, Colorado State University, 1301 Center Avenue, Fort Collins, Colorado 80523, United States*
[d] *University of Colorado Cancer Center, Aurora, Colorado 80045*

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ABSTRACT

A new prenylated alkaloid, Amoenamide A (**6**), was isolated from the fungus *Aspergillus amoenus* NRRL 35600. Previously, **6** was postulated to be a precursor of Notoamide E4 (**21**) converted from Notoamide E (**16**), which was a key precursor of the prenylated indole alkaloids in the fungi of the genus *Aspergillus*. We previously succeeded in the isolation of two pairs of antipodes, Stephacidin A (**1**) and Notoamide B (**2**), from *A. amoenus* and *A. protuberus* MF297-2 and expected the presence of other antipodes in the culture of *A. amoenus*. We here report five new antipodes (**7**-**11**) along with a new metabolite (**12**), which was isolated as a natural compound for the first time, from *A. amoenus*.

Introduction

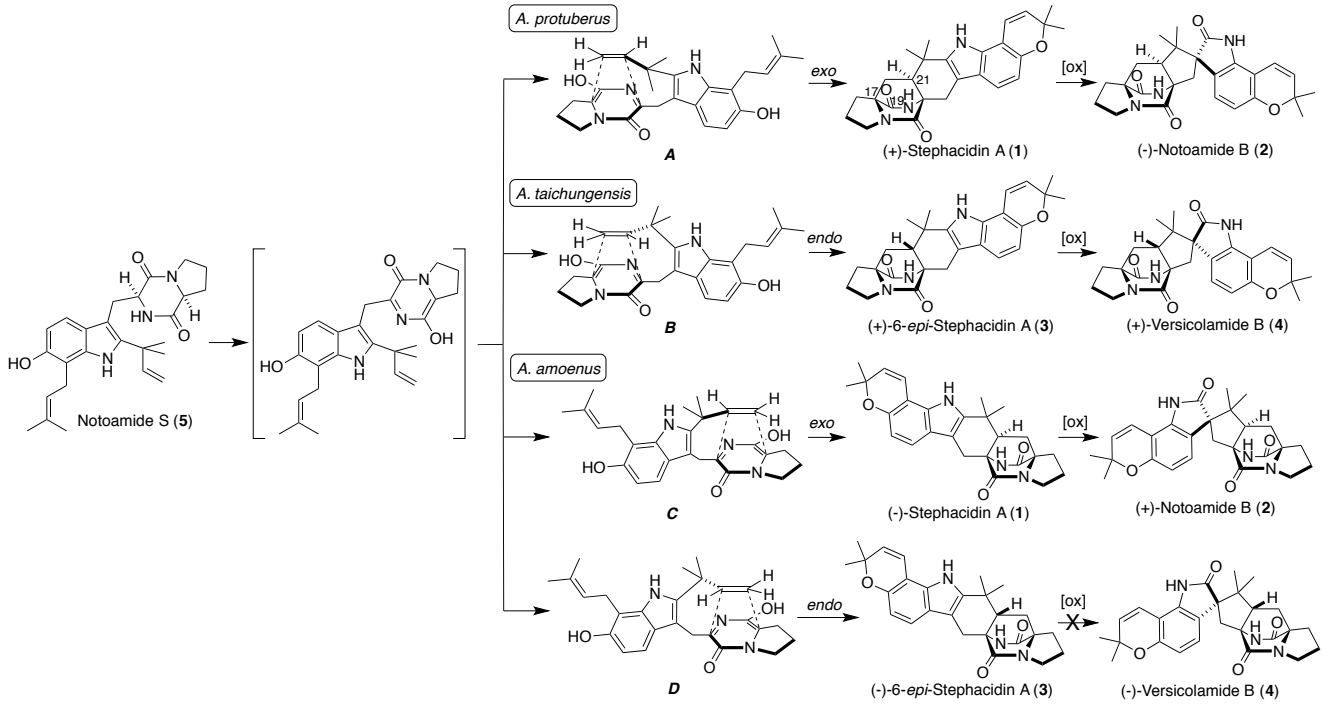
We have reported the isolation of biosynthetically interesting prenylated indole alkaloids from three fungi of the genus *Aspergillus*. (+)-Stephacidin A (**1**), (–)-Notoamide B (**2**), and their congeners were isolated from *A. protuberus* MF297-2,[1] and successively the antipodes, (–)-**1** and (+)-**2**, were obtained from *A. amoenus* (formerly *A. versicolor*) NRRL 35600[2] (Scheme 1). Recently, we reported the isolation of seven novel prenylated indole alkaloids, the Taichunamides, along with (+)-6-*epi*-Stephacidin A (**3**) and (+)-Versicolamide B (**4**) from *A. taichungensis* IBT 19404 (Scheme 1).[3] Interestingly, **1**/**2** and **3**/**4** contain a *syn*- and *anti*-bicyclo[2.2.2]diazaoctane cores, respectively (the *syn*- and *anti*-relationship is based on the H21 and bridging amide C18/N19 relative stereochemistry), and these cores are plausibly formed through an intramolecular hetero Diels–Alder reaction from a common precursor, Notoamide S (**5**)[4] (Scheme 1). To date, we have been studying the structures,[1,4,5-8] syntheses,[9-15] and bioconversions[4,13,15-17] of prenylated indole alkaloids from *A. protuberus* and the structures[2,4] and bioconversions [4,13-15] of those from *A. amoenus*. Curiously, we discovered that *A. amoenus* produced an enantiomeric mixture of **3** enriched with the (–)-isomer.[4] The presence of the enantiomerically pure (+)-**4** in *A. amoenus* suggests that the fungus possesses the oxidase, which selectively converts (+)-**3** into (+)-**4**, but does not process (–)-**3** (Scheme 1).[4] Successively, we have been studying the structures of metabolites from *A. amoenus* and here report the isolation of a new prenylated alkaloid, Amoenamide A (**6**), five new antipodes (**7**-**11**), and a new metabolite (**12**), which was isolated as a natural compound for the first time (Figure 1).

Results and Discussion

The fungus, *A. amoenus* NRRL 35600, was cultured on rice at 25 °C for a month and the metabolites were extracted with *n*-BuOH. After solvent partition, the metabolites were purified by column chromatography and HPLC to yield a new compound, Amoenamide A (**6**), five new antipodes, (–)-Notoamides F (**7**),[6] I (**8**),[6] R (**9**),[8] and U (**10**),[18] and (+)-Notoamide L (**11**),[7] and a new natural compound, (–)-6-*epi*-Notoamide I (**12**),[17] and fourteen known alkaloids, (–)-Stephacidin A (**1**), (+)- and (–)-6-*epi*-Stephacidin A (**3**), (+)-Versicolamide B (**4**), (+)-Notoamides A (**13**)[1] and B (**2**),[1] Notoamides C (**14**),[1] D (**15**),[1] E (**16**),[5] M (**17**),[7] Q (**18**),[8] and S (**6**),[4] Dehydronotoamide C (**19**),[11,19] and Speramide B (**20**)[20] (Figure 1).[21]

The molecular formula of **6** was determined to be $C_{26}H_{31}N_3O_5$ by HRESIMS. The ^{1}H NMR spectrum (DMSO-d_6) (Table 1) showed four doublet olefinic and aromatic protons (δ 5.78 (d, J = 9.7 Hz, H-26), 6.14 (d, J = 9.7 Hz, H-25), 6.73 (d, J = 8.6 Hz, H-5), and 7.63 (d, J = 8.6 Hz, H-4)), a monosubstituted double bond (δ 5.16 (dd, J = 1.0, 10.4 Hz, H-20), 5.22 (dd, J = 1.0, 17.9 Hz, H-20), and 6.13 (dd, J = 10.4, 17.9 Hz, H-21)), two exchangeable

* Corresponding author. Tel.: +81-96-371-4380; fax: +81-96-371-4380; e-mail: sachiko@kumamoto-u.ac.jp



Scheme 1. Proposed facial specificities of intramolecular hetero Diels–Alder reactions for major metabolites in three species, *A. protuberus*, *A. taichungensis*, and *A. amoenus*.

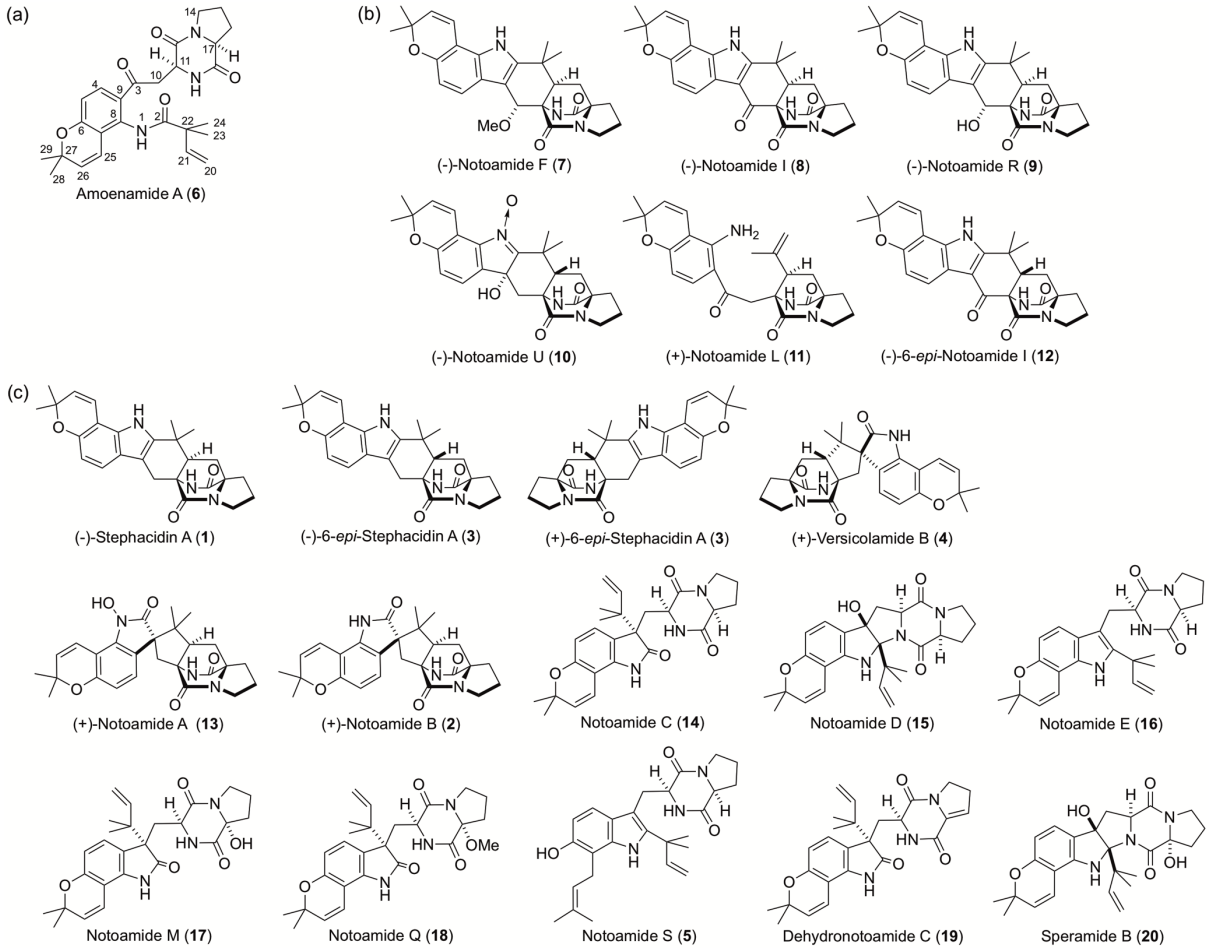


Figure 1. Structures of (a) a new compound, amoenamide A (**6**), (b) five antipodes (**7**-**11**) and a new natural compound (**12**), and (c) fourteen known compounds (**1**-**5** and **13**-**20**).

protons (δ 7.93 (s, H-19) and 9.57 (s, H-1)), two methine protons (δ 4.26 (t, J = 7.8 Hz, H-17) and 4.57 (t, J = 6.0 Hz, H-11)), and four singlet methyl groups (δ 1.29 (6H, s, H_3-23 and H_3-24), 1.39 (3H, s, H_3-28), and 1.40 (3H, s, H_3-29)), which indicated that **6** was a congener of the Notoamides. The analysis of 2D NMR spectra, including COSY, HMQC, and HMBC, showed three substructures, a 5,6-disubstituted 2,2-dimethyl-2*H*-chromene (A), a 3-substituted hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (B), and a 3-substituted 3-methylbut-1-ene (C) (Figure 2a). Key HMBC correlations showed the substructure C was connected to C-8 of the substructure A through an amide group (δ_H 9.57 (H-1), δ_C 174.7 (C-2)) (Figure 2b). The substructure B was connected to C-9 of the substructure A through a ketone group (δ_C 198.1 (C-3)). The 11*S*,17*S*-configuration for **14** were determined by a NOE correlation and chemical degradation,[1] and from the biogenetic relationship with **14**, the absolute configuration of **6** was indicated as 11*S*,17*S*. Thus, the structure of **6** was established.

Previously, we proposed that Notoamide E (**16**) would be a key biosynthetic intermediate for the Notoamides and Stephacidin A (**1**) in *A. protuberus*. In order to confirm this proposal, we performed bioconversion of ^{13}C-labeled **16**.[5] In this experiment, a new compound, Notoamide E4 (**21**), was obtained as a metabolite and we proposed a *N*-formylkynurenine derivative corresponding to **6**, was a putative precursor of **21** (Scheme 2). In the present work, we isolated natural **6** from the fungal culture, the presence of which strongly supports our hypothesis (Scheme 2).

Table 1
^{1}H and ^{13}C NMR data for **6** in DMSO-d_6

Position	δ_C	δ_H (J in Hz)	HMBC
1		9.57 s	2, 7, 9
2	174.7		
3	198.1		
4	129.8	7.63 d 8.6	3, 6, 8
5	112.7	6.73 d 8.6	6, 7, 9
6	155.7		
7	117.4		
8	132.9		
9	126.5		
10	39.9	3.04 dd 6.0, 17.7	3, 11, 12
		3.50 dd 6.0, 17.7	3, 11, 12
11	50.7	4.57 t 6.0	3, 10, 12
12	165.7		
14	44.2	3.33 m	
		3.40 m	
15	21.8	1.82 m	
		1.86 m	
16	27.5	1.90 m	
		2.15 m	
17	58.0	4.26 t 7.8	16, 18
18	169.5		
19		7.93 s	11, 12, 17
20	113.6	5.16 dd 1.0, 10.4	22
		5.22 dd 1.0, 17.9	21, 22
21	142.4	6.13 dd 10.4, 17.9	22, 23, 24
22	45.1		
23	23.6	1.29 s	2, 21, 22, 24
24	23.6	1.29 s	2, 21, 22, 23
25	118.1	6.14 d 9.7	6, 8, 27
26	129.9	5.78 d 9.7	7, 27
27	76.5		
28	27.0	1.39 s	26, 27, 29
29	27.0	1.40 s	26, 27, 28

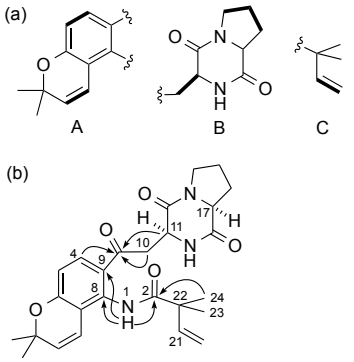
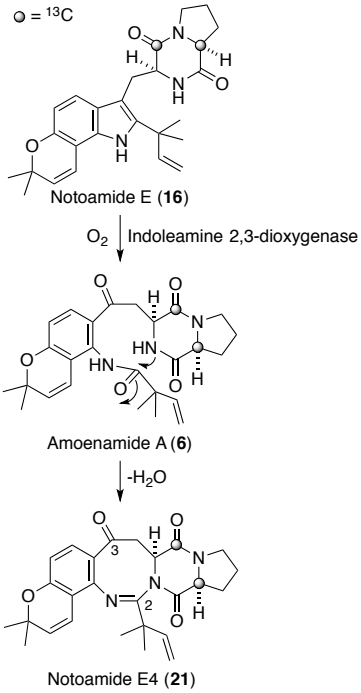


Figure 2. (a) Substructures A–C indicated by COSY (bold lines), HMQC, and HMBC spectra and (b) key HMBC correlations for **6**.



Scheme 2. Possible biosynthetic pathway from **16** to **21**.

After the isolation of the antipodes of Stephacidin A (**1**) and Notoamide B (**2**) from *A. protuberus* MF297-2[1] and *A. amoenus* NRRL 35600[2] as major metabolites, the presence of other antipodal metabolites in *A. amoenus* has also been expected to date. Herein, we succeeded in the isolation of the antipodes of previously reported natural alkaloids namely, (–)-Notoamides F (**7**),[6] I (**8**),[6] R (**9**),[8] and U (**10**),[18] and (+)-Notoamide L (**11**),[7] from *A. amoenus*. In addition, (–)-6-*epi*-Notoamide I (**12**) was isolated as a natural compound for the first time, although (±)-**12** was obtained by the bioconversion of (±)-6-*epi*-Notoamide T in *A. protuberus* MF297-2.[17] The elucidation of the biochemical basis for the stereochemical diversity of these families of prenylated indole alkaloids biosynthesized within orthologous species of *Aspergillus* fungi, specifically *A. protuberus* MF297-2, *A. amoenus* NRRL 35600, and *A. taichungensis* IBT 19404 is ongoing in our laboratories.[22-24]

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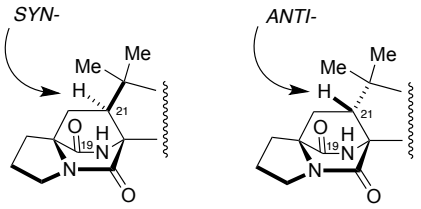

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21. The fungus, *A. amoaenus* NRRL 35600, was obtained from the basidioma of *Ganoderma australe* collected in a Hawaiian forest. The fungus was cultured on rice media (100 g × 50) in Erlenmeyer flasks (500 mL) at 25 °C for a month. The metabolites were extracted with *n*-BuOH and the concentrated aqueous solution was extracted with *n*-BuOH. The *n*-BuOH solution was evaporated and the dried material was partitioned between *n*-hexane and 90% MeOH/H_2O. The 90% MeOH/H_2O fraction (14.8 g) was subjected to ODS chromatography with 75% MeOH/H_2O to yield three fractions (fractions A (2.5 g), B (2.0 g), and C (1.1 g)) containing the prenylated indole alkaloids. Fraction A was purified by SiO_2 chromatography with *n*-hexane/CH_2Cl_2/MeOH (10:19:1) and then NH_2 chromatography with CH_2Cl_2/MeCN (1:1 and 1:3) and MeCN/H_2O (1:1) followed by HPLC (phenyl-hexyl (MeOH/H_2O) and gel filtration (MeOH)) to afford (–)-**1** (4.9 mg), (+)-**4** (0.4 mg), (–)-**8** (0.7 mg), (–)-**10** (0.5 mg), (+)-**11** (0.8 mg), and **18** (8.4 mg). Fraction B was purified by SiO_2 chromatography with *n*-hexane/CH_2Cl_2/MeOH (10:19:1) and then NH_2 chromatography with CH_2Cl_2 and CH_2Cl_2/MeCN (3:1) followed by gel filtration HPLC (MeOH) to afford **5** (17.1 mg), (–)-**12** (0.4 mg), **15** (46.9 mg), **19** (0.9 mg), and **20** (29.9 mg). Fraction C was purified by SiO_2 chromatography with *n*-hexane/CH_2Cl_2/MeOH (30:19:1 and 10:19:1) followed by HPLC (phenyl-hexyl (MeOH/H_2O), NH_2 (CH_2Cl_2/MeCN), and gel filtration (CH_2Cl_2/MeOH/H_2O)) to afford (+)-**2** (2.2 mg), (+)-**3** (0.12 mg), (–)-**3** (0.29 mg), **6** (1.1 mg), (–)-**7** (1.7 mg), (–)-**9** (0.5 mg), (+)-**13** (1.1 mg), **14** (1.6 mg), **16** (2.1 mg), and **17** (0.3 mg).
Amoenamide A (**6**): $[\alpha]_D^{20}$ –6.0° (*c* 0.91, MeOH); UV (MeOH) λ_{max} (log ε) 308 (3.04), 252 (3.56), 206 (4.90) nm; IR (film) ν_{max} 3356, 2925, 2855, 1674, 1460, 1117 cm^{-1}; HRESIMS *m/z* 488.2183 $[M+Na]^+$ (calcd for $C_{26}H_{31}N_3O_5Na$, 488.2156); ^1H and ^{13}C NMR data (DMSO-d_6), see Table 1.
(–)-Notoamide F (**7**): $[\alpha]_D^{20}$ –12° (*c* 1.4, MeOH); (+)-**7**: $[\alpha]_D^{21}$ +1.9° (*c* 0.27, MeOH).[6]
(–)-Notoamide I (**8**): $[\alpha]_D^{20}$ –58° (*c* 0.46, MeOH), $[\alpha]_D^{24}$ –69° (*c* 0.10, MeOH/$CHCl_3$ 1:1); (+)-**8**: $[\alpha]_D^{29}$ +31° (*c* 0.1, MeOH/$CHCl_3$ 1:1).[6]
(–)-Notoamide R (**9**): $[\alpha]_D^{20}$ –44° (*c* 0.19, MeOH); (+)-**9**: $[\alpha]_D^{14}$ +38° (*c* 0.5, MeOH).[8]
(–)-Notoamide U (**10**): $[\alpha]_D^{20}$ –44° (*c* 0.23, MeOH); (+)-**10**: $[\alpha]_D^{25}$ +54.1° (*c* 0.1, MeOH).[18]
(+)-Notoamide L (**11**): $[\alpha]_D^{20}$ +21° (*c* 0.17, MeOH); (–)-**11**: $[\alpha]_D^{23}$ –17° (*c* 0.77, MeOH).[7]
(–)-6-*epi*-Notoamide I (**12**): $[\alpha]_D^{20}$ –52° (*c* 0.48, MeOH).
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