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Isolation of Antipodal (−)-Versicolamide B and Notoamides L−N from a Marine-Derived *Aspergillus* sp.

Sachiko Tsukamoto,*,†,‡ Tetsuro Kawabata,† Hikaru Kato,† Thomas J. Greshock,§ Hiroshi Hirota,⊥ Tomihisa Ohta,† and Robert M. Williams*,§,||

Graduate School of Science, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan, Graduate School of Natural Science and Technology, Kanazawa University, Kanazawa 920-1192, Japan, Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523, RIKEN Genomic Sciences Center (GSC), 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama 230-0045, Japan, and the University of Colorado, Cancer Center, Aurora, Colorado 80045

sachiko@faculty.chiba-u.jp; rmw@chem.colostate.edu

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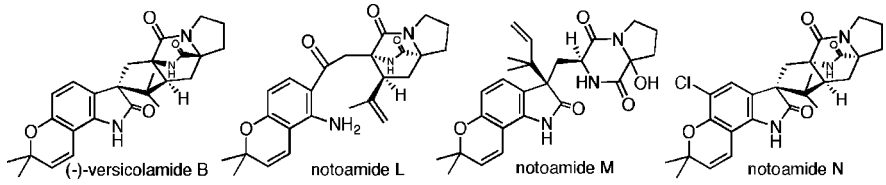
ABSTRACT



Antipodal (−)-versicolamide B and notoamides L−N were isolated from a marine-derived *Aspergillus* sp. The possible biosynthetic pathway of enantiomeric pairs of notoamide B and versicolamide B are proposed. Notoamide L is the first metabolite containing 25 carbons in the related prenylated indole alkaloids. Notoamide M is potentially a precursor to the proposed azadiene species involved in the putative intramolecular Diels−Alder reaction in the biogenesis of the bicyclo[2.2.2]diazaoctane ring system.

A number of prenylated indole alkaloids containing a diketopiperazine or a bicyclo[2.2.2]diazaoctane ring were isolated from various fungi of the genera *Aspergillus* and *Penicillium*, and the study of the biosynthetic pathways to these alkaloids has recently become an area of significant interest.[1] We isolated new alkaloids, notoamides A−D (**1**-**4**),[2] (Figure 1) from a marine-derived *Aspergillus* sp. as well as the known natural product stephacidin A (**5**).[3] Interestingly, we found that notoamide E (**6**) is a short-lived natural metabolite and biosynthetic precursor to notoamides C (**3**) and D (**4**).[4] In addition, we recently isolated notoamides F−K.[5] Further, Williams and Gloer et al. reported the isolation of antipodal (+)-notoamide B (**7**) and (−)-stephacidin A (**8**) (Figure 2) from the terrestrial organism *Aspergillus versicolor* NRRL 35600 along with a new alkaloid, (+)-versicolamide B (**9**), which possesses a novel anti relationship between C-21−C-22 and C-17−N-13 in the bicyclo[2.2.2]diazaoctane ring system.[6] With regard to the generation of the antipodal stereoisomers of notoamide B and stephacidin A, they proposed that the intramolecular Diels−Alder (IMDA) reaction occurs in a face-selective manner in the marine-

† Chiba University.
‡ Kanazawa University.
§ Colorado State University.
⊥ RIKEN GSC.
|| The University of Colorado.

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(2) Kato, H.; Yoshida, T.; Tokue, T.; Nojiri, Y.; Hirota, H.; Ohta, T.; Williams, R. M.; Tsukamoto, S. *Angew. Chem., Int. Ed.* **2007**, *46*, 2254–2256.
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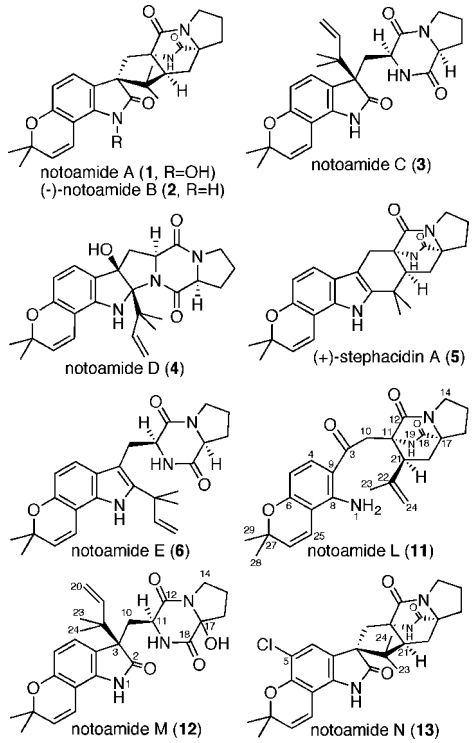

Figure 1. Structures of compounds from the marine-derived *Aspergillus* sp.

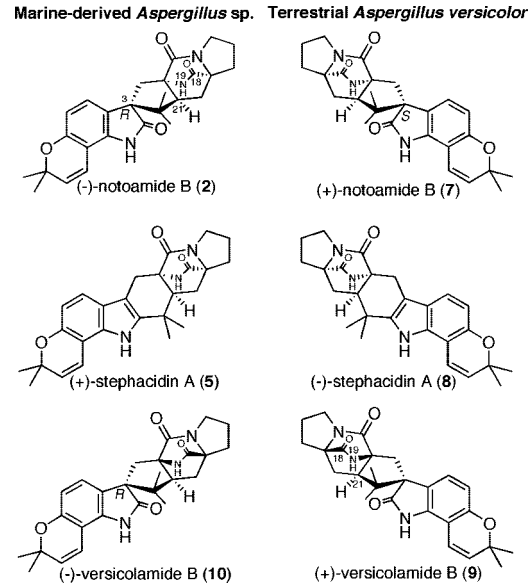

Figure 2. Structures of three pairs of enantiomeric alkaloids isolated from the marine-derived *Aspergillus* sp. and the terrestrial *Aspergillus versicolor*.

derived *Aspergillus* sp. and the terrestrial *Aspergillus versicolor*.[6] On the basis of this presumption, the presence of (−)-versicolamide B (**10**) was predicted to be a metabolite in our marine-derived *Aspergillus* sp. (Figure 2). In the continuing search for notoamide congeners, we finally succeeded in isolating **10** along with new notoamides L−N (**11**−**13**) (Figure 1). Here we report their structures and suggest another possible biosynthetic pathway that accommodates the biogenesis of the respective antipodes of notoamide B and versicolamide B.

The fractions rich in indole alkaloids, which derived from the EtOH extract of the fungal culture,[2] were further purified by HPLC to afford (−)-versicolamide B (**10**) and notoamides L−N (**11**−**13**).

The HRFABMS of **10** showed the molecular formula $C_{26}H_{29}N_3O_4$. The ^{1}H NMR spectrum of **10** (Table S1, Supporting Information) was superimposable on that of (+)-versicolamide B (**9**) and revealed that the structure and relative stereochemistry was the same as that of **9**. The specific rotation of **10** (−22°) was of the opposite sign to that of **9** (+26°), and the CD spectrum of **10** (Figure S6, Supporting Information) was also opposite to that of **9**. These data conclusively reveal that **10** is the antipodal (−)-versicolamide B, and that **9** and **10** are the third pair of antipodal natural metabolites isolated from the closely related *Aspergillus* fungi.

The molecular formula of notoamide L (**11**), $C_{25}H_{29}N_3O_4$, was established by high-resolution FABMS, and the ^{1}H NMR spectrum (Table S2, Supporting Information) showed three singlet methyl signals at δ 1.39, 1.40, and 1.64. In contrast to other prenylated indole alkaloids, one singlet methyl group is missing in **11**, and the methyl signal at δ 1.64 is observed at lower field. The ^{13}C NMR spectrum of **11** (Table S2, Supporting Information) showed a ketone carbon at δ 199.1. Usually in the family of prenylated indole alkaloids, an isoprenyl unit at C-2 or C-3 cyclizes across the diketopiperazine moiety as shown in stephacidin A (**5**) or notoamide A (**1**) or exists as a substituted group at C-2 or C-3 as shown in notoamide D (**4**) or C (**3**). For compound **11**, the HMBC spectrum showed the presence of an isopropenyl group at C-21 instead of an isoprenyl group; δ_H 1.64 (3H, s, H_3-23), 4.79 (1H, d, J = 1.0 Hz, H-24) and 4.83 (1H, d, J = 1.0 Hz, H-24)/δ_C 19.2 (CH_3, C-23), 116.2 (CH_2, C-24), and 144.7 (C, C-22). In addition, the HMBC correlations, δ 7.70 (H-4)/δ 199.1, δ 3.24 and 3.44 (H_2-10)/δ 199.1, showed that the ketone carbon existed between C-9 and C-10, being assignable to C-3. Thus, the gross structure of **11** was established. The 11*S*,17*S* configuration of **11** was indicated by the CD spectrum (Figure S12, Supporting Information),[7] and the co-occurrence of notoamides A and B (**1** and **2**) and (+)-stephacidin A (**5**) as major alkaloids indicated the 21*R* configuration for **11** on the basis of biogenetic considerations. In the structure of **11**, a C-2 carbon derived from tryptophan is lacking. Although now more than 40 members of related prenylated indole alkaloids have been reported, a metabolite containing 25 carbons such as **11** has not been isolated yet in this family. One possible biogenesis of **11** derived from (+)-stephacidin A (**5**) is shown in Scheme S1 (see Supporting Information).

(6) Greshock, T. J.; Grubbs, A. W.; Jiao, P.; Wicklow, D. T.; Gloer, J. B.; Williams, R. M. *Angew. Chem., Int. Ed.* **2008**, *47*, 3573–3577.

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Notoamide M (**12**) has the molecular formula $C_{26}H_{31}N_3O_5$, one oxygen unit more than notoamide C (**3**) or 3-*epi*-notoamide C (**14**).[4,8] The ^{1}H and ^{13}C NMR data in CD_3OD of **12** (Table S3, Supporting Information) revealed that a methine at C-17 in **3** or **14** was replaced with a quaternary carbon in **12**, which was indicated by HMBC correlations, δ 1.68 (H-15) and 1.95 (H-16)/δ 87.6 (C-17). In the ^{13}C NMR chemical shifts of three compounds,[9] differences were observed in signals for C-3 (δ 57.1 in **12**, δ 62.6 in **3** and δ 58.7 in **14**) and C-11 (δ 55.3 in **12**, δ 67.1 in **3** and δ 53.4 in **14**), and other signals except for C-15, C-16, and C-17 match well with each other. The CD spectra of **12**, **3**, and **14** in MeOH (Figures S18 and S19, Supporting Information) showed that the spectra of **3** and **14** were almost symmetrical and the spectrum of **12** was similar to that of **3**. These data suggest the 3*R* configuration of **12**. The 11*S* configuration is based on biogenetic considerations, but the configuration at C-17 remains to be determined.

The molecular formula of notoamide N (**13**) was determined as $C_{26}H_{28}ClN_3O_4$, indicating a replacement of a hydrogen atom with chlorine. The ^{1}H NMR spectrum (Table S4, Supporting Information) showed the absence of a pair of doublet aromatic signals as shown in other notoamides and the presence of a singlet aromatic signal at δ 7.19. The ^{13}C NMR spectrum of **13** was similar to that of notoamide B (**2**) except for C-5, δ 113.3 (C) in **13** and δ 109.6 (CH) in **2**. Analysis of HMBC correlations established the structure of **13** as 5-chloro-notoamide B. The CD spectrum of **13** (Scheme S25, Supporting Information) was similar to that of **2**.[2] Therefore, we have assigned the 3*R*,11*S*,17*S*,21*S* configuration to **13**.[7,10] Finally, the synthesis of **13** from synthetic notoamide B by *t*-butyl hypochlorite oxidation enabled the structure of **13** to be unambiguously assigned (see Supporting Information). Notoamide N (**13**) is the third chlorinated derivative to have been discovered in this family of prenylated indole alkaloids following the recent disclosure of malbrancheamide and malbrancheamide B.[11]

It is now established that a series of three pairs of enantiomeric prenylated indole alkaloids, (+)- and (−)-notoamide B (**7** and **2**), (+)- and (−)-stephacidin A (**5** and **8**), and (+)- and (−)-versicolamide B (**9** and **10**), are produced by distinct species of the genus *Aspergillus*. We have confirmed that all three pairs of these natural metabolites were isolated from their respective producing organisms in their optically pure forms.[12]

A biosynthetic pathway to rationalize the formation of the individual enantiomers of stephacidin A and notoamide B has been described that postulates that the respective fungi could plausibly exhibit opposite facial selectivity in a key, putative intramolecular Diels−Alder (IMDA) reaction via an achiral azadiene intermediate derived by the two-electron oxidation of notoamide E (**6**).[6] To interrogate this proposed pathway, we carried out a feeding experiment of doubly ^{13}C-labeled notoamide E in the marine-derived *Aspergillus* sp.[4] The results clearly showed that **6** is a precursor for notoamides C (**3**) and D (**4**), but not for metabolites containing a bicyclo[2.2.2]diazaoctane ring, including notoamide B (**2**) or stephacidin A (**5**), although they are the major metabolites produced from this fungus.

Here, we wish to advance an alternative pathway for the potential unified biogenesis of the antipodal stereoisomers of notoamide B (**2** and **7**) and versicolamide B (**9** and **10**) (Scheme 1). In this biogenetic hypothesis, the common precursor is deoxybrevianamide E (**15**), which we have isolated from the marine-derived *Aspergillus* fungus.[2] An *R*-selective indole oxidase in the marine-derived *Aspergillus* and a corresponding *S*-selective oxidase in the terrestrial *Aspergillus* followed by pinacol rearrangement of the isoprenyl group from C-2 to C-3 affords **16** and the corresponding 3-*epi* diastereomer (**17**), respectively. Oxidation and tautomerization of **16** would yield the optically pure azadiene intermediates **18**. In principle, **18** could afford four stereoisomers, **19** plus **20** and their corresponding C-21 epimers, although these epimers have not yet been detected. The preponderance of **19** over **20** might be due to the presence or absence of hydrogen-bonding, which could be observed in intermediate **26** or **27**, respectively, derived from **16** (Scheme S2, Supporting Information). In the biogenetic pathway postulated in Scheme 1, the enantio-divergence arises as a consequence of incipient *R*- or *S*-selective indole oxidase instead of opposite facial selectivities in the putative IMDA reaction; oxidation and prenylation reactions (**19**→**2** or **20**→**10**) to construct the pyran ring attached to the indole moiety would thus follow formation of the bicyclo[2.2.2]diazaoctane ring system. The culture of the marine-derived *Aspergillus* yields notoamide C (**3**) as a major metabolite, and 3-*epi*-notoamide C (**14**) was not detected at all (but was isolated when ^{13}C-labeled notoamide E was fed to this organism).[8] We speculate that the terrestrial *Aspergillus versicolor* might be expected to contain **14** as a major metabolite instead of **3**. Because the biogenesis of (+)- and (−)-stephacidin A (**5** and **8**) is not accommodated by this pathway, further putative precursor feeding and incorporation experiments are necessary to establish the biogenesis of stephacidin A. It also remains plausible that stephacidin A is the direct biosynthetic precursor to notoamide B via indole oxidation and pinacol rearrangement, the laboratory transformation of which we have experimentally established.[8b] With regard to the formation of a bicyclo[2.2.2]diazaoctane ring, the isolation of notoamide M (**12**) is noteworthy. To form an azadiene intermediate (**D** in Scheme 2), oxidation at C-17 of a diketopiperazine ring (A→B) followed by dehydration would be necessary. Therefore, the precursor of **12** could be notoamide C (**3**) and the isolation of **12**

(8) Although 3-*epi*-notoamide C (**14**) was not isolated from the fungal culture of the marine-derived *Aspergilus* sp. yet, **14** was obtained as a metabolite of the feeding experiment of ^{13}C-labelled notoamide E in the fungus (see ref 4) and has also been synthesized: (a) Grubbs, A. W.; Artman, G. D., III; Tsukamoto, S.; Williams, R. M. *Angew. Chem., Int. Ed* **2007**, *46*, 2257–2261. (b) Greshock, T. J.; Grubbs, A. W.; Tsukamoto, S.; Williams, R. M. *Angew. Chem., Int. Ed.* **2007**, *46*, 2262–2265.

(9) The ^{13}C NMR spectra of **3** and **14** were measured in acetone-d_6.

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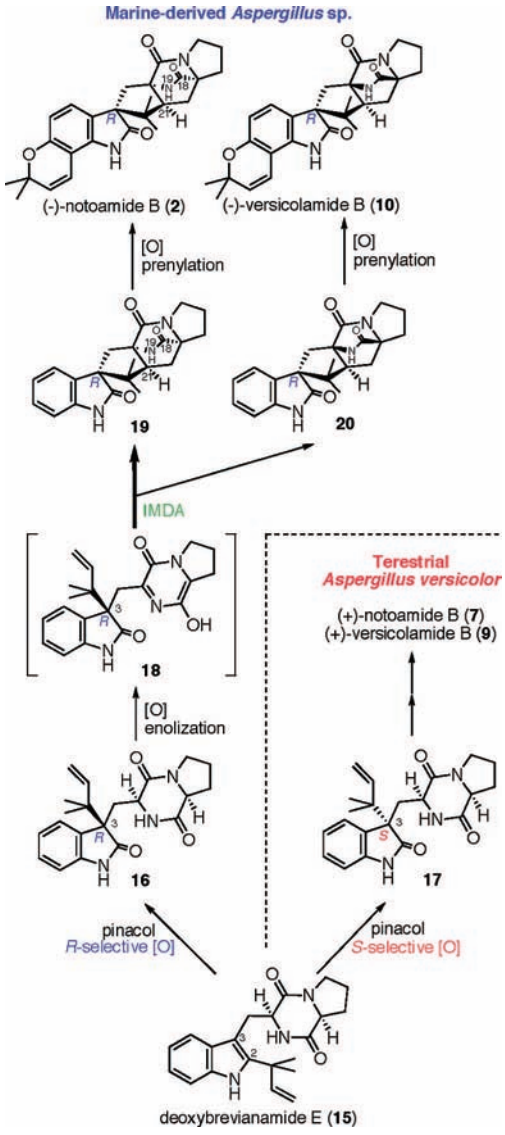
(11) Martinez-Luis, S.; Rodriguez, R.; Acevedo, L.; Gonzalez, M. C.; Lira-Rocha, A.; Mata, R. *Tetrahedron* **2006**, *62*, 1817–1822.

(12) Although the optical purity of enantiomeric pairs of notoamide B and stephacidin A were established by the chiral HPLC analysis,[6] those of versicolamide B were confirmed by enantiospecific total synthesis (unpublished data).

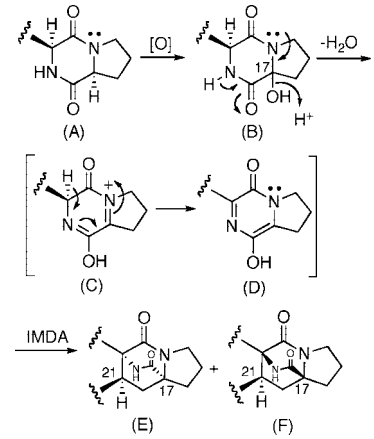
Scheme 1. Possible Biogenesis of the Enantiomeric Pairs of Notoamide B (**2**, **7**) and Versicolamide B (**9**, **10**)



provides indirect and provocative support for an oxidative manifold leading to the generation of the putative azadiene species. Alternatively, **12** could be an artifact of hydrolytic capture of the transient azadiene species (**C**).

In conclusion, we have isolated antipodal (−)-versicolamide B (**10**) along with three new notoamides L-N (**11**-**13**) from a marine-derived *Aspergillus* sp. Namely, (+)- and (−)-versicolamide B (**9** and **10**) are the third enantiomeric pair, in addition to (+)- and (−)-notoamide B (**7** and **2**) and (+)- and (−)-stephacidin A (**5** and **8**), that was isolated from distinct species of *Aspergillus*. With regard to the generation of the enantiomeric pairs of notoamide B and versicolamide B, here we propose an alternative biogenetic pathway (Scheme 1), in which a key *R*- or *S*-selective indole oxidase

Scheme 2. Possible Formation of the Bicyclo[2.2.2]diazaoctane Ring System



would exist in the respective marine-derived or in the terrestrial *Aspergillus*. The identification and characterization of such enzymes in each *Aspergillus* are currently being pursued to shed light on this possibility. Notoamide L (**11**) is the first alkaloid constituted of 25 carbons in this family of prenylated indole alkaloids, and the biogenesis of **11** comprises an as of yet undefined but alluring pathway to elucidate. Notoamide M (**12**) contains a hydroxy group at C-17, and its isolation provides indirect support for a potential mechanism that culminates in the construction of the unique bicyclo[2.2.2]diazaoctane ring system (Scheme 2). Notoamide N (**13**) is a rare chlorinated member of this family of prenylated indole alkaloids. The existence of a halogenase system in our marine-derived *Aspergillus* sp. is an important finding, which we are pursuing in the context of identifying the class of halogenase extant. Our laboratories are continuing studies to elucidate the origin of the fascinating enantio-divergence manifest in the natural production of the stephacidins, notoamides and versicolamides.

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Supporting Information Available: Isolation of **10**−**13**, synthesis of **13**, possible biosynthetic pathway of **11**, possible intermediates **26** and **27** derived from **16**. NMR spectral data tables, 1D and 2D NMR spectra, CD spectra of **10**−**13**. This material is available free of charge via the Internet at http://pubs.acs.org.

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