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Isolation of antipodal (–)-versicolamide B and notoamides L–N from a marine-derived *Aspergillus* sp

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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 twenty-five 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.

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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>.

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-derived *Aspergillus* sp. and the terrestrial *Aspergillus versicolor*.⁶ Based on 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 was further purified by HPLC to afford (–)-versicolamide B (**10**) and notoamides L-N (**11–13**).

The HRFABMS of **10** showed the molecular formula C₂₆H₂₉N₃O₄. The ¹H NMR spectrum of **10** (Table S1) 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) 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**), C₂₅H₂₉N₃O₄, was established by high-resolution FABMS, and the ¹H NMR spectrum (Table S2) 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 ¹³C NMR spectrum of **11** (Table S2) 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, H₃-23), 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 (CH₃, C-23), 116.2 (CH₂, 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 (H₂-10)/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 1*S*,17*S* configuration of **11** was indicated by the CD spectrum (Figure S12),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 forty members of related prenylated indole alkaloids have been reported, a metabolite containing twenty-five carbons such as **11** has not been isolated yet in this family. One possible biogenesis of **11** derived from (+)-stephacidin A (**5**) (see Supporting Information) is shown in Scheme S1.

Notoamide M (**12**) has the molecular formula C₂₆H₃₁N₃O₅, one oxygen unit more than notoamide C (**3**) or 3-*epi*-notoamide C (**14**).^{4, 8} The ¹H and ¹³C NMR data in CD₃OD of **12** (Table S3) 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 ¹³C 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) 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 C₂₆H₂₈ClN₃O₄, indicating a replacement of a hydrogen atom with chlorine. The ¹H NMR spectrum (Table S4) 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 ¹³C 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) 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*. It is noteworthy that we have confirmed that all three pairs of these natural metabolites were isolated from their respective producing organisms in optically pure form.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 In order to interrogate this proposed pathway, we carried out a feeding experiment of doubly ¹³C-labelled 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). 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 ¹³C-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**. Since 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 by *via* indole oxidation and pinacol rearrangement; the laboratory transformation of which that 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. In order 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** 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**), which were 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 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 twenty-five carbons in this family of prenylated indole alkaloids, and the biogenesis of **11** comprises an as 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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8. Although 3-epi-notoamide C (**14**) was not isolated from the fungal culture of the marine-derived *Aspergillus* 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 AW, Artman GD III, Tsukamoto S, Williams RM. *Angew Chem, Int Ed* 2007;46:2257–2261. (b) Greshock TJ, Grubbs AW, Tsukamoto S, Williams RM. *Angew Chem, Int Ed* 2007;46:2262–2265.
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Figure 1.
Structures of compounds from the marine-derived *Aspergillus* sp.

Marine-derived *Aspergillus* sp. Terrestrial *Aspergillus versicolor* **Figure 2.**

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

Scheme 1.
Possible biogenesis of the enantiomeric pairs of notoamide B (2, 7) and versicolamide B (9, 10).

Scheme 2.

Possible formation of the bicyclo[2.2.2]diazaoctane ring system.