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Isolation of Notoamide S and Enantiomeric 6-*epi*-Stephacidin A from the Fungus *Aspergillus amoenus*: Biogenetic Implications

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Abstract

Notoamide S has been hypothesized to be a key biosynthetic intermediate for characteristic metabolites, stephacidin A, notoamide B, and versicolamide B, in *Aspergillus* sp., but has not yet been isolated. The isolation of notoamide S and an enantiomeric mixture of 6-*epi*-stephacidin A enriched with the (–)-isomer from *A. amoenus* is reported. The presence of (+)-versicolamide B suggests that the fungus possesses only the oxidase, which converts (+)-6-*epi*-stephacidin A into (+)-versicolamide B, but not for (–)-6-*epi*-stephacidin A.

Graphical abstract

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Supporting Information

Fungal culture procedures, isolation, and spectra. This material is free of charge via the Internet at <http://pubs.acs.org>.

Notes

The authors declare no competing financial interest.

In our ongoing studies on the notoamide and stephacidin biosynthesis in two closely related fungi of the genus *Aspergillus*, we previously reported that the *A. protuberus* (formerly *Aspergillus* sp. MF297-2) produces (+)-stephacidin A, (–)-notoamide B, and (+)-versicolamide B^{1,2} and the *A. amoenus* (formerly *A. versicolor* NRRL 35600) produces the enantiomers, (–)-stephacidin A and (+)-notoamide B, but the same enantiomer of (+)-versicolamide B as produced in the *A. protuberus* (Figure 1).³ Stephacidin A, notoamide B, and versicolamide B are prenylated indole alkaloids containing a characteristic bicyclo[2.2.2]diazaoctane core structure, which is likely to arise from an intramolecular hetero Diels-Alder (IMDA) reaction (Scheme 1). In order to verify the molecular basis for the biogenesis of metabolites with this unique core structure, we performed the bioconversions of synthetic, isotopically labeled compounds, i.e. notoamide E,⁴ notoamide S,^{5,6} notoamide T,⁷ 6-*epi*-notoamide T,² and stephacidin A.⁸ Among them, notoamides S and T and 6-*epi*-notoamide T have not yet been isolated from the two fungal cultures, although notoamide S is strongly implicated to undergo the IMDA reaction to afford notoamide T and its 6-*epi* isomer through the achiral azadiene followed by cyclization and rearrangement to afford stephacidin A, notoamide B, and versicolamide B (Schemes 1 and 2). The bioconversion of notoamide S in the *A. amoenus* afforded notoamides C and D, (–)-stephacidin A, (+)-notoamide B, and (+)-versicolamide B.⁶ Notoamide T was converted into stephacidin A and notoamide B in the *A. protuberus* and *A. amoenus*⁷ and 6-*epi*-notoamide T was converted to 6-*epi*-stephacidin A and versicolamide B in the *A. protuberus*.² These incorporation experiments of notoamide T and its 6-*epi*-isomer were performed with their racemic mixtures, and interestingly the two fungi converted both exogenous as well as endogenous substrates to products. In order to confirm the presence of notoamides S and T and 6-*epi*-notoamide T as precursors in the fungal culture, we previously carefully analyzed the time-course of the metabolic profile in the culture of the *A. protuberus* but could not obtain these metabolites.

In the present study, we searched for the presence of these metabolites in the culture of the *A. amoenus* and succeeded in the isolation of notoamide S but not of notoamide T and its 6-*epi*-isomer. With respect to the metabolic profile by the *A. protuberus* and *A. amoenus*, production of the enantiomers of stephacidin A and notoamide B along with the presence of the same enantiomer of (+)-versicolamide B are enigmatic. 6-*epi*-Stephacidin A is likely the precursor of versicolamide B, and (+)-6-*epi*-stephacidin A was obtained from the culture of the *A. protuberus*.² In the present study, we elucidated the absolute configuration of 6-*epi*-stephacidin A produced by the *A. amoenus*.

The *A. amoenus* was cultured on rice medium at 25 °C for one month. The culture was extracted with *n*-BuOH and the condensed extract was partitioned between *n*-hexane and 90% MeOH-H₂O. The aqueous MeOH fraction was subjected to ODS column chromatography with MeOH/H₂O and fractions that eluted with 75% MeOH-H₂O were repeatedly purified to afford notoamide S (15.3 mg) and 6-*epi*-stephacidin A (1.22 mg).

Notoamide S and 6-*epi*-stephacidin A were identified by ¹H NMR spectra and ESIMS and the structures were corroborated by comparison to known, synthetic samples. The CD spectrum suggested that the isolated 6-*epi*-stephacidin A was the (–)-enantiomer. However, from the biosynthetic point of view, the precursor of (+)-versicolamide B should be (+)-6-

epi-stephacidin A (Scheme 2). Thus, the isolation of the (–)-enantiomer was inconsistent with the biogenetic relationship and the small molar ellipticity of the CD spectrum suggested the possibility of an enantiomeric mixture. The 6-*epi*-stephacidin A we isolated was analyzed by HPLC with a chiral column and turned out to be an enantiomeric mixture enriched with the (–)-isomer. Purification of the mixture by chiral HPLC afforded (+)- and (–)-6-*epi*-stephacidin A in a ratio of 1:2.3 and the enantiomers showed the opposite CD spectra (Figure 2). This result suggested that notoamide S was converted to both (+)- and (–)-6-*epi*-stephacidin A through (+)- and (–)-6-*epi*-notoamide T, respectively, and subsequently only (+)-6-*epi*-stephacidin A was converted into (+)-versicolamide B (Scheme 2). These observations clearly indicate that the *A. amoenus* contains an indole oxidase that transforms (+)-6-*epi*-stephacidin A to (+)-versicolamide B but does not contain a suitable indole oxidase for (–)-6-*epi*-stephacidin A. Consequently, (–)-6-*epi*-stephacidin A becomes a shunt metabolite, and the fungus does not produce (–)-versicolamide B.

Stephacidin A, notoamide B, and versicolamide B are all putatively biosynthesized from notoamide S by two-electron oxidation, tautomerization and IMDA reaction (Scheme 3). In the *A. protuberus*, (+)-stephacidin A/(–)-notoamide B and (+)-6-*epi*-stephacidin A/(+)-versicolamide B are *exo*- and *endo*- products, respectively, which are caused by the different orientation of the dienophile to the diene in the pathways **a** and **b**, respectively (Scheme 3 (A)). On the other hand, in the *A. amoenus*, (–)-stephacidin A/(+)-notoamide B and (–)-6-*epi*-stephacidin A are similarly produced in the pathways **c** and **d**, respectively (Scheme 3 (B)). In addition, the different orientation of the diene to the dienophile in the pathway **b'** leads to the production of (+)-6-*epi*-stephacidin A/(+)-versicolamide B as produced in the pathway **b**. In the *A. amoenus*, the pathway **b'** is more likely than the pathway **b**, since the positions of the diene and dienophile in the pathway **b'** are the same as those in the pathways **c** and **d**. In both fungi, *exo*-metabolites, stephacidin A and notoamide B, are produced as major metabolites compared to *endo*-metabolites, 6-*epi*-stephacidin A and versicolamide B.

In conclusion, we have successfully isolated natural notoamide S from the *A. amoenus* NRRL 35600 (formerly *A. versicolor* NRRL 35600), which was previously bioconverted into the products containing a bicyclo[2.2.2]diazaoctane core structure, (–)-stephacidin A, (+)-notoamide B, and (+)-versicolamide B.⁶ The finding of notoamide S in the culture further confirms that it is a key biosynthetic precursor of these natural products. In this study we isolated 6-*epi*-stephacidin A from the *A. amoenus* as non-racemic mixture enriched with the (–)-isomer. With the presence of the (+)-enantiomer of versicolamide B in the culture of *A. amoenus*, the result strongly suggests that the fungus possesses a highly enantio-discriminating oxidase, which selectively converts (+)-6-*epi*-stephacidin A into (+)-versicolamide B, but is unreactive toward (–)-6-*epi*-stephacidin A present (Scheme 3 (B)). We have previously reported that the biosynthetic gene clusters of *A. protuberus* and *A. amoenus* are orthologous with an overall nucleotide identity of 71%.⁹ *These phylogenetically closely related species in Aspergillus section Versicolores¹⁰ have curiously evolved enantiodivergent biosynthetic pathways to the stephacidins and notoamides but converge on the production of (+)-versicolamide B.* Efforts to clarify the underlying genetic and biochemical basis for the biogenesis of these structurally complex alkaloids are under investigation in our laboratories.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Structures of the metabolites produced by *Aspergillus protuberus* (formerly *Aspergillus* sp. MF297-2) and *A. amoenus* (formerly *A. versicolor* NRRL 35600).

Figure 2.
CD spectra of (+)- (a) and (-)-6-*epi*-stephacidin A (b) isolated from the culture of *A. amoenus* in MeOH.

Scheme 1.

Proposed Biosynthetic Pathway of Enantiomeric Alkaloids in *A. protuberus* (formerly *Aspergillus* sp. MF297-2) and *A. amoenus* (formerly *A. versicolor* NRRL 35600).

Scheme 2.

Metabolites Isolated from the Culture of *A. amoenus* NRRL 35600 and their Plausible Biosynthetic Pathway. The Compounds in Route *c* are Main Metabolites.

Scheme 3.

Proposed Mechanisms of IMDA Reactions for Metabolites in *A. protuberus* (A) and *A. amoenus* (B). The Compounds in Bold Squares are Main Metabolites and those in Plain Squares are Minor Metabolites.