Organic LETTERS

Letter

pubs.acs.org/OrgLett

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

Hikaru Kato,[†] Takashi Nakahara,[†] Kayo Sugimoto,[†] Kanae Matsuo,[†] Ippei Kagiyama,[†] Jens C. Frisvad,[‡] David H. Sherman,[§] Robert M. Williams,[∥,⊥] and Sachiko Tsukamoto*[,†]

[†]Graduate School of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862-0973, Japan

[‡]Section for Eukaryotic Biotechnology, Department of Systems Biology, Building 221, Technical University of Denmark, 2800 Kongens Lyngby, Denmark

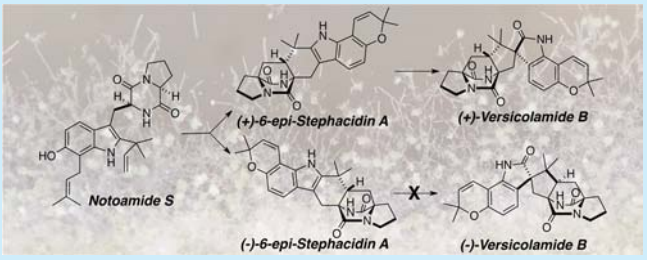
[§]Life Sciences Institute and Departments of Medicinal Chemistry, Chemistry, Microbiology & Immunology, The University of Michigan, 210 Washtenaw Avenue, Ann Arbor, Michigan 48109-2216, United States

[∥]Department of Chemistry, Colorado State University, 1301 Center Avenue, Fort Collins, Colorado 80523, United States

[⊥]University of Colorado Cancer Center, Aurora, Colorado 80045, United States

Supporting Information

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 *Aspergillus 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.



In our ongoing studies on the notoamide and stephacidin biosynthesis in two closely related fungi of the genus *Aspergillus*, we previously reported that *A. protuberus* (formerly *Aspergillus* sp. MF297-2) produces (+)-stephacidin A, (−)-notoamide B, and (+)-versicolamide B[1,2] and that *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 *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 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 *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 both *A. protuberus* and *A. amoenus*,[7] and 6-*epi*-notoamide T was converted to 6-*epi*-stephacidin A and versicolamide B in *A. protuberus*.[2] These incorporation experiments of notoamide T and its 6-*epi*-isomer

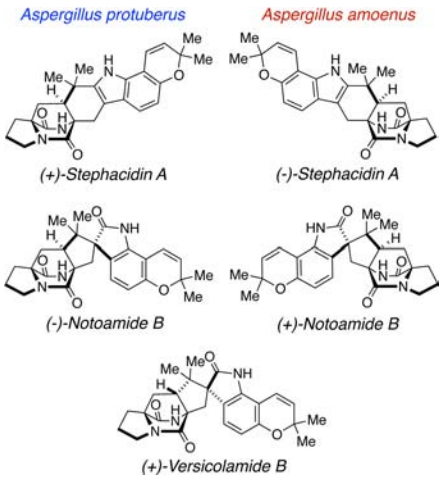
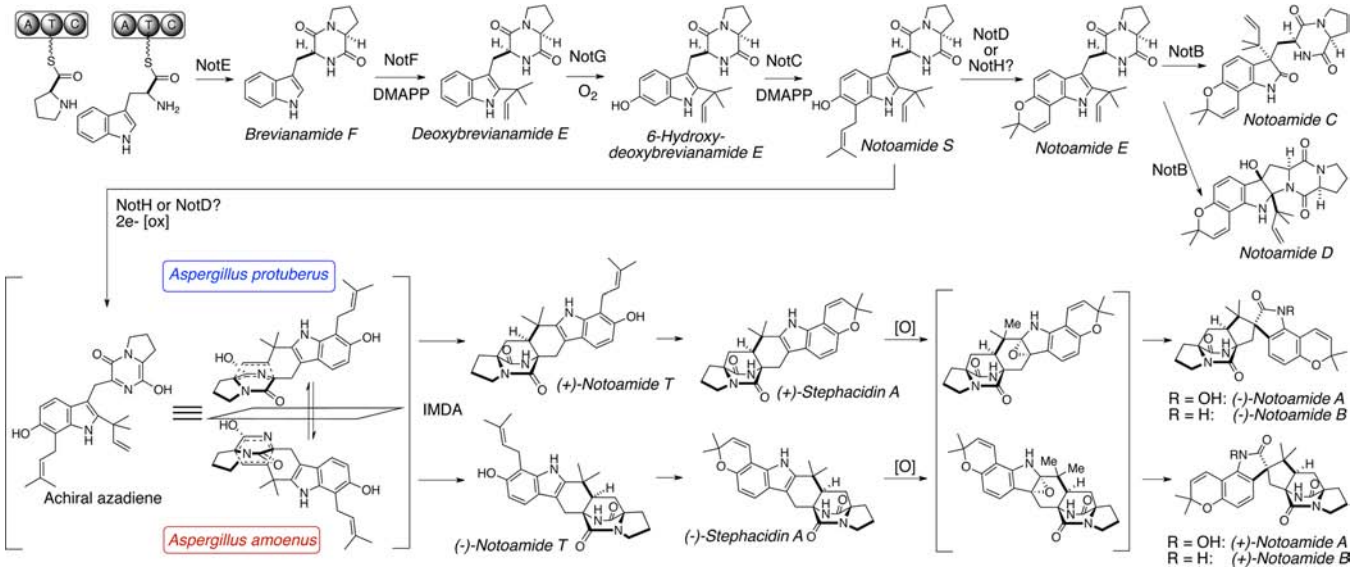


Figure 1. Structures of the metabolites produced by *A. protuberus* (formerly *Aspergillus* sp. MF297-2) and *A. amoenus* (formerly *A. versicolor* NRRL 35600).

Received: December 26, 2014
Published: January 23, 2015

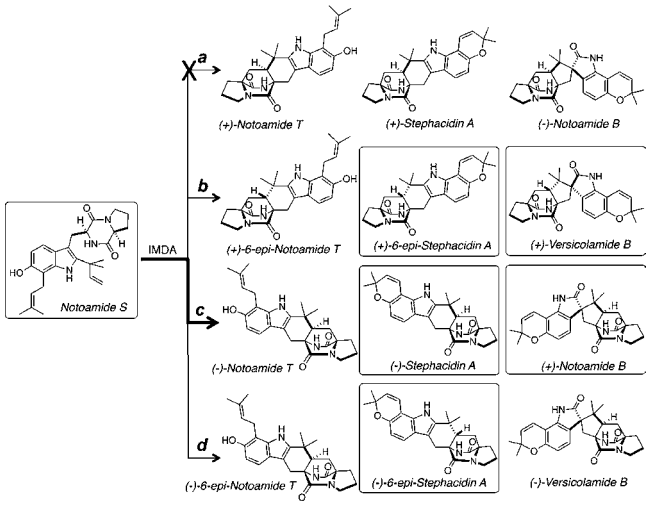
DOI: 10.1021/ol5037198
Org. Lett. 2015, 17, 700−703

Scheme 1. Proposed Biosynthetic Pathway of Enantiomeric Alkaloids in *A. protuberus* (Formerly *Aspergillus* sp. MF297-2) and *A. amoenus* (Formerly *A. versicolor* NRRL 35600)



were performed with 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 *A. protuberus* but could not obtain these metabolites.

Scheme 2. Metabolites Isolated from the Culture of *A. amoenus* and Their Plausible Biosynthetic Pathway[a]



[a]The compounds in route *c* are main metabolites.

In the present study, we searched for the presence of these metabolites in the culture of *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 of *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 *A. protuberus*.[2] In the present study, we elucidated the absolute configuration of 6-*epi*-stephacidin A produced by *A. amoenus*.

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−H_2O. The aqueous MeOH fraction was subjected to ODS column chromatography with MeOH/H_2O, and fractions that eluted with 75% MeOH−H_2O 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 ^{1}H 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 a 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 proposed 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 1:2.4, 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

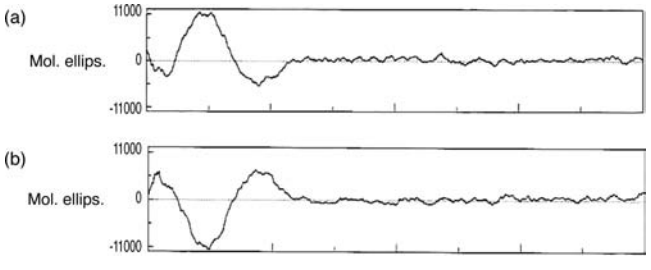
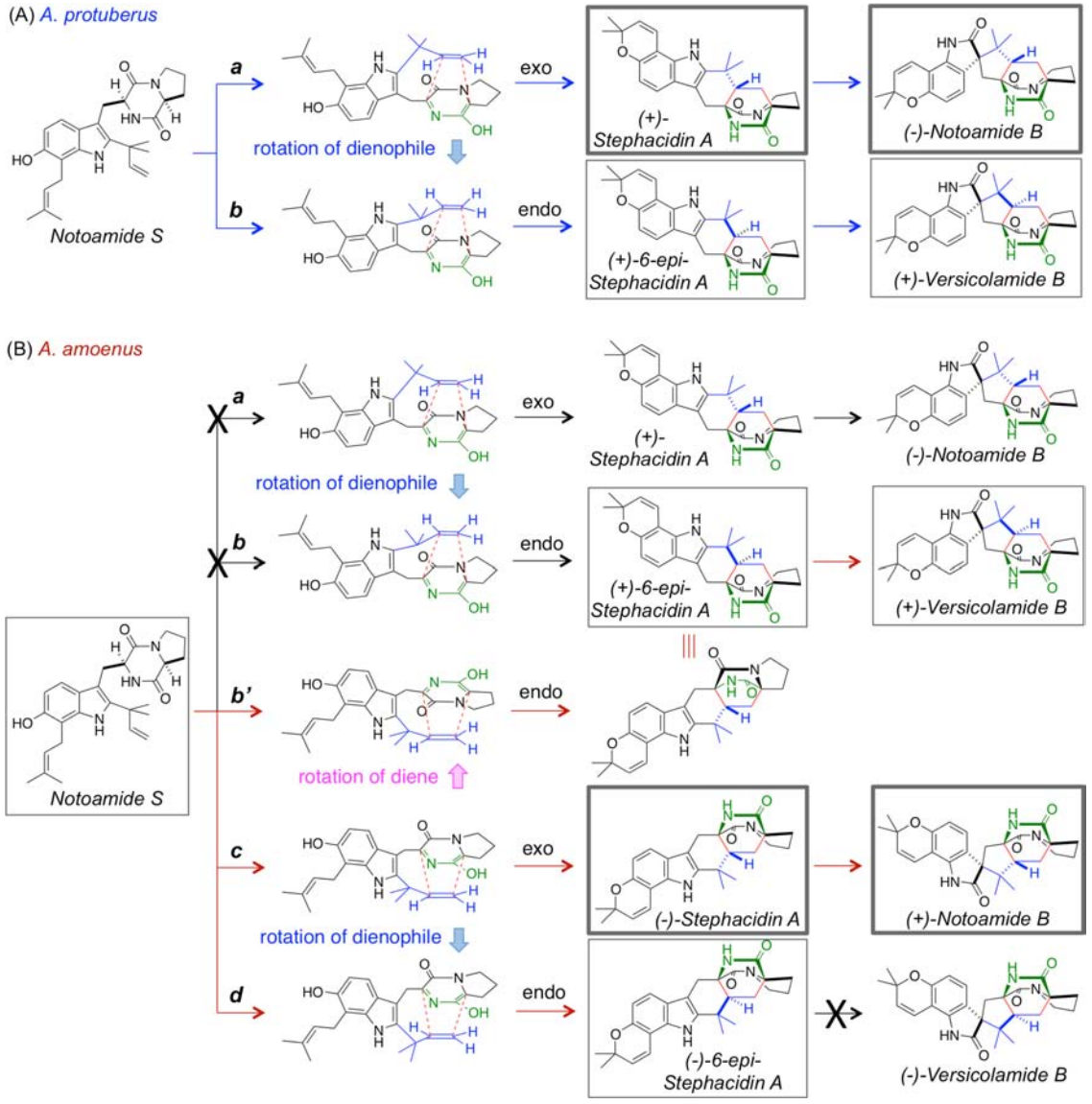


Figure 2. CD spectra of (+)- (a) and (−)-6-*epi*-stephacidin A (b) isolated from the culture of *A. amoenus* in MeOH.

DOI: 10.1021/ol5037198
Org. Lett. 2015, 17, 700−703

Scheme 3. Proposed Mechanisms of IMDA Reactions for Metabolites in *A. protuberus* (A) and *A. amoenus* (B)[a]



[a]The compounds in bold squares are main metabolites and those in plain squares are minor metabolites.

(+)- 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 *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 *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 pathways *a* and *b*, respectively (Scheme 3 (A)). On the other hand, in *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 pathway *b*′ leads to the production of (+)-6-*epi*-stephacidin A/(+)-versicolamide B as produced in pathway *b*. In *A. amoenus*, pathway *b*′ is more likely than pathway *b* since the positions of the diene and dienophile in pathway *b*′ are the same as those in 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 *A. amoenus* (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 *A. amoenus* as a nonracemic mixture enriched with the (−)-isomer. With the presence of the (+)-enantiomer of versicolamide B in the culture of *A. amoenus*, this result strongly suggests that the fungus possesses a highly enantio-discriminating oxidase, which selectively converts (+)-6-*epi*-stephacidin A into (+)-versicola-

DOI: 10.1021/ol5037198
Org. Lett. 2015, 17, 700−703

mide B but is unreactive toward the (−)-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 Versicolores*[10] *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.

■ ASSOCIATED CONTENT

Supporting Information

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

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: sachiko@kumamoto-u.ac.jp.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was financially supported in part by Grants-in-Aid for Scientific Research (Nos. 23108518 and 25108719 to S.T. and 24710252 to H.K.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan and a grant from the Nagase Science and Technology Foundation (to S.T.). Financial support from the National Institutes of Health (R01 CA070375 to R.M.W. and D.H.S.) is gratefully acknowledged.

■ REFERENCES

(1) 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.
(2) Kato, H.; Nakahara, T.; Yamaguchi, M.; Kagiyama, I.; Finefield, J. M.; Sunderhaus, J. D.; Sherman, D. H.; Williams, R. M.; Tsukamoto, S. *Tetrahedron Lett.* **2015**, *56*, 247−251.
(3) 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.
(4) Tsukamoto, S.; Kato, H.; Greshock, T. J.; Hirota, H.; Ohta, T.; Williams, R. M. *J. Am. Chem. Soc.* **2009**, *131*, 3834−3835.
(5) McAfoos, T. J.; Li, S.; Tsukamoto, S.; Sherman, D. H.; Williams, R. M. *Heterocycles* **2010**, *82*, 461−472.
(6) Li, S.; Finefield, J. M.; Sunderhaus, J. D.; McAfoos, T. J.; Williams, R. M.; Sherman, D. H. *J. Am. Chem. Soc.* **2012**, *134*, 788−791.
(7) Sunderhaus, J. D.; McAfoos, T. J.; Finefield, J. M.; Kato, H.; Li, S.; Tsukamoto, S.; Sherman, D. H.; Williams, R. M. *Org. Lett.* **2013**, *15*, 22−25.
(8) Finefield, J. M.; Kato, H.; Greshock, T. J.; Sherman, D. H.; Tsukamoto, S.; Williams, R. M. *Org. Lett.* **2011**, *13*, 3802−3805.
(9) Li, S.; Anand, K.; Tran, H.; Yu, F.; Finefield, J. M.; Sunderhaus, J. D.; McAfoos, T. J.; Tsukamoto, S.; Williams, R. M.; Sherman, D. H. *MedChemComm* **2012**, *3*, 987−996.
(10) Jurjevi, Z.; Peterson, S. W.; Horn, B. W. *IMA Fungus* **2012**, *3*, 59−79.

DOI: 10.1021/ol5037198
Org. Lett. 2015, 17, 700−703