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Study on the biosynthesis of the notoamides: Pinacol-type rearrangement of the isoprenyl unit in deoxybrevianamide E and 6-hydroxydeoxybrevianamide E

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Abstract

Two reverse-prenylated indole alkaloids, deoxybrevianamide E and 6-hydroxydeoxybrevianamide E, are proposed as biosynthetic precursors for advanced metabolites isolated from the marine-derived *Aspergillus* sp. In order to uncover the role of the alkaloids in the biosynthetic pathway, the feeding experiments of the [¹³C]₂-[¹⁵N]-labeled deoxybrevianamide E and 6-hydroxydeoxybrevianamide E were performed to afford the metabolites, which were produced by oxidation and successive pinacol-type rearrangement of the isoprenyl units.

Keywords

Aspergillus sp; biosynthesis; prenylated indole alkaloids

Introduction

The brevianamides,¹ paraherquamides,² stephacidins,³ malbrancheamides,⁴ marcfortines,⁵ asperparalines,⁶ and notoamides⁷ are secondary metabolites produced by various genera of fungi, including *Aspergillus* and *Penicillium* species and exhibit a wide range of biological activities. These prenylated indole alkaloids are comprised of three different building blocks, tryptophan, a cyclic amino acid, and one or two isoprene units, and show a range of interesting structural features, such as a dioxopiperazine or oxopiperazine within a bicyclo[2.2.2]diazaoctane ring system. Among the families of prenylated indole alkaloids, the notoamides are secondary metabolites produced by the marine-derived *Aspergillus* sp., which was isolated from the mussle, *Mytilus edulis galloprovincialis*, collected off the Noto Peninsula in the Sea of Japan. At the beginning of our chemical investigation, four new prenylated indole alkaloids, notoamides A–D (**1–4**), along with the known alkaloids, sclerotiamide (**5**), stephacidin A (**6**), and deoxybrevianamide E (**7**), were isolated from the fungal culture (Figure 1).^{7a} Based on the structural relationship between these metabolites,

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Supplementary data

Supplementary data (experimental details for the synthesis of isotopically labeled [¹³C]₂-[¹⁵N]-deoxybrevianamide E (**7**) and labeled substrates **10** and **16**) associated with this article can be found, in the online version, at doi: 10.1016/j.tetlet.2011.xx.xxx.

we speculated that a possible biosynthetic pathway existed from **7** to **1–6**.⁸ In this biogenesis, we postulated that notoamide E (**8**) would serve as a key intermediate to other alkaloids, although **8** had not yet been detected in the culture. Closer examination of the fungal metabolites revealed that **8** appears in the culture on day five and is completely consumed by day six.^{7e} This result strongly suggested that the rapid disappearance of **8** was due to the conversion of this metabolite into other downstream metabolites, such as **1–6**. Through a feeding experiment of doubly ¹³C-labeled **8** with the marine-derived *Aspergillus* sp., we found significant ¹³C incorporation of **8** into **3** and **4**, as well as 3-*epi*-notoamide C (**9**) (Figure 1) and three structurally unique metabolites, notoamides E2, E3, and E4.^{7e} Interestingly, **9** and notoamides E2, E3, and E4 were not present in the normal culture of *Aspergillus* sp. when cultivated on the standard nutrient-rich medium. Surprisingly, the yield of **9** was nearly four times that of **3** in the feeding experiment. This result strongly suggested that the excess amount of the precursor, [¹³C]₂-**8**, altered the metabolite profile of *Aspergillus* sp. and resulted in the conversion of **8** into the new alkaloids, **9** and notoamides E2, E3, and E4. Unexpectedly, formation of any labeled bridged bicyclo[2.2.2]diazaoctane-containing alkaloids, such as **1**, **2**, **5**, and **6**, was completely halted, although they are typically the major metabolites produced by this fungus. From this result, we proposed an alternative pathway for the formation of the bicyclo[2.2.2]diazaoctane-containing alkaloids, in which the formation of the core bridging bicycle is formed via an intramolecular Diels-Alder (IMDA) reaction, which precedes the construction of the pyranoindole moiety. In this pathway, **7** is first converted to **10** by an *R*-selective indole oxidase. Oxidation and tautomerization of **10** would yield azadiene intermediate **11**, followed by the IMDA reaction to afford the bicyclo[2.2.2]diazaoctane-containing alkaloid **12**. Subsequent oxidation and prenylation would afford **2** through its precursor **13** (Figure 2). In order to verify this hypothesis, we decided to perform a feeding experiment of [¹³C]₂-[¹⁵N]-**7** with the marine-derived *Aspergillus* sp. Additionally, the isolation of a minor alkaloid, notoamide J (**14**),^{7b} from the marine-derived *Aspergillus* sp. strongly attracted our interest toward determining the role of this alkaloid in the biosynthetic pathway of the notoamides. Since **14** contains a hydroxy group at the C-6 position of the indole moiety, **14** is expected to be a precursor of the pyranoindole alkaloids (Figure 3). However, the absolute configuration at the C-3 position of **14** is different from that of the major metabolite **3**. Therefore, to determine the biosynthetic role of **14**, we performed the feeding experiment of its presumed precursor, [¹³C]₂-[¹⁵N]-6-hydroxydeoxybrevianamide E (**15**).

Results and Discussion

[¹³C]₂-[¹⁵N]-Deoxybrevianamide E (**7**) was synthesized⁹ and fed to the marine-derived *Aspergillus* sp. Triply labeled **7** (60 mg, 170 μmol) was dissolved in a sterile trace element solution and provided to the fungal culture for 14 days.¹⁰ Purification of the fungal metabolites afforded a mixture of [¹³C]₂-[¹⁵N]-labeled **10** and **16** (0.34 mg, 0.93 μmol) in the ratio of 1:1 (Figure 3). Non-labeled **10** and **16** were previously synthesized¹¹ and the ¹H NMR spectra of synthetic non-labeled **10** and **16** and the labeled isolated metabolites matched. Significant ¹³C incorporation was observed by ¹³C NMR spectroscopy at C-12 and C-18 of [¹³C]₂-[¹⁵N]-labeled **10** (δ173.5 and 166.5) and [¹³C]₂-[¹⁵N]-labeled **16** (δ169.8 and 165.4). From analysis of the ESI mass spectrum, incorporation of intact labeled **7** into a mixture of [¹³C]₂-[¹⁵N]-labeled **10** and **16** was determined to be 9.7 and 11%, respectively.¹² Unexpectedly, no labeled bridged bicyclo[2.2.2]diazaoctane-containing alkaloids such as **12** were obtained. To further examine the downstream metabolites of **10** and **16**, synthetic [¹³C]₂-[¹⁵N]-labeled **10** and **16**¹³ were fed individually to the *Aspergillus* sp. culture in the same way described for **7**. However, no labeled metabolites were obtained from the putative precursor incorporation studies and the precursors were recovered, which indicated that **10** and **16** were dead-end (shunt) metabolites in the biosynthesis of the notoamides (Figure 3).

[¹³C]₂-[¹⁵N]-6-Hydroxydeoxybrevianamide E (**15**, 57.5 mg, 157 μmol) was synthesized¹⁴ and fed to the same *Aspergillus* sp.¹⁵ Analysis of the metabolites showed incorporation of triply labeled-**15** only into [¹³C]₂-[¹⁵N]-notoamide J (**14**) (0.16 mg, 0.41 μmol). ¹³C-Enrichment was detected at δ165.4 and 169.9, which correspond to the respective C-12 and C-18 positions of **14**. Examination of the ESI mass spectrum showed 12.3% incorporation of intact triply labeled **15** into **14**. The configuration at the C-3 position was determined to be *S* on the basis of the CD spectrum, which matched natural **14**. The absence of other advanced labeled metabolites in this feeding experiment suggests that **14** is a shunt metabolite in the notoamide biosynthesis.

In conclusion, we performed precursor incorporation experiments of several synthetic [¹³C]₂-[¹⁵N]-labeled putative biosynthetic substrates to clarify the biosynthesis of the notoamides in a marine-derived *Aspergillus* sp. Although we expected to obtain the bridged bicyclo[2.2.2]diazaoctane-containing alkaloids such as **12** or **2** (Figure 2), the feeding experiments of the labeled-**7**, **10**, **16**, or **15** did not afford any labeled alkaloids containing the core bridging bicycle (Figure 3). [¹³C]₂-[¹⁵N]-Deoxybrevianamide E (**7**) incorporated into labeled **10** and **16** in a 1:1 ratio. On the other hand, [¹³C]₂-[¹⁵N]-6-hydroxydeoxybrevianamide E (**15**) incorporated into labeled notoamide J (**14**). Among the prenylated indole alkaloids isolated from the marine-derived *Aspergillus* sp. that contain the dioxopiperazine ring, notoamides C (**3**), M,^{7d} and Q^{7f} display the 3*R*-configuration, while only **14** contains the 3*S*-configuration. Since **3** is a major alkaloid isolated from the fungal culture, the results of the present feeding experiments of the labeled-**7** and **15** were unexpected. The difference of the metabolism between **7** and **15** would be due to the presence of the hydroxy group at the indole ring in **15**, and the hydroxy group may play an important role in the pinacol-type rearrangement of the reverse-prenyl group from C-2 to C-3. Surprisingly, three antipodal metabolites containing the bridged bicyclo[2.2.2]diazaoctane ring, (+)-notoamide B ((+)-**2**), (–)-stephacidin A ((–)-**6**), and (+)-versicolamide B ((+)-**17**) were isolated from the closely related terrestrial-derived *Aspergillus versicolor* (Figure 4), and (+)-**2** and (+)-**17** possess 3*S* configuration, which is opposite to those of (–)-**2** and (–)-**17** isolated from the marine-derived *Aspergillus* sp.^{7c} It suggests that the terrestrial-derived *Aspergillus versicolor* contains the metabolic system in which the biosynthesis of the prenylated indole alkaloids proceeds in a parallel yet enantiomeric series of alkaloids relative to the marine-derived *Aspergillus* sp.¹⁶ We recently found that the precursor incorporation experiment of labeled **15** in the terrestrial-derived *Aspergillus versicolor* afforded **14** with the 3*S* configuration,¹⁴ which is the same result obtained from the marine-derived *Aspergillus* sp. reported in this paper. Efforts to further elucidate the biosynthetic pathway of the notoamides is currently underway in our laboratory.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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9. For the preparation of [¹³C]₂-[¹⁵N]-Deoxybrevianamide E (7) see supporting information.
10. Culture of *Aspergillus* sp. was maintained on agar plates (20 g malt extract, 5 g peptone and 20 g agar per liter of 50% seawater) in an incubator at 25°C. The spores from the agar plates was cultured in two 500 mL flasks containing a medium (100 mL × 8) composed of 50% seawater with 2.0% malt extract and 0.5% peptone at 27°C for 7 days. The mycelial cells were filtered by a cheese cross and rinsed with 100 mL of sterile water. [¹³C]₂-[¹⁵N]-Deoxybrevianamide E (7) (60 mg, 170 μmol) was dissolved in acetone (1 mL) containing Tween 80 (0.2 mL), and the sample was dried up. The precursor/Tween 80 residue was added to three 500 mL flasks containing the sterile trace element solution (150 mL × 12) composed of 35 mM NaNO₃, 5.7 mM K₂HPO₄, 4.2 mM MgSO₄, 1.3 mM KCl, 36 μM FeSO₄·7H₂O, 25 μM MnSO₄·H₂O, 7 μM ZnSO₄·7H₂O and 1.5 μM CuCl₂·2H₂O, and the mixtures were sonicated for 15 minutes to aid in micelle formation. The fungal cells were added to the media and incubated at 27°C for 14 days, and the flasks were swirled daily to ensure even distribution of the precursor. The culture broth was extracted with *n*-BuOH, and the fungal cells were extracted with MeOH. The both solutions were combined and evaporated. The extract was partitioned between *n*-hexane and 90% MeOH-H₂O. The latter fraction was evaporated, and the residue was purified by ODS column chromatography with 30, 70, and 90% MeOH-H₂O and MeOH. The fractions eluted with 70 and 90% MeOH-H₂O were combined and purified by ODS HPLC with 65% MeOH-H₂O to afford a mixture of [¹³C]₂-[¹⁵N]-**10** and **16** (0.34 mg, 0.93 μmol). **10**: ¹H NMR (acetone-*d*₆) δ: 7.27 (d, *J* = 7.8 Hz, 1H), 7.11 (dt, *J* = 7.8, 1.4 Hz, 1H), 6.76 (dt, *J* = 7.8, 1.4 Hz, 1H), 6.75 (d, *J* = 7.8 Hz, 1H), 6.48 (s, 1H), 6.33 (dd, *J* = 17.4, 10.6 Hz, 1H), 4.94 (dd, *J* = 17.4, 1.4 Hz, 1H), 4.88 (dd, *J* = 10.6, 1.4 Hz, 1H), 4.04 (m, 1H), 3.76 (m, 1H), 3.47 (m, 1H), 3.37 (m, 1H), 2.78 (dd, *J* = 13.3, 5.0 Hz, 1H), 2.54 (dd, *J* = 13.3, 7.3 Hz, 1H), 2.20 (m, 1H), 1.90–1.80 (m, 2H), 1.27 (s, 3H), 1.25 (s, 3H). ¹³C NMR (acetone-*d*₆) δ: 173.5 and 166.5 (d). ESIMS *m/z* 371 [M + H]⁺. **16**: ¹H NMR (acetone-*d*₆) δ: 7.16 (d, *J* = 6.9 Hz, 1H), 7.00 (dt, *J* = 7.8, 1.4 Hz, 1H), 6.65 (dt, *J* = 7.3, 0.9 Hz, 1H), 6.61 (d, *J* = 7.8 Hz, 1H), 6.53 (s, 1H), 6.51 (dd, *J* = 17.4, 10.6 Hz, 1H), 4.94 (dd, *J* = 17.4, 1.8 Hz, 1H), 5.01 (dd, *J* = 10.6, 1.8 Hz, 1H), 4.39 (dt, *J* = 8.3, 2.3 Hz, 1H), 4.16 (dt, *J* = 7.8, 2.8 Hz, 1H), 3.40 (ttt, *J* = 13.3, 6.0, 2.8 Hz, 1H), 3.16 (ttt, *J* = 12.4, 7.8, 4.6 Hz, 1H), 2.96 (dt, *J* = 11.4, 7.3 Hz, 1H), 2.62 (ttt, *J* = 13.3,

7.0, 5.5 Hz, 1H), 2.01 (m, 1H), 1.95 (m, 1H), 1.72 (m, 1H), 1.60 (m, 1H), 1.39 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (acetone- d_6) δ : 169.8 and 165.4 (d). ESIMS m/z 371 $[\text{M} + \text{H}]^+$.

11. For the preparation of **10** and **16** see supporting information.
12. The percentage of ^{13}C -enrichment in **10** and **16** from $[\text{C}^{13}]_2\text{-}[\text{N}^{15}]\text{-7}$ was calculated according to the method outlined in: Lambert JB, Shurvell HB, Lightner DA, Cooks RG. Organic Structural Spectroscopy. Prentice Hall Upper Saddle River, NJ 1998:447–448. These calculations are based on the comparison of the mass spectrum of the labeled material to the mass spectrum of the unlabeled material.
13. For the preparation of $[\text{C}^{13}]_2\text{-}[\text{N}^{15}]\text{-10}$ and **16** see supporting information.
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15. The feeding experiment of $[\text{C}^{13}]_2\text{-}[\text{N}^{15}]\text{-6-hydroxydeoxybrevianamide E (15)}$ (57.5 mg, 149 μmol) with *Aspergillus* sp. was performed in the same way described for $[\text{C}^{13}]_2\text{-}[\text{N}^{15}]\text{-deoxybrevianamide E (7)}$ ¹⁰ to afford $[\text{C}^{13}]_2\text{-}[\text{N}^{15}]\text{-notoamide J (14)}$. CD(MeOH) $[\theta]_{219} -6997$, $[\theta]_{231} 0$, $[\theta]_{239} +3778$, $[\theta]_{253} 0$, $[\theta]_{267} -1396$, $[\theta]_{325} 0$. ^1H NMR (acetone- d_6) δ 9.59 (br s, 1H), 7.00 (d, $J = 8.2$ Hz, 1H), 6.49 (d, $J = 8.2$, 1.8 Hz, 1H), 6.47 (d, $J = 1.8$ Hz, 1H), 6.28 (br s, 1H), 6.11 (dd, $J = 17.4$, 11.0 Hz, 1H), 5.08 (dd, $J = 11.0$, 1.3 Hz, 1H), 5.01 (dd, $J = 17.4$, 1.3 Hz, 1H), 3.98 (m, 1H), 3.32 – 3.44 (m, 2H), 3.26 (m, 1H), 3.10 (m, 1H), 1.77 – 1.94 (m, 3H), 1.10 (s, 3H), 1.05 (s, 3H). ^{13}C NMR (acetone- d_6) δ : 169.9 and 165.4 (d); ESIMS m/z 385 $[\text{M} - \text{H}]^-$.
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Figure 1.
Result of the feeding experiment of doubly ^{13}C -labeled **8** with the marine-derived fungus *Aspergillus* sp.

Figure 2.
Possible biogenesis of the bicyclo[2.2.2]diazaoctane-containing alkaloid **2** from **7**.

Figure 3.
Result of the feeding experiment of [^{13}C] $_2$ -[^{15}N]-**7**, **10**, **16**, and **15**.

Marine-derived *Aspergillus* sp.Terrestrial *Aspergillus versicolor* **Figure 4.**

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