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Studies on the Biosynthesis of the Notoamides: Synthesis of an Isotopomer of 6-Hydroxydeoxybrevianamide E and Biosynthetic Incorporation into Notoamide J

Jennifer M. Finefield[†], David H. Sherman[‡], Sachiko Tsukamoto[§], and Robert M. Williams^{†,*}

[†]Department of Chemistry, Colorado State University, 1301 Center Avenue, Fort Collins, Colorado 80523, U.S.A.

[‡]Life Sciences Institute and Department of Medicinal Chemistry, The University of Michigan, 210 Washtenaw Avenue, Ann Arbor, Michigan 48109-2216

[§]Medical and Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto, 862-0973, Japan

Abstract

6-Hydroxydeoxybrevianamide E is proposed as a biosynthetic precursor to several advanced metabolites isolated from both marine-derived *Aspergillus* sp. and a terrestrial-derived *Aspergillus versicolor*. To verify the role of this reverse-prenylated indole alkaloid as an intermediate along the biosynthetic pathway, [¹³C]₂-[¹⁵N]-6-hydroxydeoxybrevianamide E was synthesized and fed to *Aspergillus versicolor*. Analysis of the metabolites showed incorporation of the intermediate only into the natural product notoamide J.

Introduction

The brevianamides,¹ paraherquamides,² stephacidins,³ and notoamides⁴ are secondary metabolites produced by various genera of fungi, which include (among others) *Aspergillus* and *Penicillium* species. These prenylated indole alkaloids exhibit a range of interesting structural features, such as a core bicyclo[2.2.2]diazaoctane ring system and a complex, oxidized amino acid skeleton. A variety of bioactivities are also exhibited by many members of these families of alkaloids, including insecticidal, antitumor, anthelmintic, and antibacterial properties. Structurally, these natural products are comprised of tryptophan, a cyclic amino acid, and one or two isoprene units. The synthesis and biosynthesis of these natural products have been extensively investigated,⁵ however, a detailed understanding of the assembly and modification of the advanced metabolites is still relatively unknown.

Over the past several years, the number of new prenylated indole alkaloids isolated from fungi of both marine and terrestrial origin has greatly increased. Recently, Gloer and

rmw@lamar.colostate.edu.

Supporting Information Available: Spectroscopic data and experimental details for the preparation of all new compounds, as well as copies of ¹H NMR and ¹³C NMR spectra. This material is available free of charge via the Internet at <http://pubs.acs.org>.

coworkers reported the isolation of versicolamide B (**1**), stephacidin A (**2**), and notoamide B (**3**) from a terrestrial-derived fungus, *Aspergillus versicolor* NRRL 35600 (Figure 1).^{4c} In separate work, Tsukamoto and coworkers reported the isolation of the same prenylated indole alkaloids from a closely related, albeit, marine-derived *Aspergillus* sp.^{4a} Surprisingly, the marine-derived fungus and the terrestrial-derived fungus were found to produce, in parallel, the corresponding enantiomers of **1**, **2**, and **3**. This raised the question as to whether these two fungal strains follow similar, if not identical, biosynthetic pathways.

With the growing number of prenylated indole alkaloids isolated from various fungal cultures, it is often the minor/trace metabolites that provide key insights into the biosynthetic pathway of these unique natural products. Since 2007, Tsukamoto and coworkers have isolated over twenty secondary metabolites from the marine-derived *Aspergillus* sp., the majority of which are minor metabolites.⁴ Oddly, some of these prenylated indole alkaloids - specifically deoxybrevianamide E and notoamide J - could serve as potential precursors to the more advanced metabolites, including (+)-**1** and (+)-**3**, from the closely related terrestrial-derived *Aspergillus versicolor* NRRL 35600.

Notoamide J (**4**) is a novel secondary metabolite that contains two notable structural features, both of which may allow **4** to serve as an advanced intermediate along the biosynthetic pathway to other notoamides.^{4b,6} This compound is unique since it possesses a free hydroxyl at the C-6 position of the indole, in contrast to the pyranindole moiety found on a majority of the stephacidins and notoamides. This feature might enable **4** to serve as a biosynthetic precursor to the many natural products containing the pyranindole ring system. With the oxindole core in place, one can envision how **4** might give rise to the *spiro*-oxindole ring system through a proposed biosynthetic intramolecular Diels-Alder (IMDA) reaction. Formation of the bicyclo[2.2.2]diazaoctane ring system from the oxindole precursor via an IMDA has been corroborated by our research group through the biomimetic, asymmetric total synthesis of (+)- and (-)-versicolamide B.⁷ As shown in Scheme 1, we propose that **4** could arise biosynthetically from the common precursor deoxybrevianamide E (**5**), which was recently isolated from the marine-derived *Aspergillus* sp. by Tsukamoto.^{4a} Hydroxylation at the C-6 position of **5** would afford 6-hydroxydeoxybrevianamide E (**6**), a direct precursor to notoamide J. From here, oxidation, followed by a pinacol-type rearrangement could afford notoamide J. This laboratory recently validated the oxidative rearrangement of **6** to yield **4** in the course of the total synthesis of notoamide J (**4**).⁶

Efforts to uncover the biosynthetic pathway of these secondary metabolites have been further aided by the development of whole genome sequencing. In collaboration with Sherman et al., we have recently described the genome-based characterization of two prenylation steps in the biosynthetic pathway of the marine-derived *Aspergillus* sp.⁸ The first prenyl transferase reaction is the reverse-prenylation of brevianamide F (*cyclo*-L-Trp-L-Pro) and results in the formation of the known metabolite and proposed biosynthetic precursor, deoxybrevianamide E (**5**). As stated above, **5** could undergo hydroxylation of the indole ring at the C-6 position to afford **6**, the proposed notoamide J precursor. Contrary to our original hypothesis, the next step was found to be C-7 normal prenylation of **6** to give notoamide S (**7**), not notoamide J (**4**).⁸

The diverse metabolite profile of both the major and minor metabolites produced by *Aspergillus* sp., combined with the results from genome mining of the same fungal culture, has sparked interest in determining whether the metabolites produced by *Aspergillus versicolor* follow a similar, if not identical, biosynthetic pathway. In line with the pathway established by Sherman et al., it was postulated that 6-hydroxydeoxybrevianamide E would serve as a precursor to notoamide S in *Aspergillus versicolor*. On the other hand, based on

the isolation of notoamide J from *Aspergillus* sp., it was possible that 6-hydroxydeoxybrevianamide E would undergo an oxidative rearrangement and yield notoamide J in the terrestrial-derived fungal culture. In an attempt to provide further insight into the early stages of this perplexing biosynthetic pathway, we decided to rely on traditional feeding studies. Herein, we report the synthesis of the isotopically labeled proposed precursor [^{13}C] $_2$ -[^{15}N]-6-hydroxydeoxybrevianamide E (**8**) and the results of the ensuing feeding experiment with terrestrial-derived *Aspergillus versicolor* NRRL 35600.

Results and Discussion

In order to investigate the role of **6** in the biosynthesis of the notoamides, [^{13}C] $_2$ -[^{15}N]-labeled 6-hydroxydeoxybrevianamide E (**8**) was synthesized according to methods recently outlined by our laboratory in the total synthesis of notoamide J.⁶ As shown in Scheme 2, the known reverse-prenylated 6-OBoc indole (**9**) was converted to gramine **10** by treatment with dimethylamine and formaldehyde. Somei-Kametani coupling⁹ of **10** with ^{13}C -labeled glycine benzophenone imine (derived from [^{13}C]-glycine¹⁰), followed by imine hydrolysis, afforded the desired ^{13}C -labeled tryptophan derivative **11**.^{4e} Coupling of **11** with labeled [^{13}C]-[^{15}N]-Boc-*L*-proline¹⁰ in the presence of HATU and $^i\text{Pr}_2\text{NEt}$ provided **12**. Double Boc deprotection with TFA, followed by cyclization with 2-hydroxypyridine in refluxing toluene yielded a mixture of diastereomers of the triply-labeled dioxopiperazine **8**. The *cis*-diastereomer was isolated and added to cultures of *A. versicolor* in a precursor incorporation experiment.

Fungal extracts from the precursor incorporation experiment were analyzed by ^{13}C -NMR spectroscopy, electrospray mass spectroscopy and LC-MS. ^{13}C -Enrichment was detected at 165.8 ppm and 169.4 ppm, which correspond to the respective C-12 and C-18 positions of notoamide J. Further analysis of the HRMS revealed that this ^{13}C -enriched metabolite displayed a molecular formula of $\text{C}_{19}[^{13}\text{C}]_2\text{H}_{25}\text{N}_2[^{15}\text{N}]\text{O}_4$, which also corresponds to triply labeled notoamide J. Analysis of the electrospray mass spectrum showed 8.4% incorporation of intact triply-labeled 6-hydroxydeoxybrevianamide E (**8**) into **4**.^{11,12} Additional analysis of the fungal extract by LC-MS showed the production of several unlabeled advanced metabolites, such as **1**, **2**, and **3**, as well as notoamides C and D (Table 1). We observed that 32% of **8** was consumed, based on recovery of intact starting material. Upon closer inspection of the fungal extract, with the exception of recovered starting material **8** and labeled notoamide J, no other ^{13}C -enrichment was detected by ^{13}C -NMR spectroscopy or LC-MS, thus it is concluded that **8** was stereospecifically converted to a single oxindole compound, notoamide J.

The incorporation of **8** into notoamide J only provides a small glimpse into the biosynthetic pathway of the notoamides and strophacidins. The lack of ^{13}C -incorporation into more advanced metabolites may implicate notoamide J as a shunt (dead-end) metabolite. One possibility for the biosynthetic formation of notoamide J is through a pinacol-type rearrangement via a promiscuous oxidase, the product of which cannot be further metabolized by the organism. The presence of an indole 2,3-oxidase is also supported by the results from two notoamide E incorporation studies, in which doubly ^{13}C -labeled notoamide E was fed to the Tsukamoto marine-derived *Aspergillus* sp., as well as terrestrial-derived *Aspergillus versicolor*, wherein labeled notoamide C, 3-*epi*-notoamide C and notoamide D were formed.^{4e,13} Results from those precursor incorporation studies revealed that notoamide E underwent an oxidative rearrangement, and in the process, unexpectedly altered the metabolite profile of *Aspergillus* sp., but not *Aspergillus versicolor*.⁸ Fortunately, the excess amount of **8** did not alter the normal metabolic profile of *A. versicolor*, as evident from the isolation of both major and minor metabolites, as well as the considerable amount of recovered starting material. It is also postulated that the lack of formation of both

notoamide J diastereomers (notoamide J and 3-*epi*-notoamide J) is a result of **8** not altering the metabolite profile, as is believed to be the case for the formation of 3-*epi*-notoamide C in the feeding studies with ^{13}C -notoamide E.^{4e}

In summary, the natural minor metabolite, notoamide J (**4**), was produced in cultures of *Aspergillus versicolor* NRRL 35600 during a precursor incorporation study with [^{13}C]₂-[^{15}N]-6-hydroxydeoxybrevianamide E (**8**). Incorporation of intact triply labeled **8** was determined to be 8.4%. While notoamide J has not yet been isolated from the terrestrial culture under normal growth conditions (ie., without precursors added), the results from this precursor incorporation study demonstrate that an indole oxidase capable of forming notoamide J from **8** is present in *Aspergillus versicolor*. Efforts to further elucidate the biosynthesis of the notoamides and stephacidins are currently underway by our group.

Experimental Section

General methods

^1H and ^{13}C NMR spectra were obtained using 300 MHz or 400 MHz spectrometers. The chemical shifts are given in parts per million (ppm) relative to TMS at δ 0.00 ppm or to residual CDCl_3 δ 7.26 ppm for proton spectra and relative to CDCl_3 at δ 77.23 ppm for carbon spectra. IR spectra were recorded on an FT-IR spectrometer as thin films. Mass spectra were obtained using a high/low resolution magnetic sector mass spectrometer. Flash column chromatography was performed with silica gel grade 60 (230–400 mesh). Unless otherwise noted materials were obtained from commercially available sources and used without further purification. Dichloromethane (CH_2Cl_2), tetrahydrofuran (THF), toluene (PhMe), *N,N*-dimethylformamide (DMF), acetonitrile (CH_3CN), triethylamine (Et_3N), and methanol (MeOH) were all degassed with argon and passed through a solvent purification system containing alumina or molecular sieves.

Synthesis of [^{13}C]₂-[^{15}N]-6-hydroxydeoxybrevianamide E

Compounds **9** and **10** were synthesized by a known method established within the literature.⁶

[^{13}C]-Ethyl 2-amino-3-(6-((*tert*-butoxycarbonyl)oxy)-2-(2-methylbut-3-en-2-yl)-1H-indol-3-yl)propanoate (11**)**—Gramine **10** (740 mg, 2.06 mmol), [^{13}C]-*N*-(Diphenylmethylene)glycine ethyl ester (553 mg, 2.06 mmol), tributylphosphine (304 μL , 1.24 mmol) and acetonitrile (10 mL) were combined and stirred for 20 hours at reflux under Ar. The reaction was concentrated and purified via flash column chromatography in 10% ethyl acetate in hexanes to afford 1.19 g of a yellow amorphous solid, which was dissolved in THF (16 mL). 1M HCl (5.2 mL) was added and the reaction mixture stirred for 30 minutes at room temperature. The solvent was removed under reduced pressure and the residue was rediluted with saturated aqueous NaHCO_3 until basic. The mixture was extracted with CH_2Cl_2 (100 mL x2), dried over Na_2SO_4 and concentrated. The crude residue was purified by flash column chromatography (3:1 hexanes/ethyl acetate; 5:95 MeOH/ CH_2Cl_2) to give 610 mg (71%) of **11** as a yellow oil. ^1H -NMR (300 MHz, CDCl_3) δ 8.49 (bs, 1H), 7.42 (d, J = 8.4 Hz, 1H), 7.04 (d, J = 2.1 Hz, 1H), 6.81 (dd, J = 8.7, 2.1 Hz, 1H), 5.99 (dd, J = 17.4, 10.2 Hz, 1H), 5.06–5.00 (m, 2H), 4.12–4.00 (m, 2H), 3.73 (ddd, J = 5.1, 9.9, 5.1 Hz, 1H), 3.25–3.17 (m, 1H), 2.98–2.89 (m, 1H), 1.52 (s, 9H), 1.41 (d, J = 2.1 Hz, 6H), 1.11 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ 175.6, 153.1, 146.5, 146.1, 141.6, 134.2, 127.9, 119.0, 113.3, 112.0, 106.8, 103.7, 83.4, 61.0, 56.4, 55.6, 39.3, 31.3, 27.9, 14.4, 14.2; Enriched ^{13}C -peak: 175.6; IR (neat) 3388, 2973, 1730, 1469, 1238, 1149, 888 cm^{-1} ; HRMS (ESI/APCI) calcd for $\text{C}_{22}[\text{C}^{13}]\text{H}_{33}\text{N}_2\text{O}_5$ ($\text{M}+\text{H}$) 418.2418, found 418.2422.

[¹³C]₂[¹⁵N]-(2R)-tert-butyl 2-((3-(6-((tert-butoxycarbonyl)oxy)-2-(2-methylbut-3-en-2-yl)-1H-indol-3-yl)-1-ethoxy-1-oxopropan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (12)—¹³C-Amine **11** (610 mg, 1.46 mmol) and [¹³C]-[¹⁵N]-N-Boc-L-proline (317 mg, 1.46 mmol) were stirred in acetonitrile (15 mL) at 0°C. HATU (832 mg, 2.19 mmol) and ⁱPr₂N^tEt (1.0 mL, 5.84 mmol) were added and the reaction stirred for 3 hours at room temperature. The reaction was quenched with 1M HCl (30 mL) and extracted with CH₂Cl₂ (3 × 50 mL). The combined organic layer was washed with brine (50 mL), dried over Na₂SO₄, and concentrated under reduced pressure. The crude residue was purified by flash column chromatography and eluted with 1:1 hexanes/EtOAc to afford 860 mg (95%) of **12** as a yellow amorphous solid. ¹H-NMR (300 MHz, CDCl₃) δ 8.40 (bs, 1H), 7.42-7.34 (m, 1H), 7.02 (d, *J* = 1.2 Hz, 1H), 6.83-6.81 (m, 1H), 6.09-5.98 (m, 1H), 5.13-5.06 (m, 2H), 4.80-4.69 (m, 1H), 4.22-3.74 (m, 3H), 3.47-2.95 (m, 4H), 2.00-1.67 (m, 2H), 1.66-1.16 (m, 28H), 1.12-0.92 (m, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 172.6, 172.4, 171.9, 170.7, 169.5, 169.2, 154.9, 152.8, 146.6, 145.7, 141.6, 134.1, 128.1, 118.5, 118.2, 113.5, 112.4, 105.4, 105.2, 103.7, 83.3, 80.6, 77.0, 61.4, 53.8, 53.0, 47.3, 39.3, 28.4, 27.9, 27.6, 14.1, 13.8; ¹³C-enriched peaks: 172.6, 172.4; IR (neat) 3342, 2984, 1750, 1644, 1481, 1447, 1140 cm⁻¹; HRMS (ESI/APCI) calcd for C₃₁[¹³C]₂H₄₇N₂[¹⁵N] NaO₈ (M+Na) 639.3293, found 639.3293.

[¹³C]₂[¹⁵N]-(3S,8aS)-3-((6-hydroxy-2-(2-methylbut-3-en-2-yl)-1H-indol-3-yl)methyl) hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (8)—TFA (0.3 M) was slowly added to a solution of **12** (400 mg, 0.65 mmol) in CH₂Cl₂ (0.3 M) at 0°C. The reaction stirred for 3 hours at room temperature. The mixture was quenched with saturated NaHCO₃ to a pH 9 and extracted with EtOAc (3 × 20 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The residue was dissolved in toluene (0.2 M) and 2-hydroxypyridine (8.6 mg, 0.09 mmol) was added. The reaction refluxed for 14 hours under Ar atmosphere, cooled to room temperature, and concentrated under reduced pressure. The residue was rediluted with CH₂Cl₂ (10 mL) and washed with 1M HCl (20 mL). The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The crude residue was purified via flash column chromatography eluting with 3:97 MeOH/CH₂Cl₂ to afford 68 mg (40%) of the desired *cis*-diastereomer as cream foam. The *trans*-diastereomer was isolated in 38% yield (64 mg) as yellow foam (overall: 52% for two steps). *Cis*: ¹H NMR (300 MHz, 20:1 CDCl₃/CD₃OD) δ 8.05 (bs, 1H), 7.22 (d, *J* = 8.7 Hz, 1H), 6.74 (d, *J* = 2.1 Hz, 1H), 6.61 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.08 (dd, *J* = 17.4, 10.5 Hz, 1H), 5.15-5.09 (m, 2H), 4.38 (d, *J* = 11.4 Hz, 1H), 4.09-4.01 (m, 1H), 3.72-3.54 (m, 3H), 3.16-3.06 (m, 1H), 2.38-1.81 (m, 6H), 1.49-1.44 (m, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 169.7, 166.3, 153.0, 146.1, 140.3, 135.7, 123.2, 118.5, 112.5, 110.2, 104.1, 97.1, 60.7, 45.6, 39.1, 28.5, 28.1, 28.0, 26.2, 26.1, 22.7; ¹³C-enriched peaks: 169.7, 166.3; IR (neat) 3353, 2925, 1664, 1457 cm⁻¹; HRMS (ESI/APCI) calcd for C₁₉[¹³C]₂H₂₆N₂[¹⁵N]O₃ (M+H) 371.2006, found 371.2004. *Trans*: ¹H NMR (300 MHz, (CD₃)₂CO) δ 7.91 (bs, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 6.81 (d, *J* = 2.4 Hz, 1H), 6.64 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.22 (dd, *J* = 17.4, 10.5 Hz, 1H), 5.67-5.63 (m, 1H), 5.14-5.03 (m, 2H), 4.42-4.38 (m, 1H), 4.20-4.17 (m, 1H), 3.64-3.42 (m, 3H), 3.07-2.98 (m, 1H), 2.00-1.82 (m, 3H), 1.56 (s, 3H), 1.54 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 169.7, 166.3, 166.1, 152.8, 146.0, 140.2, 135.7, 123.2, 118.4, 112.3, 110.2, 104.1, 97.1, 65.1, 64.9, 60.5, 45.7, 39.1, 29.9, 28.1, 26.2, 22.7, 14.4; ¹³C-enriched peaks: 169.7, 166.3; IR (neat) 3358, 2920, 1662, 1456 cm⁻¹; HRMS (ESI/APCI) calcd for C₁₉[¹³C]₂H₂₅N₂[¹⁵N]O₃Na (M+Na) 393.1826, found 393.1826.

General procedure for the precursor incorporation experiment

A culture of *Aspergillus versicolor* NRRL 35600 was obtained from the Department of Agriculture in Peoria, IL. This culture was transferred to malt extract agar slants and

allowed to incubate for fourteen days. Potato dextrose broth was prepared by dissolving 48 g of the medium and 6 g tryptose in 2L of doubly distilled H₂O (DDH₂O). The solution was heated to aid in dissolving the medium, which was then transferred to Fernbach flasks (4 × 500 mL) and autoclaved. Spores of *A. versicolor* were added to the broth from the agar slants. The Fernbach flasks were covered and gently placed in the incubator for fourteen days. [¹³C]₂-[¹⁵N]-6-Hydroxy-deoxybrevianamide E (**8**) (59 mg, 0.159 mmol) was dissolved in 0.5 mL DMSO and added to 75 mL of hot DDH₂O containing 10 mL of 1% TWEEN 80. The solution was allowed to cool to room temperature and was added to 350 mL of a sterile trace element solution (35 mM NaNO₃, 5.7 mM K₂HPO₄, 4.2 mM MgSO₄·7H₂O, 1.3 mM KCl, 36 μM FeSO₄·7H₂O, 25 μM MnSO₄·H₂O, 7 μM ZnSO₄·7H₂O, and 1.5 μM CuCl₂·2H₂O). The fungus broth was decanted and the fungal cells were washed with 100 mL sterile DDH₂O. The precursor/trace element solution (110 mL) was added to each flask using a syringe and needle. The fungal cells were incubated at 25°C for fourteen days and each flask was swirled daily to ensure even distribution of the labeled compound.

Isolation and purification

The trace element solution was decanted, and the fungus was pureed in a blender with 1:1 MeOH-CHCl₃. The puree was transferred to a 2 L Erlenmeyer flask, diluted to 1.2 L with 1:1 MeOH-CHCl₃, and placed in the shaker for 24 h. Celite (30 g) was added to the flask and allowed to shake for an extra 10 min. The suspension was filtered through Whatman #2 paper and the filtrate was stored at 4°C. The mycelia “cake” was diluted with 600 mL 1:1 MeOH-CHCl₃ and placed on the shaker for an additional 48 h. The suspension was filtered through Whatman #2 paper, and the combined filtrates were concentrated under vacuum. The residue was dissolved in 250 mL H₂O and extracted with EtOAc (3 × 300 mL). The organic layer was concentrated and partitioned between MeCN and hexanes. The layers were separated, and the MeCN layer was concentrated under vacuum.^{4c,e,5b} The crude material was purified via silica gel flash column chromatography (1% MeOH in DCM – 3% MeOH in DCM) to afford three fractions that were each analyzed by ¹³C NMR spectroscopy. Further purification was carried out on those fractions containing ¹³C-labeled material via preparative thin layer chromatography (1000 μm, 3% MeOH in DCM x5) to afford triply-labeled 6-hydroxydeoxybrevianamide E (40 mg, 0.108 mmol) and notoamide J (1.4 mg, 0.003 mmol).

Notoamide J (4)—HRMS (ESI/APCI) calcd for C₁₉[¹³C]₂H₂₆N₂[¹⁵N]O₄ (M+H) 387.1955, found 387.1951. [α]_D²² –141 (c 0.0014, MeOH) lit. [α]_D¹⁹ –156 (c 0.067, MeOH)^{4b}

Calculation of percent incorporation from mass spectra

The percentage of ¹³C-enrichment in notoamide J from isotopically labeled 6-hydroxydeoxybrevianamide E was calculated according to the method by Lambert *et al.*¹¹ These calculations are based on the comparison of the mass spectrum of the labeled material (fixed ¹⁵N) to the mass spectrum of the unlabeled material. For these experiments, electrospray mass spectroscopy was used, thus the base peak was the M(¹⁵N)+H. (See Supporting Information)

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Antipodal metabolites isolated from *Aspergillus* sp. and *Aspergillus versicolor*.

Scheme 1.

Two possible biosynthetic pathways for the formation of (+)-versicolamide B and (+)-notoamide B from **5**.

Scheme 2.

Synthesis of **8** and result of the feeding study with *Aspergillus versicolor*.

Table 1

Intact incorporation of [^{13}C] $_2$ -[^{15}N]-6-hydroxydeoxybrevianamide E (**8**) into isolated metabolites.

Metabolite	% ^{13}C -incorporation	Notes
notoamide J	8.4%	1.4 mg
notoamide C	0%	0.6 mg
notoamide D	0%	0.9 mg
(-)-stephacidin A	0%	1.1 mg
(+)-notoamide B	0%	1.2 mg
(+)-versicolamide B	0%	1.5 mg