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# Biosynthesis of Indole Diterpenes, Emindole, and Paxilline: Involvement of a Common Intermediate

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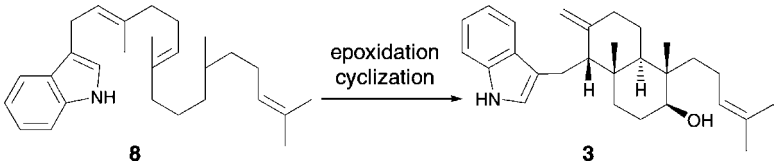
## ABSTRACT




**The key step for construction of the carbon skeleton in the indole diterpenes, paxilline, and emindole DA was examined. Intact incorporation of multiply $^2$H-labeled 3-geranylgeranylindole into two different fungal metabolites proves 3-geranylgeranylindole to be a biosynthetic intermediate. These results give evidence that indole diterpenes are biosynthesized via epoxidation of a common intermediate, and the subsequent cationic cyclization, analogous to those in the steroid biosynthesis.**

Indole diterpenes[1] isolated from fungi show structural diversity and various bioactivities such as tremogenic,[1] insecticidal,[2] and pollen growth inhibitory activity.[3] Tremogenic mycotoxins such as paxilline (**1**)[4] share a common carbon framework as shown in emindole SB (**2**) (Figure 1).[5] On the other hand, there are a number of structural variations such as emindoles DA (**3**),[6] SA[7] (2′-epimer of **3**), PA (**4**),[8] nominine (**5**),[9] emeniveol (**6**),[3] and aflavinine (**7**)[10] (Figure 1). On the basis of the carbon skeletons, it is proposed that these metabolites are biosynthesized by epoxidation of a common intermediate, 3-geranylgeranylindole (**8**) and subsequent cyclization[11] (Scheme 1) similar to cationic cyclization in the biosynthesis of terpenes and steroids. Other structural types of indole diterpenes represented by petromindole (**9**)[12] and radarin C (**10**)[13] are also found in nature (Figure 1). These compounds would be biosynthesized via terminal epoxide of **8**.

In the biosynthetic studies of **1** and its structurally related metabolites,[11,14] involvement of tryptophan for the construc-

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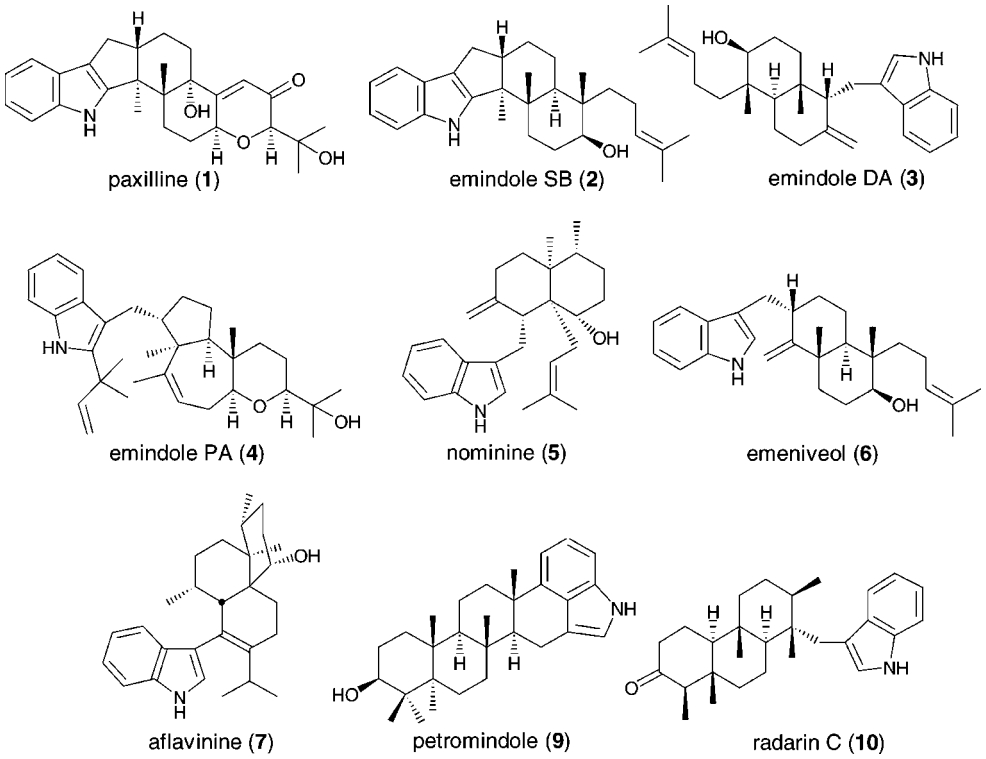
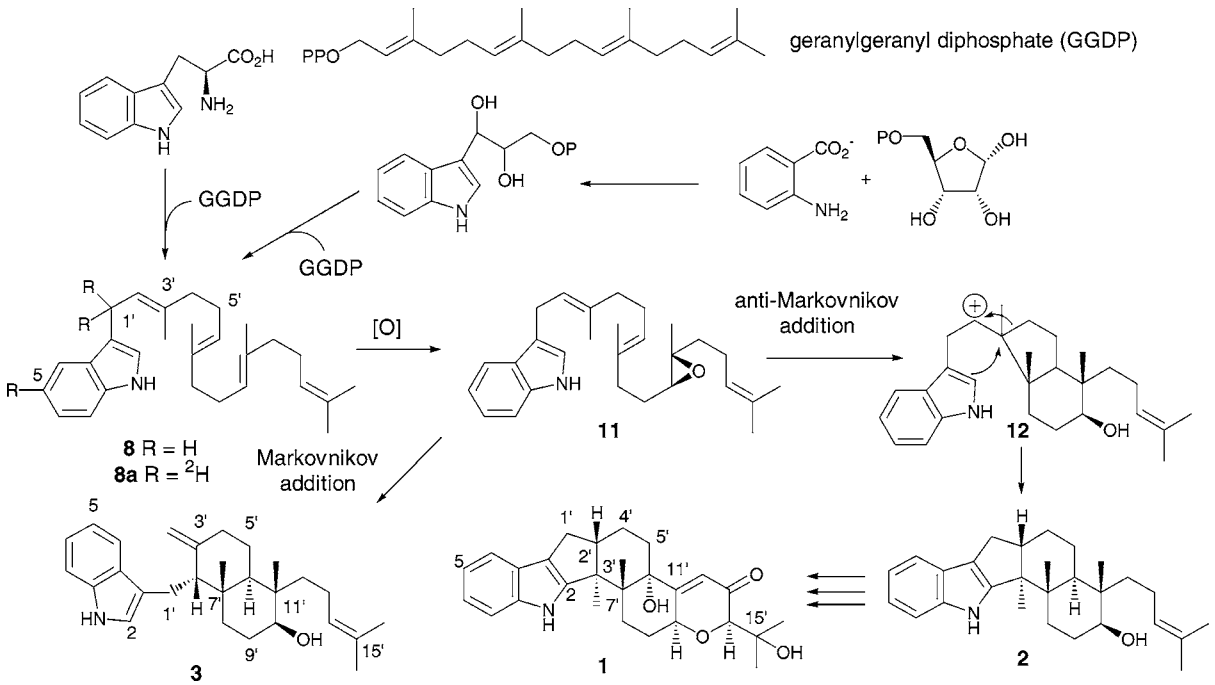



**Figure 1.** Representative structures of indole diterpenoids.

tion of the indole diterpene core was proposed (Scheme 1). Alternatively, in the biosynthetic study of nolispirodulic acid,[15] which has the same carbon skeleton as that of **1**, a series of feeding experiments demonstrated that the indole

**Scheme 1**[a]




[a] Proposed biosynthetic pathway of indole diterpenes, paxilline (**1**), and emindoles SB (**2**) and DA (**3**). To avoid confusion, the numbering of compounds **1** and **3** is the same as that in **8**.

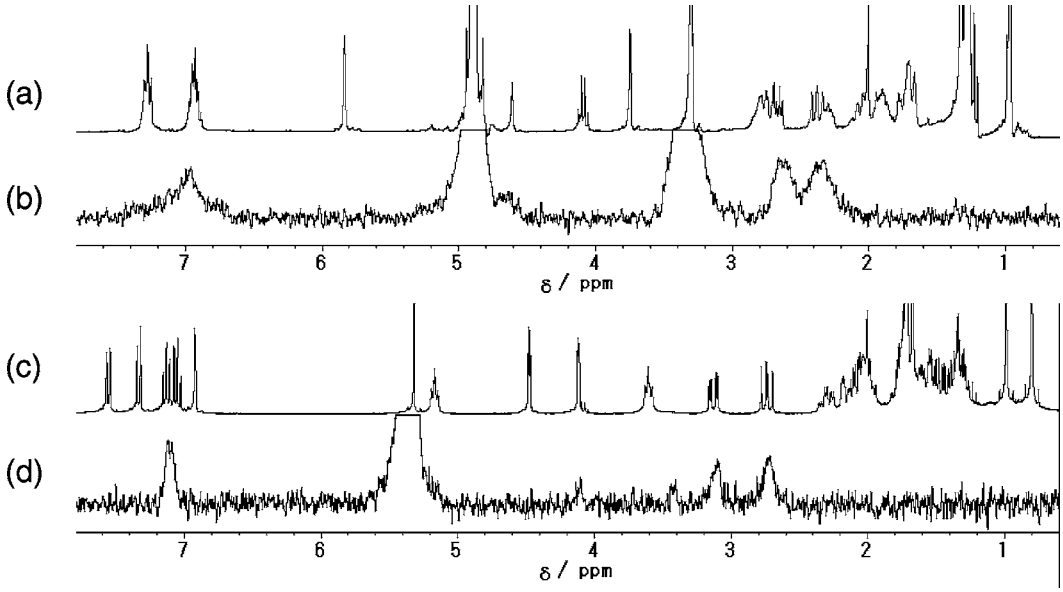
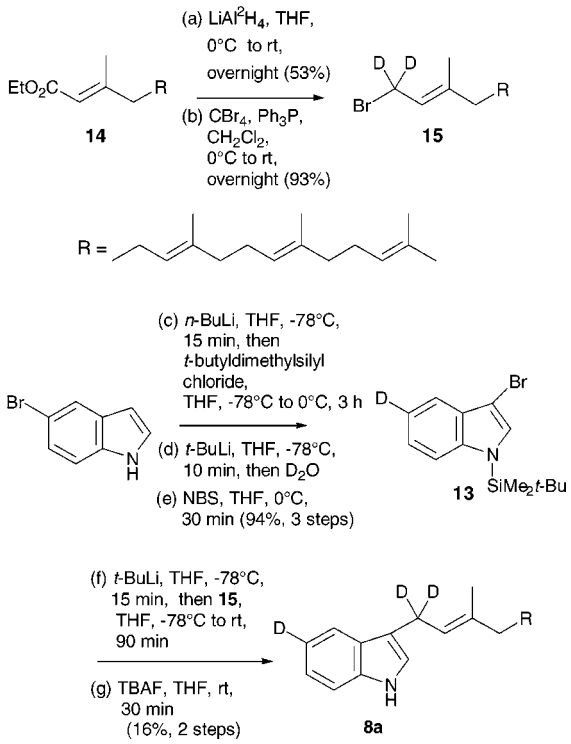



**Figure 2.** NMR spectra of paxilline (**1**) and emindole DA (**3**). (a, c) $^{1}$H NMR of **1** and **3**, respectively; (b, d) $^{2}$H NMR of **1** and **3**, derived from [5,1′,1′-$^{2}H_{3}$]-3-geranylgeranylindole **8a**, respectively.

diterpene core is constructed via anthranilic acid and D-ribose, indicating the intermediacy of indole-3-glycerol phosphate (Scheme 1). In 2001, Scott and co-workers identified the biosynthetic gene cluster of paxilline (**1**) in *Penicillium paxilli.*[16] Gene disruption and chemical complementation of the intermediate to the mutants lacking *paxG* and *paxP* proved that two cytochrome P450 monooxygenases (PaxP and PaxQ) are responsible for final conversion from paspaline to **1**.[17] The presence of a gene encoding geranylgeranyl diphosphate synthase in the biosynthetic gene cluster of **1** indicates that geranylgeranylated compound **8** is a plausible intermediate. Rainier and Smith demonstrated a biomimetic synthesis of emindole DA (**3**),[18] supporting this proposal. Recently, an alternative cyclization of a model epoxide to afford a paxilline-like product has been reported by Clark and co-workers.[19] Matsuda and co-workers have reported biomimetic synthesis of petromindole with plant origin lupeol synthase.[20] This enzymatic synthesis provided further support for epoxidation and cyclization of 3-geranylgeranylindole (**8**) to give indole diterpenes. Although these biomimetic syntheses showed the feasibility of this route, direct evidence of the intermediacy of **8** is still lacking. Our interest in cationic cyclization to form complex natural products[21] prompted us to explore the substrate of enzymes responsible for epoxidation and cyclization of indole diterpenes. Here, we report the first direct evidence of the common intermediacy of 3-geranylgeranylindole (**8**) in the biosynthesis of indole diterpenes **3** and **1**.

To exclude the possibility of degradation and reincorporation of the labeled compound, the deuterium labels were

**Scheme 2**

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introduced at two different positions of **8**. Starting from commercially available 5-bromoindole, lithiation followed by quenching with $^2H_2O$ gave 5-$^2$H-indole, which was converted to bromide **13**[22] in two steps in 94% yield. Ester **14**[23] was reduced with $LiAl^2H_4$ to give an alcohol which was converted to bromide **15** in 49% yield using standard conditions (Scheme 2). The resulting bromide **15** was treated with 3-lithioindole[21] derived from **13** followed by desilylation to afford **8a** in acceptable yield (16%). Feeding experiments of the labeled precursor were carried out with two different fungi producing indole diterpenes. The labeled compound **8a** was administered into *P. paxilli* (ATCC 26601), which produces paxilline (**1**). The $^2$H NMR spectrum in $CH_3OH$ of the resultant paxilline (**1**) exhibited three signals at 6.87, 2.51, and 2.28 ppm corresponding to deuteriums at 5-H and diastereotopic 1′-$CH_2$ with nearly the same integral ratio (1.0:1.1:1.0) (Figure 2c,d). Similar experiment with an emindole DA-producing fungus, *Emericella desertorum* (IFO 80840), afforded $^2$H-labeled **3**. The $^2$H NMR spectrum in $CH_2Cl_2$ of **3** from **8a** showed three signals at 7.12, 3.10, and 2.71 ppm corresponding to deuteriums at 5-H and diastereotopic 1′-$CH_2$ with nearly the same integral ratio (1.0:0.8:0.8) (Figure 2a,b). These observations indicate that **8a** was incorporated into **1** and **3** in an intact manner via epoxide **11** (Scheme 1). Specific incorporation of **8a** into **1** and **3** was relatively low (0.82 and 0.16%, respectively, as determined from the $^2$H NMR spectra with reference to natural abundance solvent peaks of $CH_2Cl_2$ and $CH_3OH$). With the authentic **8** in hand, the mycelial extracts of both fungi were examined by HPLC. None of the precursor **8** was detected in the extracts. This observation indicated that **8** is not accumulated in the mycelia and rapidly converted into paxilline (**1**) and emindole DA (**3**).

To identify the enzymes responsible for cyclization of indole diterpenes, we are currently working on functional analysis of the corresponding gene products.

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