



Title	Biosynthesis of Indole Diterpene Lolitrems : Radical-Induced Cyclization of an Epoxyalcohol Affording a Characteristic Lolitremane Skeleton
Author(s)	Jiang, Yulu; Ozaki, Taro; Harada, Mei; Miyasaka, Tadachika; Sato, Hajime; Miyamoto, Kazunori; Kanazawa, Junichiro; Liu, Chengwei; Maruyama, Jun-ichi; Adachi, Masaatsu; Nakazaki, Atsuo; Nishikawa, Toshio; Uchiyama, Masanobu; Minami, Atsushi; Oikawa, Hideaki
Citation	Angewandte chemie-international edition, 59(41), 17996-18002 <a href="https://doi.org/10.1002/anie.202007280">https://doi.org/10.1002/anie.202007280</a>
Issue Date	2020-10-05
Doc URL	<a href="http://hdl.handle.net/2115/82870">http://hdl.handle.net/2115/82870</a>
Rights	This is the peer reviewed version of the following article: Angewandte chemie-international edition 59(41) October 5 2020, pp.17996-18002, which has been published in final form at <a href="https://onlinelibrary.wiley.com/doi/10.1002/anie.202007280">https://onlinelibrary.wiley.com/doi/10.1002/anie.202007280</a> . This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.
Type	article (author version)
File Information	Angew. Chem.-Int. Edit.59-41_17996-18002.pdf



[Instructions for use](#)

# Biosynthesis of Indole Diterpene Lolitrems: Radical-Induced Cyclization of an Epoxyalcohol Affording a Characteristic Lolitremane Skeleton

Yulu Jiang,<sup>[a]</sup> Taro Ozaki,<sup>[a]</sup> Mei Harada,<sup>[b,c]</sup> Tadachika Miyasaka,<sup>[d]</sup> Hajime Sato,<sup>[b,c]</sup> Kazunori Miyamoto,<sup>[b]</sup> Junichiro Kanazawa,<sup>[b]</sup> Chengwei Liu,<sup>[a]</sup> Jun-ichi Maruyama,<sup>[e]</sup> Masaatsu Adachi,<sup>[f]</sup> Atsuo Nakazaki,<sup>[d]</sup> Toshio Nishikawa,<sup>[d]</sup> Masanobu Uchiyama,<sup>[b,c]</sup> Atsushi Minami,<sup>\*[a]</sup> and Hideaki Oikawa<sup>\*[a]</sup>

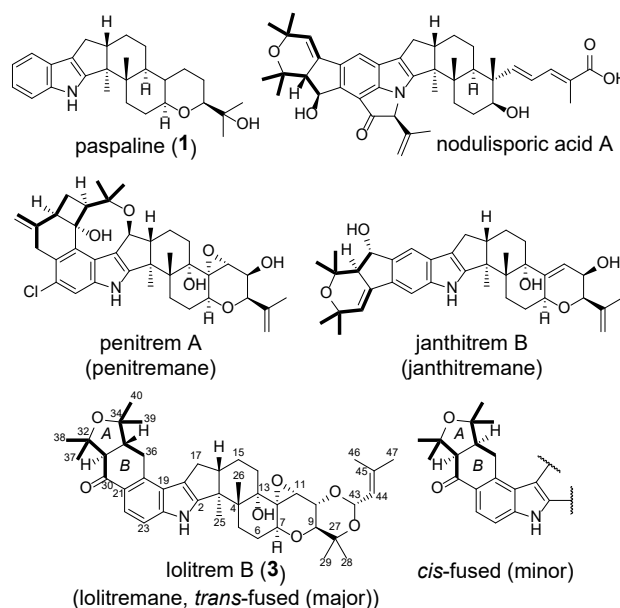
- [a] Y. Jiang, Dr. T. Ozaki, Dr. C. Liu, Dr. A. Minami, Prof. Dr. H. Oikawa  
Department of Chemistry  
Faculty of Science, Hokkaido University  
Sapporo 060-0810, Japan  
E-mail: [aminami@sci.hokudai.ac.jp](mailto:aminami@sci.hokudai.ac.jp), [hoik@sci.hokudai.ac.jp](mailto:hoik@sci.hokudai.ac.jp)  
Dr. C. Liu Present address: College of Life Sciences, Northeast Forestry University, Harbin 150040, China.
- [b] M. Harada, Dr. H. Sato, Dr. K. Miyamoto, Dr. J. Kanazawa, Prof. Dr. M. Uchiyama  
Graduate School of Pharmaceutical Sciences  
The University of Tokyo  
7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
- [c] M. Harada, Dr. H. Sato, Prof. Dr. M. Uchiyama  
Cluster for Pioneering Research (CPR), Advanced Elements Chemistry Laboratory  
RIKEN  
2-1 Hirosawa, Wako, Saitama 351-0198, Japan
- [d] T. Miyasaka, Dr. A. Nakazaki, Prof. Dr. T. Nishikawa  
Graduate School of Bioagricultural Sciences  
Nagoya University  
Chikusa, Nagoya 464-8601, Japan
- [e] Prof. Dr. J. Maruyama  
Department of Biotechnology, Graduate School of Agricultural and Life Sciences  
The University of Tokyo  
1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
- [f] Dr. M. Adachi  
Graduate School of Pharmaceutical Sciences  
Tohoku University  
6-3. Aoba, Aramaki, Aoba-ku, Sendai 980-8578, Japan

Supporting information for this article is given via a link at the end of the document.

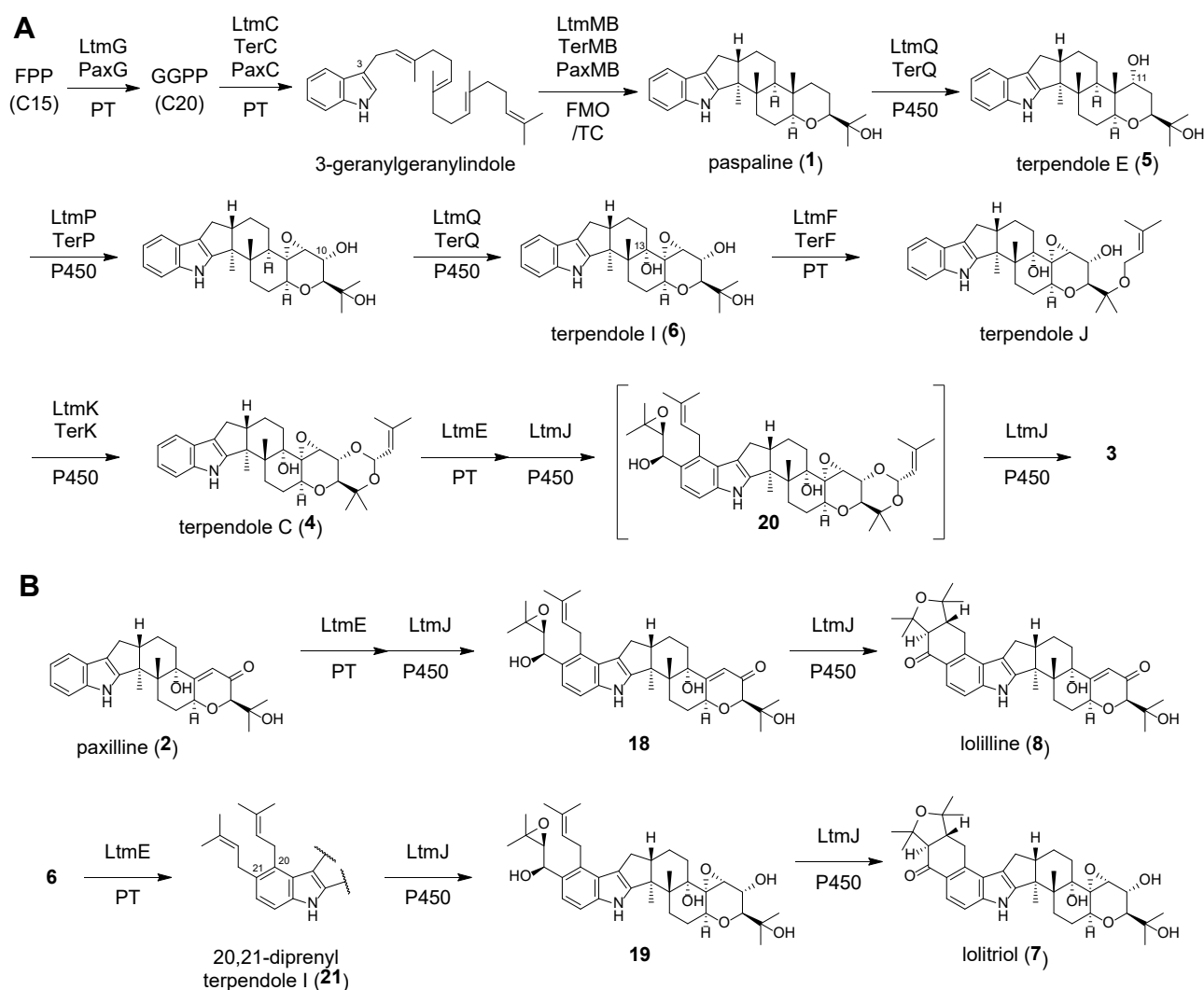
**Abstract:** Lolitrems are tremorgenic indole diterpenes that exhibit a unique 5/6 bicyclic system of the indole moiety. Although genetic analysis has indicated that prenyltransferase LtmE and cytochrome P450 LtmJ are involved in the construction of this unique structure, the detailed mechanism remains to be elucidated. Herein, we reconstitute the biosynthetic pathway for lolitrems employing a recently established genome-editing technique for the expression host *Aspergillus oryzae*. Heterologous expression and bioconversion of the various intermediates revealed that LtmJ catalyzes multistep oxidation to furnish the lolitremane core. We also isolated the key reaction intermediate with an epoxyalcohol moiety. This observation allowed us to establish the mechanism of radical-induced cyclization, which was firmly supported by density functional theory calculations and a model experiment with a synthetic analog.

## Introduction

Indole diterpenes (IDTs) are representative fungal secondary metabolites with remarkable structural diversity and a wide range of biological activities, such as inhibition of calcium-activated potassium channels, tremorgenic activity, progesterone receptor



**Figure 1.** Representative fungal indole diterpenes.



**Figure 2.** Biosynthetic pathway for terpendoles and lolitrem B. A) The biosynthetic pathway for **3**. B) The biosynthetic pathway for **8** and **7** from **2** and **6**. PT: prenyltransferase; FMO: flavin-dependent monooxygenase; TC: terpene cyclase.

agonistic activity, and anti-MRSA activity.<sup>[1,2]</sup> To understand the mechanism of their structural diversification, extensive studies have been conducted on their biosynthesis. Among more than a hundred natural indole terpenes, paspaline (**1**)-derived IDTs are the largest group, comprising 70% of all isolated IDTs (Figure 1).

The core structure of IDTs is diversified mainly by cytochromes P450, such as PaxPQ, involved in the biosynthesis of paxilline (**2**), or their homologs.<sup>[3,4]</sup> In the late stage of biosynthesis, prenylation of the indole ring and further modification also contribute to structural diversification. Previously, we elucidated the biosynthetic pathway of penitrem A and shearinines/janthitrem, in which prenyltransferases and flavoproteins are involved in the construction of a bicyclic system on indole rings (Figure 1).<sup>[5,6]</sup> However, little is known about the biosynthesis of lolitrem, which have a unique 5/6 bicyclic system (Figure 1). Lolitrem are tremorgenic mycotoxins produced by the fungal endophyte *Epichloë festucae* var. *lolii*. A major congener, lolitrem B (**3**), is known as a causative agent of ryegrass staggers.<sup>[7,8]</sup> Among IDTs, lolitrem/terpendoles are known as one of the most diverse

classes of compounds, including more than 30 congeners. Most lolitrem are structurally characterized by the presence of *trans*-fused 5/6 bicyclic systems (A/B rings), while minor congeners can also have *cis*-fused systems (Figure 1).<sup>[2]</sup> The biosynthetic gene cluster of lolitrem, which is composed of ten genes and splits into three loci,<sup>[9,10]</sup> contains four genes, *ltmGCMB* (Figure S1), which are responsible for the biosynthesis of **1**.<sup>[11]</sup> In the biosynthesis of terpendole C (**4**), which has an identical structure to the IDT core of **3**, a key intermediate **1** undergoes a series of modifications catalyzed by TerPQFK to yield **4** via terpendole E (**5**), J, and I (**6**) as intermediates (Figure 2A),<sup>[12]</sup> suggesting that the orthologous enzymes LtmPQFK are responsible for the same conversions in lolitrem biosynthesis.<sup>[11]</sup> Prenyltransferase LtmE and cytochrome P450 LtmJ are specific to the biosynthesis of lolitrem and are likely involved in the construction of their A/B rings.<sup>[11]</sup> However, the exact mechanism is still unknown. In this study, we elucidated the mechanism of P450-catalyzed oxidative cyclization employing heterologous expression, density functional theory (DFT) calculation, and a model experiment with a synthetic analog.

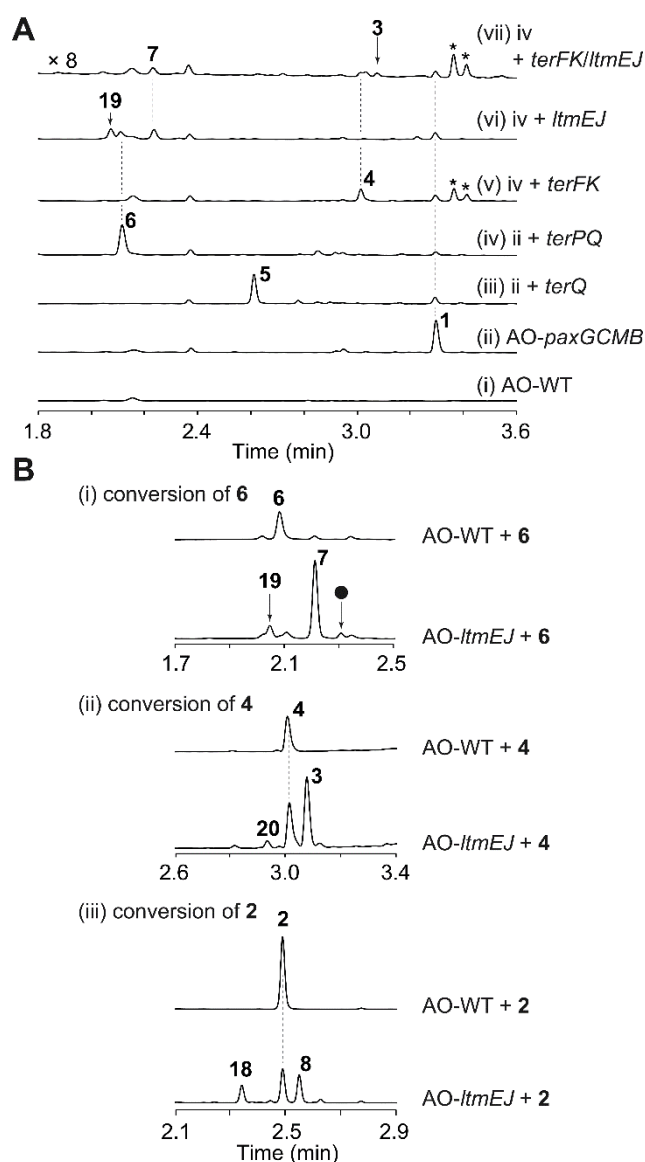
## Results and Discussion

Based on the biosynthetic studies of **2**,<sup>[3]</sup> we initially introduced four genes, *paxG*, *paxC*, *paxM*, and *paxB*, into *Aspergillus oryzae* NSPID1 employing CRISPR/Cas9-based genome editing to produce the putative intermediate **1** (Figure S2, Table S1).<sup>[13]</sup> This screening-free method readily enabled us to obtain the AO-*paxGCMB* which produced **1** (133 mg kg<sup>-1</sup>), as we reported previously<sup>[3]</sup> (Figure 3A-ii). Given the expected transformant, cytochrome P450 genes *terP* and/or *terQ* were introduced into AO-*paxGCMB*. The obtained transformants, AO-*paxGCMB/terQ* and AO-*paxGCMB/terPQ*, produced **5** (109 mg kg<sup>-1</sup>) and **6** (148 mg kg<sup>-1</sup>), respectively (Figure 3A-iii and iv). Two additional genes, *terF* and *terK*, were introduced. The obtained transformant, AO-*paxGCMB/terPQFK*, which produced **4** (52 mg kg<sup>-1</sup>), confirmed that TerF and TerK exerted the expected activities (Figure 3A-v). Finally, we introduced *ltmE* and *ltmJ*. The resulting AO-*paxGCMB/terPQ/ltmEJ* and AO-*paxGCMB/terPQFK/ltmEJ* produced lolitriol (**7**)<sup>[14]</sup> (6 mg kg<sup>-1</sup>) and lolitrem B (**3**) (0.3 mg kg<sup>-1</sup>), respectively (Figure 3A-vi and vii). These results showed that LtmE and LtmJ are responsible for the construction of the A/B rings of lolitrems. The low production of **4** and **3** can be explained by the substrate promiscuity of prenyltransferase TerF (Figure 3A-v and vii). Competition between several enzymes is also likely (e.g., **6** is a substrate for both TerF and LtmE).

Considering that more than 10 lolitremanes/lolitrems congeners have been isolated in nature, we hypothesized that LtmEJ can accept a diverse array of IDTs. To investigate their substrate scope, we constructed the transformant AO-*ltmEJ*. Indole terpenes **6**, **4**, and **2** were converted to naturally occurring lolitrems **7**, **3**, and lolilline (**8**),<sup>[15]</sup> respectively (Figure 3B). Among the compounds tested, paspalinine (**9**) was converted to the putative lolitremane **10**, while 13-desoxypaxilline (**11**) was not converted (Figure 4A, B, S3), suggesting that the 13-hydroxy group was important for substrate recognition. This hypothesis was supported by the observation that terpendole D (**12**) and emindole SB (**13**) were not converted in the same condition (Figure 4C, D). Interestingly, it was possible to apply the same empirical rule to indole sesquiterpenes (ISTs): lecanindole B (**14**), which has a 9-hydroxy group, was also converted to the putative product **15**, while prelecanindole (**16**) was not (Figure 4E, F, S3). These results indicated that LtmE and LtmJ can be applied to the modification of IDTs/ISTs with 13/9-hydroxy groups (Figure S4). Conversion of **5** was extremely low, but MS spectra and chromatograph profiles indicated that lolicine A (**17**) was formed (Figure 4G, S3).<sup>[14]</sup> Insufficient conversion of **5** suggested the other structural features may also be important for their recognition by the enzymes. In the case of **6**, we also detected a trace amount of isomeric metabolites (Figure 3B), suggesting that the products with *cis*-fused A/B rings were formed in the same condition.

LC-MS analysis of the bioconversion of **2**, **6**, and **4** revealed that products **8**, **7**, and **3** were always accompanied by the compounds **18**, **19**, and **20**, respectively, whose deduced molecular weights were two mass units larger than those of the corresponding lolitrems (Figures 3B and S3). Similar compounds were also observed in the cases of **5**, **9**, and **14** (Figure 4A, E, G, S3). We determined the planar structures of **18** and **19** to be

epoxyalcohols (Scheme 1A, Figure S5, Table S2), suggesting that **20** also had the same partial structure. Comparing the chemical shifts of <sup>1</sup>H and <sup>13</sup>C NMR spectra with those of synthetic analogs, we determined the relative stereochemistry to be *syn* (Tables S2–S4).<sup>[16]</sup> Although the NMR spectra of **18**, which was previously identified by the bioconversion of **2** by AO-*spdE/ltmJ*,<sup>[17]</sup> were nearly identical to those of sespelline (**S4**), an unnatural IDT produced by the bioconversion of **2** by AO-*spdE/spdJ*,<sup>[17]</sup> the difference in their retention times suggested a diastereomeric relationship (Figure S6).

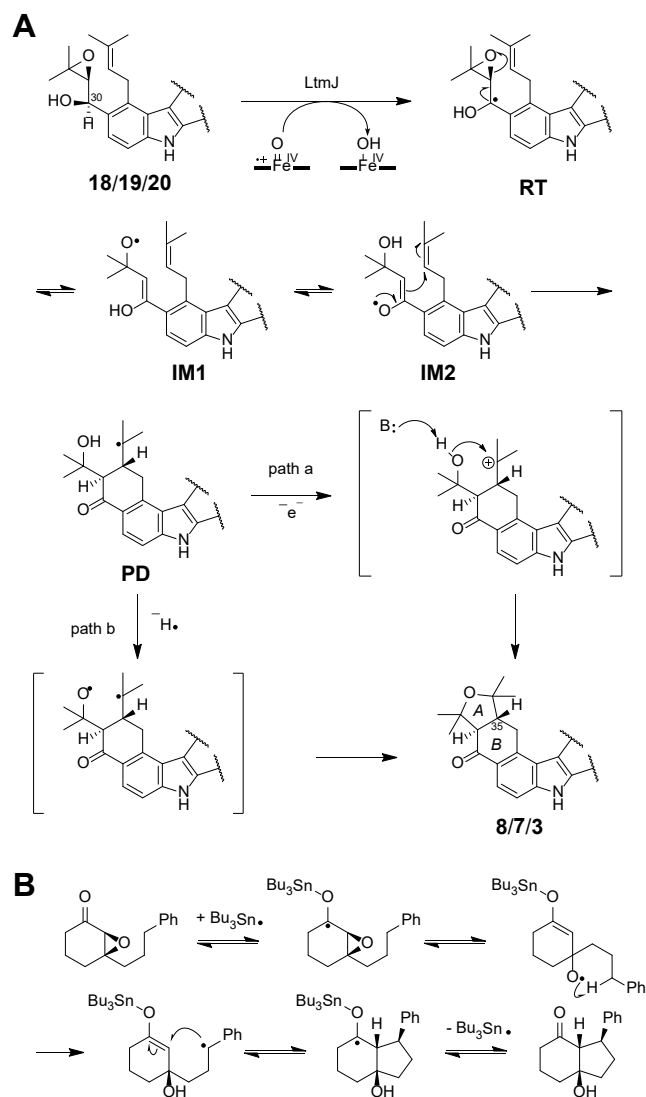


**Figure 3.** HPLC analyses of IDTs produced by the transformants. A) Traces of the metabolites extracted from the transformants. The peaks marked with asterisks indicate putative monoprenylated derivatives of **4**. B) Bioconversion of **6**, **4**, and **2** by AO-WT or AO-*ltmEJ*. All chromatograms were extracted at 265 nm. The black circle indicates a putative 35-epimer of **7** based on its MS spectrum.

Subsequently, we conducted bioconversion of **18** and **19** by AO-*ltmJ* and obtained **8** and **7**, respectively (Figures S6 and S7).

## RESEARCH ARTICLE

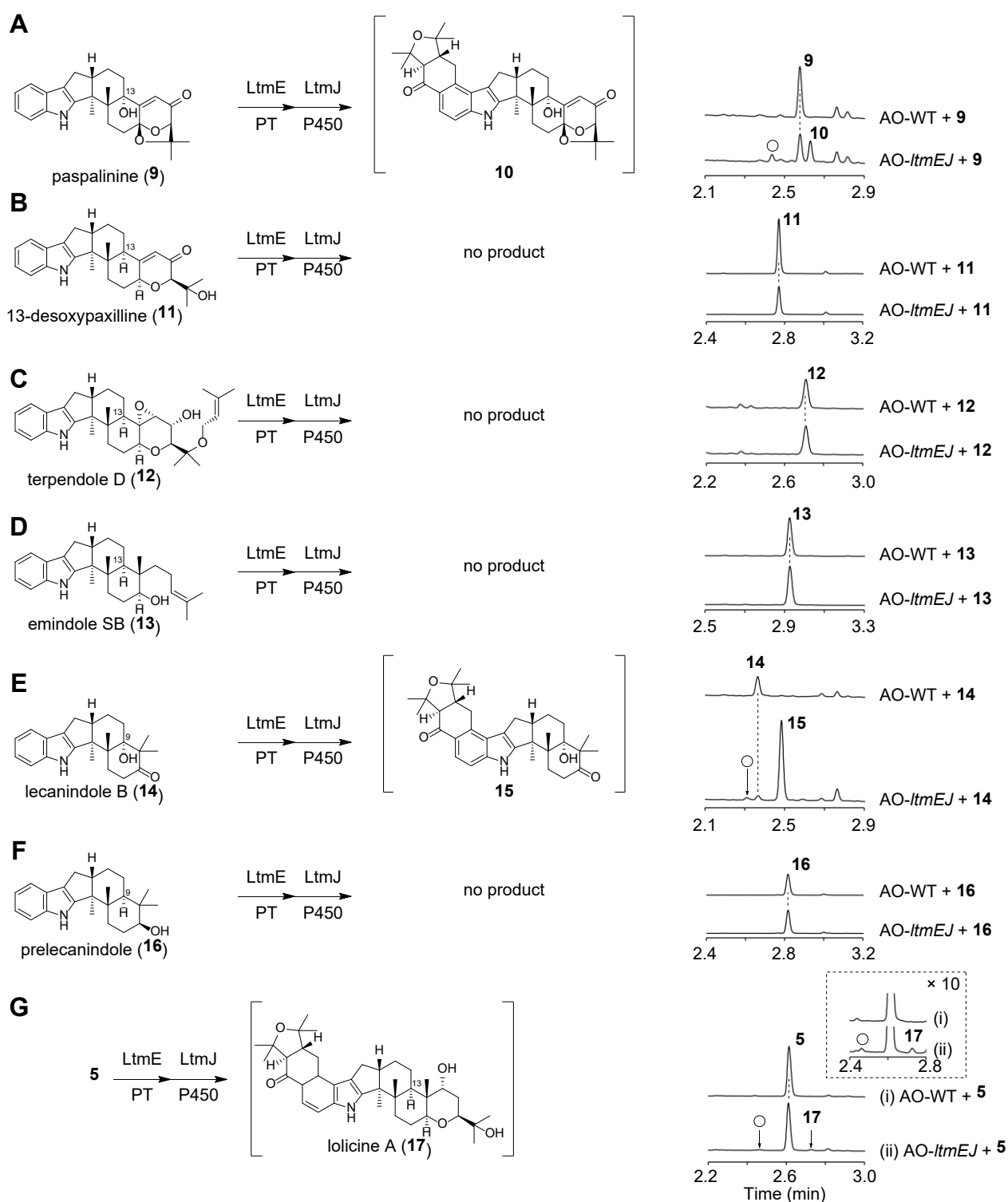
In addition, 20,21-diprenylterpendole I (**21**), obtained by the bioconversion of **6** by AO-*ltmE*, was also converted to **7** by AO-*ltmJ* (Figure S8, Table S2). These results unequivocally indicated that **18** and **19** are the intermediates of 3-step oxidation catalyzed by LtmJ (Figure 2B). Interestingly, **S4** was not converted to **8** or other related compounds (Figure S6), suggesting that the stereochemistry of epoxyalcohol is important for recognition by LtmJ.



**Scheme 1.** Proposed mechanism of A) LtmJ-catalyzed reaction and B) the related radical cyclization.

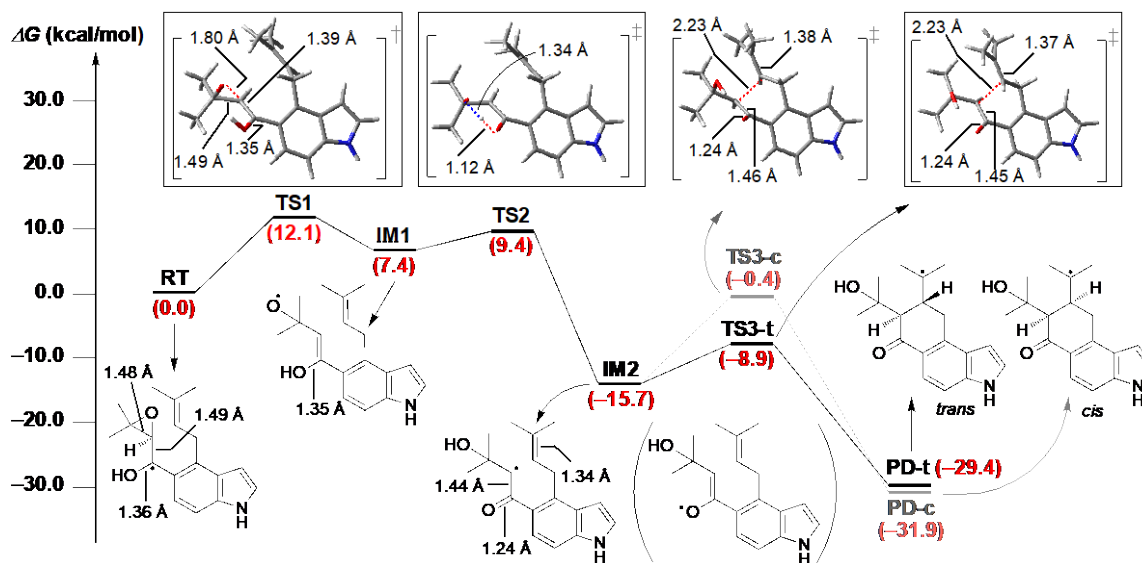
Epoxide opening-triggered cyclizations with epoxide hydrolases are often found in natural product biosynthesis.<sup>[18,19]</sup> However, the successful conversion of epoxyalcohol into a bicyclic ketone in bioconversion with AO-*ltmJ* strongly suggests that P450 monooxygenase LtmJ is solely responsible for the formation of a bicyclic system via epoxyalcohol. Therefore, we propose a mechanism of oxidative cyclization, shown in Scheme 1A. Based on previous studies that reported radical-induced epoxide ring opening,<sup>[20]</sup> we hypothesized that H30 would be abstracted by LtmJ to generate substrate radical **RT**, which would be rapidly converted to give alkoxy radical **IM1**.<sup>[21]</sup> Radical-induced epoxide opening was used for the isomerization of  $\alpha,\beta$ -epoxyketones to carbocycles (Scheme 1B).<sup>[22]</sup> A subsequent 1,5-H shift would give the isomeric alkoxy radical **IM2**,<sup>[23]</sup> which would undergo intramolecular addition to the double bond in a 6-*exo* manner. The resulting tertiary radical **PD** would undergo one electron oxidation to give a tertiary cation,<sup>[24–28]</sup> which would be trapped by the tert-hydroxy group, furnishing the A/B rings (Scheme 1A, path a). Alternatively, the tertiary radical would couple the oxy radical which would be generated by abstraction of second hydrogen from the hydroxy group (Scheme 1A, path b).

To examine the feasibility of this hypothesis, we next performed DFT calculations. The results of several examinations indicated that the most thermodynamically and kinetically favorable pathway for radical-induced 6-membered ring (B-ring) formation is the multistep reaction cascade shown in Figure 5, which is essentially the same framework as that described above: 1) all activation barriers are low enough for the reactions to proceed smoothly at ambient temperature (no step with a barrier greater than 20 kcal mol<sup>-1</sup>), 2) the first radical-induced epoxide ring-opening reaction is the rate-determining step with the highest energy barrier (12.1 kcal mol<sup>-1</sup>) in this cyclization cascade, and 3) the overall exothermicity is very large (nearly 30 kcal mol<sup>-1</sup>). The initial epoxide ring-opening reaction of **RT** via **TS1** is an endothermic process due to the instability of oxy radicals, affording an allylic alkoxy radical (**IM1**), which smoothly undergoes a 1,5-H shift via **TS2** with very low activation energy (2.0 kcal mol<sup>-1</sup>) to yield the more stable radical intermediate (**IM2**) with a large exothermicity. Judging by the elongated C–C bond distance (1.44 Å) and the shortened C–O bond distance (1.24 Å) of the enol moiety, we consider **IM2** to be an  $\alpha$ -ketoalkyl radical, in which the secondary carbon radical is stabilized by the resonance with an  $\alpha$ -carbonyl  $\pi$  bond rather than an enoyl (vinyloxy) radical structure (shown in parentheses in Figure 5). The next step branches into two possible routes to afford *cis*- and *trans*-1-tetralone derivatives (**PD-c** and **PD-t**). Our computational study suggested that the intramolecular *trans*-annulation of **IM2** is kinetically more favorable than the *cis*-pathway by 8.5 kcal/mol<sup>-1</sup>, in line with the experimental findings that a selective formation of *trans*-fused A/B rings with/without only a trace amount of *cis*-fused congeners. Essentially the same results were obtained when we used a model substrate **22** with a benzene core (Figure S9).

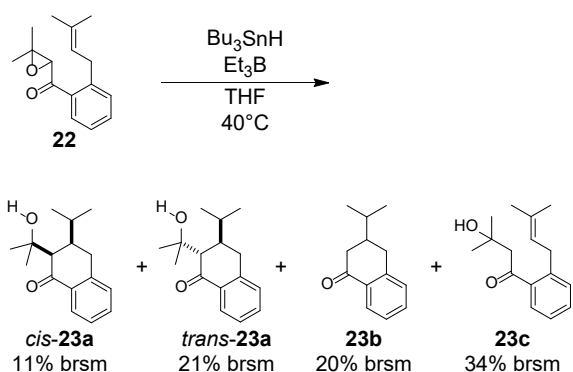


**Figure 4.** HPLC profiles of feeding experiments to AO-*ltmEJ* with **9** (A), **11** (B), **12** (C), **13** (D), **14** (E), **16** (F), and **5** (G). All chromatograms were extracted at 265 nm. White circles indicate putative intermediates with epoxyalcohol moiety.





**Figure 5.** Computed reaction pathway from RT to PD. Potential energies relative to RT are shown in parentheses ( $\text{kcal mol}^{-1}$ ; Gibbs free energies calculated at the UM06-2X/6-31G(d) level).



**Scheme 2.** A model experiment of conversion of **22** to **23a**. Reagents and conditions:  $\text{Bu}_3\text{SnH}$  (4 equiv),  $\text{Et}_3\text{B}$  (1 equiv), THF,  $40^\circ\text{C}$ , 1 h. *cis*-**23a** and *trans*-**23a** were obtained as inseparable mixtures with **22** and **23c**, respectively. The yields were calculated from the ratios of integration values of  $^1\text{H}$  NMR spectra of these mixtures and described based on the recovered starting material (brsm) (49% of **22** was recovered).

To validate the proposed cyclization mechanism of the 6-membered ring from epoxyalcohols **18** and **19**, we designed a model experiment using a simplified prenyl benzene (**22**) possessing epoxyketone at the ortho position, as it has been reported that reaction of epoxyketone with a tin radical induces ring opening of the epoxide via a ketyl radical, a proposed intermediate in the enzymatic reaction (Scheme 1B). After several experiments, we found that epoxyketone **22** was heated with  $\text{Bu}_3\text{SnH}$  in the presence of  $\text{Et}_3\text{B}$  at  $40^\circ\text{C}$  for 1 h to give the expected product **23a** along with **23b** and **23c** (Scheme 2). A careful separation and NMR analysis enabled us to determine the yield of the products as shown in Scheme 2. The relative stereochemistry of *trans*-**23a** was determined by extensive NMR

analysis of the alcohol obtained by stereoselective reduction of *trans*-**23a**<sup>[29]</sup>. Product **23b** was formed from **23a** by a retro aldol reaction, while product **23c** was formed by hydrogen abstraction of the enolate radical intermediate from  $\text{Bu}_3\text{SnH}$ . The conditions of low temperature and short reaction time despite the low conversion were due to suppression of the retro aldol reaction.<sup>[25]</sup> The formation of **23a** and **23b** thus strongly supports the proposed enzyme reaction mechanism.

## Conclusion

In this study, we achieved total biosynthesis of **3** and elucidated the mechanism of the construction of characteristic A/B rings. Bioconversion of various indole terpenes suggested that both LtmE and LtmJ show relaxed substrate specificities, which account for the diversity of the reported lolitremes. As a synthetic route for **5**, a key intermediate in the biosynthesis, has already been established,<sup>[30]</sup> other structurally related terpendoles can be synthesized in either natural or unnatural forms. Bioconversion of those synthetic analogs with AO-*LtmEJ* would be effective for the biosynthesis of lolitrem derivatives.<sup>[31]</sup>

In fungal metabolite biosynthesis, we often observe the conversion of secondary alcohol to ketone via a carbonyl radical, such as **RT**. An intriguing point of LtmJ-catalyzed oxidative cyclization is that **RT** did not end up forming ketone. Instead, rapid epoxide opening preceded O-rebound to give enol radical **IM1**. This branch point completely changed the LtmJ-catalyzed reaction pathway to one distinct from conventional P450-catalyzed reactions. Our proposed pathway is firmly supported by DFT calculations and chemical demonstration of the last oxidative cyclization. Our findings add a radical-induced mechanism to the

repertoire of biosynthetic cascade cyclization, which is usually initiated by acid-catalyzed epoxide opening.

## Acknowledgements

This work was supported by JSPS KAKENHI (B) (No. 19H02891 (H.O.), No. 19H02896 (T.N.)), JSPS KAKENHI (S) (No. 17H06173 (M.U.)), JSPS Grant-in-Aid for Scientific Research on Innovative Areas (No. 16H06446 (A.M.), No. 19H04635 (T.O.), No. 17H05430 (M.U.), No. 17H06406 (T.N.), No. 19H04644 (J.M.)). Allotment of computational resource (Project G19012) from HOKUSAI BigWaterfall (RIKEN) is gratefully acknowledged. We are grateful to Dr. H. Osada and Dr. T. Motoyama (RIKEN) for the helpful discussion about terpendole biosynthesis and Prof. D. Takemoto (Nagoya University) for the genomic DNA sample of a lolitrem producer. We also thank S. Yamaguchi (Nagoya University) for the preparation of compound **22**.

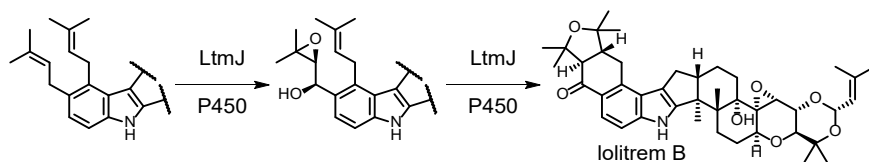
**Keywords:** biosynthesis • radical reactions • natural products • cytochrome P450 • lolitrem

- [1] A. Minami, C. Liu, H. Oikawa, *Heterocycles* **2016**, *92*, 397-421.
- [2] C. Liu, A. Minami, T. Ozaki, H. Oikawa in *Comprehensive Natural Products III: Chemistry and Biology*, (Eds.: T. P. Begley, H.-W. Liu), Elsevier Science, **2020**, vol. 2.
- [3] K. Tagami, C. Liu, A. Minami, M. Noike, T. Isaka, S. Fueki, Y. Shichijo, H. Toshima, K. Gomi, T. Dairi, et al., *J. Am. Chem. Soc.* **2013**, *135*, 1260-1263.
- [4] K. Tagami, A. Minami, R. Fujii, C. Liu, M. Tanaka, K. Gomi, T. Dairi, H. Oikawa, *ChemBioChem* **2015**, *16*, 2076-2080.
- [5] C. Liu, K. Tagami, A. Minami, T. Matsumoto, J. C. Frisvad, H. Suzuki, J. Ishikawa, K. Gomi, H. Oikawa, *Angew. Chem. Int. Ed.* **2015**, *54*, 5748-5752; *Angew. Chem.* **2015**, *127*, 5840-5844.
- [6] C. Liu, A. Minami, T. Dairi, K. Gomi, B. Scott, H. Oikawa, *Org. Lett.* **2016**, *18*, 5026-5029.
- [7] R. T. Gallagher, A. D. Hawkes, P. S. Steyn, R. Vleggaar, *J. Chem. Soc. Chem. Commun.* **1984**, 614-616.
- [8] R. T. Gallagher, E. P. White, P. H. Mortimer, *N. Z. Vet. J.* **1981**, *29*, 189-190.
- [9] C. A. Young, M. K. Bryant, M. J. Christensen, B. A. Tapper, G. T. Bryan, B. Scott, *Mol. Genet. Genomics* **2005**, *274*, 13-29.
- [10] C. A. Young, S. Felitti, K. Shields, G. Spangenberg, R. D. Johnson, G. T. Bryan, S. Saikia, B. Scott, *Fungal Genet. Biol.* **2006**, *43*, 679-693.
- [11] S. Saikia, D. Takemoto, B. A. Tapper, G. A. Lane, K. Fraser, B. Scott, *FEBS Lett.* **2012**, *586*, 2563-2569.
- [12] T. Motoyama, T. Hayashi, H. Hirota, M. Ueki, H. Osada, *Chem. Biol.* **2012**, *19*, 1611-1619.
- [13] C. Liu, A. Minami, T. Ozaki, J. Wu, H. Kawagishi, J. I. Maruyama, H. Oikawa, *J. Am. Chem. Soc.* **2019**, *141*, 15519-15523.
- [14] S. C. Munday-Finch, A. L. Wilkins, C. O. Miles, *J. Agric. Food Chem.* **1998**, *46*, 590-598.
- [15] S. C. Munday-Finch, A. L. Wilkins, C. O. Miles, H. Tomoda, S. Omura, *J. Agric. Food Chem.* **1997**, *45*, 199-204.
- [16] M. Adachi, K. Higuchi, N. Thasana, H. Yamada, T. Nishikawa, *Org. Lett.* **2012**, *14*, 114-117.
- [17] K. Kudo, C. Liu, T. Matsumoto, A. Minami, T. Ozaki, H. Toshima, K. Gomi, H. Oikawa, *ChemBioChem* **2018**, *19*, 1492-1497.
- [18] A. Minami, H. Oguri, K. Watanabe, H. Oikawa, *Curr. Opin. Chem. Biol.* **2013**, *17*, 555-561.
- [19] J. J. Fernández, M. L. Souto, M. Norte, *Nat. Prod. Rep.* **2000**, *17*, 235-246.
- [20] P. Dowd, W. Zhang, *Chem. Rev.* **1993**, *93*, 2091-2115.
- [21] D. H. R. Barton, R. S. H. Motherwell, W. B. Motherwell, *J. Chem. Soc. Perkin Trans. 1* **1981**, 2363-2367.
- [22] V. H. Rawal, V. Krishnamurthy, A. Fabre, *Tetrahedron Lett.* **1993**, *34*, 2899-2902.
- [23] S. Kim, J. S. Koh, *J. Chem. Soc. Chem. Commun.* **1992**, 1377-1378.
- [24] D. D. M. Wayner, D. J. McPhee, D. Griller, *J. Am. Chem. Soc.* **1988**, *110*, 132-137.
- [25] D. Zhu, M.-J. Seo, H. Ikeda, D. E. Cane, *J. Am. Chem. Soc.* **2011**, *133*, 2128-2131.
- [26] Y.-H. Chooi, Y. J. Hong, R. A. Cacho, D. J. Tantillo, Y. Tang, *J. Am. Chem. Soc.* **2014**, *136*, 4426-4436.
- [27] H.-C. Lin, Y. Tsunematsu, S. Dhingra, W. Xu, M. Fukutomi, Y.-H. Chooi, D. E. Cane, A. M. Calvo, J. K. Watanabe, Y. Tang, *J. Am. Chem. Soc.* **2013**, *135*, 16805-16808.
- [28] A. Tazawa, Y. Ye, T. Ozaki, C. Liu, Y. Ogasawara, T. Dairi, Y. Higuchi, N. Kato, K. Gomi, A. Minami, H. Oikawa, *Org. Lett.* **2018**, *20*, 6178-6182.
- [29] *cis-23a* was isolated as the corresponding alcohol by reduction of the mixture with NaBH<sub>4</sub> in MeOH. *trans-23a* was isolated from the mixture by exclusive reduction of **23c** with NaBH(OAc)<sub>3</sub> in THF. The details are given in ESI.
- [30] T. Teranishi, T. Murokawa, M. Enomoto, S. Kuwahara, *Biosci. Biotechnol. Biochem.* **2015**, *79*, 11-15.
- [31] R. Tanifuji, A. Minami, H. Oguri, H. Oikawa, *Nat. Prod. Rep.* **2020**, DOI 10.1039/C9NP00073A.



## Entry for the Table of Contents

Insert graphic for Table of Contents here. ((Please ensure your graphic is in **one** of following formats))



Lolitrem B is a tremorgenic indole diterpene produced by a fungal endophyte. During its biosynthesis, LtmJ, a P450 monooxygenase, catalyzes a 3-step oxidation of a diprenylated intermediate to construct the 5/6 bicyclic system. Given the reaction intermediate with an epoxyalcohol, we establish the mechanism of radical-induced cyclization, supported by density functional theory calculations and a model experiment.

Institute and/or researcher Twitter usernames: ((optional))