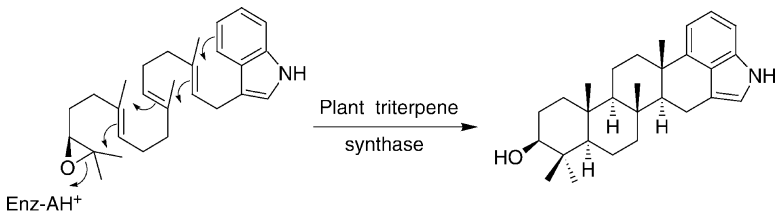
Communication

Enzymatic Synthesis of an Indole Diterpene by an Oxidosqualene Cyclase: Mechanistic, Biosynthetic, and Phylogenetic Implications

Quanbo Xiong, Xiuwen Zhu, William K. Wilson, A. Ganesan, and Seiichi P. T. Matsuda

J. Am. Chem. Soc., **2003**, 125 (30), 9002-9003• DOI: 10.1021/ja036322v • Publication Date (Web): 08 July 2003



ω-Oxidogeranylgeranylindole — Indole diterpene

More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 1 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 07/08/2003

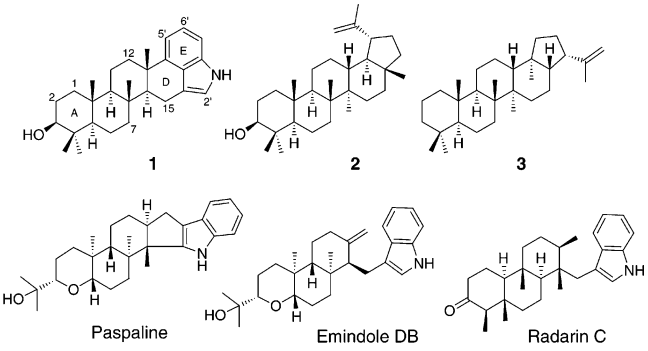
Enzymatic Synthesis of an Indole Diterpene by an Oxidosqualene Cyclase: Mechanistic, Biosynthetic, and Phylogenetic Implications

Quanbo Xiong,[†] Xiuwen Zhu,[§] William K. Wilson,[†] A. Ganesan,*[,§] and Seiichi P. T. Matsuda*[,†,||]

Department of Chemistry and Department of Biochemistry and Cell Biology, Rice University, Houston, Texas 77005, and Department of Chemistry, University of Southampton, Southampton SO17 1BJ, UK

Received May 24, 2003; E-mail: matsuda@rice.edu; ganesan@soton.ac.uk

Indole diterpenes comprise a structurally diverse family of secondary fungal metabolites with potent biological properties.[1] The biosynthesis of indole diterpenes is believed to proceed by condensation of geranylgeranyl pyrophosphate with a tryptophan precursor to give 3-geranylgeranylindole, followed by epoxidation and polyene cyclization.[1a,2] In contrast to the conversion of 2,3-oxidosqualene to triterpenes,[3] most indole diterpenes, including paspalines and emindoles, are cyclized from a 6,7-epoxide and differ regio- and stereochemically from pentacyclic triterpenes.[1,2] A notable exception to this pattern was provided by the recent isolation of petromindole (**1**)[4] from the soil fungus *Petromyces muricatus*.[5] Orienting **1** in a steroidal perspective reveals the similarities between petromindole and pentacyclic triterpenes, e.g., lupeol (**2**) and hopene (**3**), which all share the same substituents in rings A and B and the same relative stereochemistry in rings A−D. These parallels suggested that petromindole formation may be catalyzed by a fungal enzyme closely related to plant triterpene synthases. Remarkably, we found that lupeol synthase can convert 3-(*ω*-oxidogeranylgeranyl)indole (**4**) to petromindole. Described herein are mechanistic, biosynthetic, and phylogenetic implications of this unusual cyclization.[6]



The putative precursor (**4**) of petromindole was synthesized by an efficient method for alkylating indole regioselectively at C-3.[7] To explore the enzymatic conversion of **4** to **1**, we considered the numerous cyclases that transform squalene or oxidosqualene[8] into approximately 100 different carbocyclic skeleton types.[9] Seeking a biosynthetically flexible enzyme available in an experimentally convenient system, we chose LUP1, a lupeol synthase from *Arabidopsis thaliana*. LUP1 has been heterologously expressed in yeast with high catalytic activity,[10] converting oxidosqualene to a mixture of lupeol, *β*-amyrin, germanicol, taraxasterol, *ψ*-taraxasterol, and lupane-3*β*,20-diol.[10b] In all six products, rings A and B have the same substitution pattern and relative stereochemistry as petromindole, yet the broad product profile suggests biosynthetic flexibility in the E-ring region of the LUP1 active site. This elasticity might accommodate the differences between **4** and oxidosqualene, notably the misplacement of the C-14 methyl, the modified locus for post-cyclization proton loss, and the presence of the indole system.

Racemic **4** was incubated[11] with freshly lysed cells of a yeast mutant expressing LUP1 but lacking lanosterol synthase.[12] The isolated crude product from this in vitro reaction was chromatographed on silica gel to give unreacted **4**, triterpenes, sterols, and a polar UV-active fraction corresponding in TLC mobility to petromindole. GC and GC−MS analysis of this polar material as the TMS derivative indicated a mixture of **1** and monocyclized products **5** and **6** in a 1:5:1 ratio. No other indole-containing species were detected by NMR or GC−MS. Petromindole was separated on reversed phase TLC from **5** and **6**, which were each isolated by reversed phase HPLC. Structures of **5** and **6** were established from GC−MS and 1D and 2D NMR spectra, which were compatible with data reported for the analogous monocyclic triterpenes, camelliol[13] and achilleol.[14] Compounds **5** and **6** appear to be products of incomplete cyclization that resulted from misfolding of **4**. Perhaps **5** and **6** arise partially by reverse annulation from **7**, which may resist further cyclization owing to the displaced C-14 methyl (see Supporting Information).

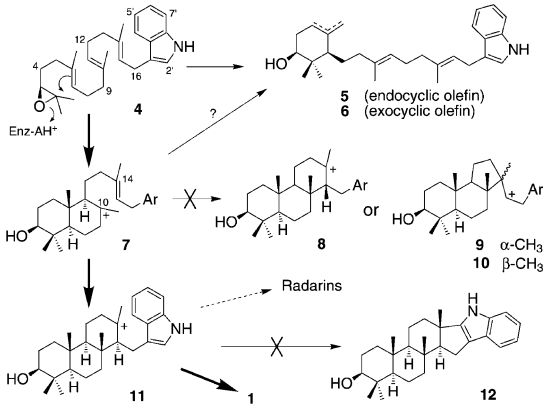
Petromindole from the LUP1 reaction was identified on the basis of the virtual identity of its GC−MS, ^{1}H NMR, and chiral HPLC behavior with that of an authentic standard. In chiral HPLC comparisons with a racemate isolated from a biomimetic cyclization of *N*-pivaloyl-**4**,[15] petromindole from the LUP1 incubation and the authentic standard coeluted with the less mobile enantiomer. This analysis showed the LUP1 product to be a single enantiomer and thus excluded the possibility of its formation by nonenzymatic cyclization. On the basis of the known stereospecificity of oxidosqualene cyclases,[16] these results established that the LUP1 product and thus also the authentic sample from *P. muricatus* have the 3*S* configuration.

The mechanism proposed below for cyclization of **4** to **1** underscores the fundamental biosynthetic differences between petromindole and most other indole diterpenes. Substrate **4** evidently undergoes protonation, epoxide ring opening, and chair−chair bicyclization to cation **7** by the same route observed in dammarenyl cation formation, whereas ring B of most indole diterpenes originates via cyclization of an internal epoxide without formation of a carbocyclic A ring.[1a,2] Ring C of petromindole is formed by annulation of C-10 onto the *re* face of the $\Delta^{14(15)}$ double bond as judged by the resulting *syn* stereochemistry of the C-10 and C-14 methyls. Molecular modeling (see Supporting Information) and biomimetic reactions of non-indole geranylgeraniol derivatives[17] indicate the Markovnikov product **11** to be strongly favored over **10**.[18] For most other indole diterpenes, ring C is formed by *si* addition to give intermediates resembling **8** or **9**.[1a,2] The endothermicity of *si* addition is apparently offset through coupling with an

[†] Department of Biochemistry and Cell Biology, Rice University.
[§] Department of Chemistry, University of Southampton.
[||] Department of Chemistry, Rice University.

10.1021/ja036322v CCC: $25.00

exothermic reaction, e.g., ring enlargement to a paspaline intermediate[1a,2a] or deprotonation to emindoles.[1a,19] Petromindole is apparently formed by cyclization of cation **11** to indole at C-4′. This regioselectivity corresponds to anticipated constraints of the LUP1 active-site cavity, as judged by the shape of lupeol. By contrast, we detected products resembling **12** in a biomimetic cyclization of *N*-pivaloyl-**4**,[20] and other indole diterpenes also arise from C-2′ annulation. Thus, petromindole biosynthesis differs markedly from that of other indole diterpenes in substrate folding, regioselectivity of annulation, and formation of rings A and B.



The foregoing mechanistic comparisons, together with the observed ability of lupeol synthase to catalyze the formation of **1**, support our hypothesis that the native petromindole synthase is a modified oxidosqualene cyclase distant from other indole diterpene synthases. However, one class of indole diterpenes, the radarins,[21] seems to share petromindole's biosynthetic pathway from substrate **4** to cation **11**, which then undergoes a backbone rearrangement instead of D-ring formation (see Supporting Information). Petromindole and radarins may not be the only examples of indole diterpenes derived from **4**, as only a small fraction of fungal metabolites have been described.[1c]

The putative petromindole synthase could have evolved from a fungal lanosterol synthase or, as we propose, a pentacyclic triterpene synthase. Nonsterol triterpenes are ubiquitous plant components but are found in some fungi.[22] Like LUP1 in our incubations, an adventitious fungal triterpene synthase[23] may have initially produced traces of **1** as an aberrant byproduct from **4**.[24] If **1** conferred a competitive advantage, selective pressures could have induced evolution of an efficient petromindole synthase. Although no indole diterpene synthases have yet been characterized, petromindole synthase should be identifiable by its postulated homology to oxidosqualene cyclases. Ongoing genome sequencing projects will likely accelerate progress in understanding the evolutionary relationships of the fungal cyclases.

Acknowledgment. We thank the Chemistry Department (University of Southampton) for a Ph.D. studentship and the Robert A. Welch Foundation (C-1323) and NSF (MCB-0209769) for funding. A sample of naturally isolated petromindole was generously provided by Professor Ken-ichi Kawai (Hoshi University, Japan). Geranylgeraniol was a kind gift of Takasago Chemical Co.

Supporting Information Available: Details of substrate synthesis, enzymatic and biomimetic cyclization, molecular modeling, NMR signal assignments, and GC−MS and NMR spectra of **1**, **5**, and **6** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Indole diterpenes differ structurally in the number of rings, nature of the carbocyclic skeleton, degree of oxygenation, and extent of indole substitution. Reviews: (a) Structural types and biosynthesis: Nozawa, K. *Proc. Jpn. Assoc. Mycotoxicol.* **1993**, *37*, 17−21. (b) Tremorgenic mycotoxins: Steyn, P. S.; Vleggaar, R. *Fortschr. Chem. Org. Naturst.* **1985**, *48*, 1−80. (c) Antiinsectan fungal metabolites: Gloer, J. B. *Acc. Chem. Res.* **1995**, *28*, 343−350.

(2) Isotopic labeling studies of the cyclization mechanism: (a) Acklin, W.; Weibel, F.; Arigoni, D. *Chimia* **1977**, 63. (b) Laws, I.; Mantle, P. G. *J. Gen. Microbiol.* **1989**, *135*, 2679−2692. (c) Byrne, K. M.; Smith, S. K.; Ondeyka, J. G. *J. Am. Chem. Soc.* **2002**, *124*, 7055−7060.

(3) (a) Wendt, K. U.; Schulz, G. E.; Corey, E. J.; Liu, D. R. *Angew. Chem., Int. Ed.* **2000**, *39*, 2812−2833. (b) Schulz-Gasch, T.; Stahl, M. *J. Comput. Chem.* **2003**, *24*, 741−753.

(4) Ooike, M.; Nozawa, K.; Udagawa, S.-I.; Kawai, K.-I. *Chem. Pharm. Bull.* **1997**, *45*, 1694−1696.

(5) Udagawa, S.; Uchiyama. S.; Kamiya, S. *Mycotaxon* **1994**, *52*, 207−214.

(6) Onocerin appears to represent another case of enzymatic cyclization of a linear isoprenoid bearing a terminal epoxide and a cyclic substituent: Rowan, M. G.; Dean, P. D. G. *Phytochemistry* **1972**, *11*, 3111−3118.

(7) Zhu, X.; Ganesan, A. *J. Org. Chem.* **2002**, *67*, 2705−2708.

(8) The presence of the 3β-hydroxyl in **1** and the need for enzymatic deprotonation at C-4′ (near the deprotonation site for lupeol) suggested an oxidosqualene cyclase rather than a squalene−hopene cyclase.

(9) Matsuda, S. P. T. In *Biochemical Principles and Mechanisms of Biosynthesis and Biodegradation of Polymers*; Steinbüchel, A., Ed.; Wiley-VCH: Weinheim, 1998; pp 300−307.

(10) (a) Herrera, J. B.; Bartel, B.; Wilson, W. K.; Matsuda, S. P. T. *Phytochemistry* **1998**, *49*, 1905−1911. (b) Segura, M. J.; Meyer, M. M.; Matsuda, S. P. T. *Org. Lett.* **2000**, *2*, 2257−2259.

(11) Cultures of yeast expressing LUP1 were centrifuged, and the cell pellet was suspended in 0.1 M sodium phosphate buffer (pH 6.2) and lysed in a French press. To the resulting 40% slurry (260 mL) was added a solution of racemic **4** (40 mg in 20 mL of 4% Tween 80). After 24-h incubation at 23 °C, the reaction was terminated by addition of ethanol (560 mL). Denaturated protein was removed by centrifugation, and the supernatant was concentrated in vacuo and extracted with ethyl ether. The ether extract was evaporated to a residue representing the crude product. Additional details are given in Supporting Information.

(12) Deletion of lanosterol synthase reduced experimental complexity by precluding its potential cyclization of **4** as well as formation of biosynthetic precursors of ergosterol.

(13) Barrero, A. F.; Alvarez-Manzaneda Roldan, E. J.; Alvarez-Manzaneda Roldan, R. *Tetrahedron Lett.* **1989**, *30*, 3351−3352.

(14) Akihisa, T.; Arai, K.; Kimura, Y.; Koike, K.; Kokke, W. C. M. C.; Shibata, T.; Nikaido, T. *J. Nat. Prod.* **1999**, *62*, 265−268.

(15) Chiral HPLC analyses and isolation of racemic **1** from reaction of $AlCl_3$ with *N*-pivaloyl-**4** in CH_2Cl_2 are described in Supporting Information.

(16) Barton, D. H.; Jarman, T. R.; Watson, K. C.; Widdowson, D. A.; Boar, R. B.; Damps, K. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1134−1138.

(17) (a) van Tamelen, E. E.; Marson, S. A. *Bioorg. Chem.* **1982**, *11*, 219−249. (b) Kulcitki, V.; Ungur, N.; Vlad, P. F. *Tetrahedron* **1998**, *54*, 11925−11934. (c) Heinemann, C.; Demuth, M. *J. Am. Chem. Soc.* **1999**, *121*, 4894−4895. (d) Johnson, W. S.; Telfer, S. J.; Cheng, S.; Schubert, U. *J. Am. Chem. Soc.* **1987**, *109*, 2517−2518. The outcome may be affected by the presence of electron-withdrawing groups, at least in the case of free-radical cyclizations (ref. (c)).

(18) The observed petromindole formation with LUP1 is notable in view of a recent report suggesting that replacement of the central 1,6 methyl substitution pattern of squalene by a 1,5 pattern (reflecting the difference between head-to-head and head-to-tail condensation of isoprene units) may block cyclization by a triterpene synthase: (a) Abe, I.; Tanaka, H.; Noguchi, H. *J. Am. Chem. Soc.* **2002**, *124*, 14514−14515. However, the 1,5 methyl arrangement does not adversely affect the energetics of nonenzymatic cyclization, as judged by biomimetic reactions of ω-oxidogeranylgeraniol derivatives, whose tricyclic products resemble pentacyclic triterpenes more than typical indole diterpenes.[17]

(19) Although most indole diterpenes are formed via skeletal rearrangement of a five-membered C ring, emindoles may be synthesized via Markovnikov C-ring cyclization: Nozawa, K.; Yuyama, M.; Shoichi, N.; Kawai, K.-I.; Udagawa, S.-I. *J. Chem. Soc., Perkin Trans. 1* **1988**, 2155−2160. A biomimetic synthesis of emindole SA supports this proposal: Rainier, J. D.; Smith, A. B., III. *Tetrahedron Lett.* **2000**, *41*, 9419−9423.

(20) In view of the extensive enzymatic guidance needed for the regio- and stereospecific cyclization of **4** to **1**, petromindole and its analogues may be more easily prepared by enzymatic reactions than biomimetic synthesis.

(21) Laakso, J. A.; Gloer, J. B.; Wicklow, D. T.; Dowd, P. F. *J. Org. Chem.* **1992**, *57*, 138−141.

(22) For example, the filamentous fungus *Aspergillus nidulans* synthesizes β-amyrin: Gealt, M. A. *J. Gen Microbiol.* **1983**, *129*, 543−546.

(23) Triterpene synthases evolved after the divergence of fungi and plants but may have been incorporated into fungal parasites from their plant hosts by horizontal gene transfer. General review: Katz, L. A. *Int. J. Syst. Evol. Microbiol.* **2002**, *52*, 1893−1900.

(24) Substrate **4** could have arisen from unnatural ω-epoxidation of geranylgeranylindole, the fungal precursor of indole diterpenes. The absence of any reported monocyclized indole diterpenes similar to **5** and **6** suggests that **1** is not simply an aberrant metabolite in *P. muricatus*.

JA036322V