DFT Study on the Biosynthesis of Preasperterpenoid A: Role of Secondary Carbocations in the Carbocation Cascade

Hajime Sato,*a,b Mami Yamazaki,a and Masanobu Uchiyama*,b,c,d

*a Graduate School of Pharmaceutical Sciences, Chiba University; 1–8–1 Inohana, Chuo-ku, Chiba 260–8675, Japan: 
*b Cluster of Pioneering Research (CPR), Advanced Elements Chemistry Laboratory, RIKEN; 2–1 Hirosawa, Wako, Saitama 351–0198, Japan: 
*c Graduate School of Pharmaceutical Sciences, The University of Tokyo; 7–3–1 Hongo, Bunkyo-ku, Tokyo 113–0033, Japan: and 
*d Research Initiative for Supra-Materials (RISM), Shinshu University; 3–15–1 Tokida, Ueda, Nagano 386–8567, Japan.

Received January 14, 2020; accepted February 22, 2020

Preasperterpenoid A, featuring a 5/7/(3)6/5 pentacyclic structure, is a C25 sesterterpenoid produced by Penicillium verruculosum. The results of density functional calculations on putative biosynthetic carbocation cyclization/rearrangements leading to preasperterpenoid A revealed a highly concerted four-step cyclization mechanism. Interestingly, two secondary carbocation structures were obtained as minima, but appeared almost as shoulders in the energy profile, and may represent essentially transient structures during the highly concerted reaction.

Key words terpene; biosynthesis; density functional theory; secondary carbocation

Introduction

Terpene/terpenoid natural products exhibit a wide range of chemical structures and biological activities, and are of interest in pharmaceutical, agrochemical, and material sciences. Their structural diversity and complexity are generated in nature via enzyme-initiated tandem carbocationic cascade reactions.1,2) Recently, computational chemistry has been extensively used for mechanistic investigations of their biosynthesis, because terpene synthases catalyze domino-type multiple reactions in their active sites, and isolation of intermediates is extremely challenging. Our previous quantum-chemical studies on terpene-forming carbocation rearrangements3–5) have shown that, in most cases, terpene cyclization proceeds via a sophisticated multi-step carbocationic cascade and the pathways to major enzymatic products involve stable carbocations such as allyl cation, tertiary (3°) carbocation, and cyclopropylcarbinyl cation as intermediates. On the other hand, secondary carbocation intermediates have been proposed in some cases, but whether or not these cations are actually viable intermediates is uncertain. In this study, we report density functional theory (DFT) calculations on sesterterpene-forming carbocation cascades in the preasperterpenoid A biosynthetic pathway. The primary focus of the present study is to investigate the feasibility of the putative involvement of secondary (2°) carbocations III and V in the biosynthetic pathway (Fig. 1).

Preasperterpenoid A is produced by Penicillium verruculosum preasperterpenoid A synthase (PvPS),6–8) which belongs to the different clade from other sesterterpene synthases (STS),9) such as NfSS,3,4) EvQS,10) AtTPS30,11) and Br580.12) PvPS synthesizes preasperterpenoid A as a single product with the characteristic 5/7/(3)6/5 pentacyclic structure (PD), containing five chiral carbons with the appropriate C–C bond connectivity and stereochemistry by cyclizing the achiral and acyclic C25 substrate (SM), geranylgeranylpyrophosphate (GFPP). Thus, it is of considerable interest to unveil the biosynthetic pathway/mechanism leading to this chiral, congested, polycyclic and structurally complex molecule.

Fig. 1. Proposed Biosynthetic Pathway of Preasperterpenoid A (Taken from Ref. 6)

*To whom correspondence should be addressed. e-mail: hajime.sato@chiba-u.jp; uchiyama@mol.f.u-tokyo.ac.jp

© 2020 The Pharmaceutical Society of Japan
Results and Discussion

Density functional theory (DFT) calculations were employed herein to comprehensively evaluate the proposed preasperterpenoid A biosynthetic pathway, starting from IM1. The full reaction pathway for the conversion of GFPP (SM) into IM7, and the energy diagram (relative energies with respect to IM1) are shown in Figs. 2 and 3, respectively. Since the stereochemistry at C3 of IM7 is experimentally inaccessible, we computed two possible pathways: path α (H3α at IM7) and path β (H3β at IM7). The energy diagram (Fig. 2) immediately suggests that both pathways to preasperterpenoid A show similar energy profiles and are thermodynamically and kinetically favored (all energy barriers are less than 10 kcal mol\(^{-1}\) and the overall exothermicity is very large at ca. 40 kcal mol\(^{-1}\)). However, overall, path α is energetically more favorable than path β.

Our calculation results provide several new insights into the complicated ring formation/skeletal reaction processes and conformational changes (the Cartesian coordinates of the 3D structures of all species are given in the supporting information). We will hereafter focus on path α. Enzyme-initiated dissociation of diphosphate from GFPP (SM) affords an allylic carbocation (IM1) that is partially stabilized by a cation–π interaction with the C10–C11 double bond. Cation-mediated double-annulation proceeds smoothly to form a 5/11 bicyclic tertiary carbocation intermediate (IM2). This is similar to other terpene cyclization cascades.\(^5,13\) Then, 1,2-alkyl shift proceeds with an activation energy of +6.5 kcal mol\(^{-1}\) to give a 6-membered-ring secondary carbocation intermediate (IM3) with +4.9 kcal mol\(^{-1}\) endothermicity. This energy loss is a result of the intrinsically unstable secondary carbocation and of the rather small energy gain to
form a through-space interaction with the distal C18–C19 double bond (21.4 kcal mol\(^{-1}\) based on Natural Bond Orbital (NBO) analysis). However, the unfavorable energy loss of the ring-expansion reaction is compensated by the successive cation-mediated annulation with a very small activation energy (+0.9 kcal mol\(^{-1}\)) leading to 5/6/11 tricyclic tertiary carbocation intermediate (IM4) with large exothermicity (−9.7 kcal mol\(^{-1}\)). Subsequently, 1,5-hydrogen shift takes place to give the hydrogen-bridged cycloalkonium intermediate (IM5), which is immediately converted to a relatively stable secondary carbocation intermediate (IM6). Although IM6 is a homoallyl cation, the p-orbital at carbocation center is not aligned with the π system of the C6–C7 double bond, but rather is aligned with the C10–C14 bond, leading a strong hyperconjugative interaction (1.64 Å), which is consistent with previous work.\(^{14}\) Then, asynchronous concerted annulation hyperconjugative interaction (1.64 Å), which is consistent with previous experimental findings. In this context, it should be noted that B3LYP tends to yield more minima for reactions that are concerted with other levels of theory.\(^{15}\) We therefore think these reactions can be regarded as essentially concerted reactions, and thus we can say that preasperterpenoid A is synthesized via four highly concerted steps.

**Conclusion**

The carbocation cyclization/rearrangement pathway from GFPP to preasperterpenoid A was delineated on the basis of DFT calculations, which indicate that the core framework of preasperterpenoid A is constructed via tandem carbocationic representations of all IM and TS.

**Experimental**

All calculations were carried out using the Gaussian 16 package.\(^{16}\) Structure optimizations were done with the B3LYP\(^{27}\) density functional theory method and the 6-31+G(d,p) basis set without any symmetry restrictions. Vibrational frequency calculations at the same level of theory as the optimization were performed to verify that each local minimum has no imaginary frequency and that each TS has only a single imaginary frequency. Intrinsic reaction coordinate (IRC) calculations\(^{18–21}\) for all TSs were performed with GRRM1\(^{22–26}\) based on Gaussian 16. Single-point energies were calculated at the mPW1PW91/6-31+G(d,p) level\(^{27}\) based on the structure optimized by the B3LYP method. Discussion is focused on relative Gibbs free energy energies (\(G_{\text{rel}}\)) based on single-point energy and frequency calculation at the mPW1PW91 level, since B3LYP underestimates the relative energies of cyclic structures versus acyclic structures.\(^{28,29}\) This level of theory has previously been validated for a wide variety of terpene-forming reactions.

**Acknowledgments**

This work was supported by JSPS KAKENHI (S) (No. 17H06173 (M.U.), JSPS Grant-in-Aid for Scientific Research on Innovative Areas (No. 17H05430 (M.U.), and JP16H06454 (M.Y.). Allotment of computational resource (Project G19I02) from HOKUSAI BigWaterfall (RIKEN) is gratefully acknowledged.

**Conflict of Interest**

The authors declare no conflict of interest.

**Supplementary Materials**

The online version of this article contains supplementary materials.

**References**

1. Christianson D. W., *Chem. Rev.*, **117**, 11570–11648 (2017).
2. Dickschat J. S., *Nat. Prod. Rep.*, **33**, 87–110 (2016).
3. Narita K., Sato H., Minami A., Kudo K., Gao L., Liu C., Ozaki T., Kodama M., Lei X., Taniguchi I., Monde K., Yamazaki M., Uchiyama M., Okawa H., *Org. Lett.*, **19**, 6696–6699 (2017).
4. Sato H., Narita K., Minami A., Yamazaki M., Wang C., Suemune H., Nagano S., Tomita T., Okawa H., Uchiyama M., *Sci. Rep.*, **8**, 2473–2481 (2018).
5. Sato H., Teramoto K., Masumoto Y., Terzuka N., Sakai K., Ueda S., Totsuka Y., Shinada T., Nishiyama M., Wang C., Kuzuyama T., Uchiyama M., *Sci. Rep.*, **5**, 18471–18476 (2015).
6. Huang X., Huang H., Li X., Sun X., Huang H., Lu Y., Lin Y., Long Y., She Z., *Org. Lett.*, **15**, 721–723 (2013).
7. Suzuki T., Rinkel J., Okada M., Abe I., Dickschat J. S., *Chem. Eur. J.*, **23**, 10053–10057 (2017).
8. Huang J.-H., Lv J.-M., Wang Q.-Z., Zou J., Lu Y.-J., Wang Q.-L., Chen D.-N., Yao X.-S., Gao H., Hu D., *Org. Biomol. Chem.*, **17**, 248–251 (2019).
9. Minami A., Ozaki T., Liu C., Okawa H., *Nat. Prod. Rep.*, **35**, 1330–1346 (2018).
10. Sato H., Mitsushita T., Yamazaki M., Abe I., Uchiyama M., *Angew. Chem. Int. Ed.*, **57**, 14752–14757 (2018).
11. Huang A. C., Koutsar S. A., Hong Y. J., Medema M. H., Bond A. D., Tantillo D. J., Osbourn A., *Proc. Natl. Acad. Sci. U.S.A.*, **114**, E6005–E6014 (2017).
12. Huang A. C., Hong Y. J., Bond A. D., Tantillo D. J., Osbourn A., *Angew. Chem. Int. Ed.*, **57**, 1291–1295 (2017).
13. Hong Y. J., Tantillo D. J., *Org. Biomol. Chem.*, **13**, 10273–10278 (2015).
14. Tantillo D. J., *Chem. Soc. Rev.*, **39**, 2847–2850 (2014).
15. Castiñeira Reis M., Lopez C. S., Nieto Faza O., Tantillo D. J., *Chem. Soc. Rev.*, **45**, 2159–2170 (2019).
B., Petrone A., Henderson T., Ranasinghe D., Zakrzewski V. G., Gao J., Rega N., Zheng G., Liang W., Hada M., Ehara M., Toyota K., Fukuda R., Hasegawa J., Ishida M., Nakajima T., Honda Y., Kitao O., Nakai H., Vreven T., Throssell K., Montgomery J. A., Jr., Peralta J. E., Ogliaro F., Bearpark M. J., Heyd J. J., Brothers E. N., Kudin K. N., Staroverov V. N., Keith T. A., Kobayashi R., Normand J., Raghavachari K., Rendell A. P., Burant J. C., Iyengar S. S., Tomasi J., Cossi M., Millam J. M., Klene M., Adamo C., Cammi R., Ochterski J. W., Martin R. L., Morokuma K., Farkas O., Reisman J. B., Fox D. J. Gaussian, Inc., Wallingford CT, 2016.

17) Becke A. D., J. Chem. Phys., 98, 5648–5652 (1993).
18) Fukui K., Acc. Chem. Res., 14, 363–368 (2002).
19) Page M., Doubleday C., McIver J. W. Jr., J. Chem. Phys., 93, 5634–5642 (1990).
20) Ishida K., Morokuma K., Komornicki A., J. Chem. Phys., 66, 2153–2156 (1977).
21) Schlegel H. B., Gonzalez C., J. Phys. Chem., 94, 5523–5527 (1990).
22) Maeda S., Osada Y., Morokuma K., Ohno K. GRRM 11, Version 11.03, 2012.
23) Maeda S., Ohno K., Morokuma K., Phys. Chem. Chem. Phys., 15, 3683–3701 (2013).
24) Ohno K., Maeda S., Chem. Phys. Lett., 384, 277–282 (2004).
25) Maeda S., Ohno K., J. Phys. Chem. A, 109, 5742–5753 (2005).
26) Ohno K., Maeda S., J. Phys. Chem. A, 110, 8933–8941 (2006).
27) Adamo C., Barone V., J. Chem. Phys., 108, 664–675 (1998).
28) Matsuda S. P. T., Wilson W. K., Xiong Q., Org. Biomol. Chem., 4, 530–543 (2006).
29) Hong Y. J., Tantillo D. J., J. Org. Chem., 83, 3780–3793 (2018).