

/ *Review*Theoretical Study of Natural Product Biosynthesis
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The biosynthetic pathways of natural products are complicated, and it is difficult to fully elucidate their details using experimental chemistry alone. In recent years, efforts have been made to elucidate the biosynthetic reaction mechanisms by combining computational and experimental methods. In this review, we will discuss the biosynthetic studies using computational chemistry for various terpene compounds such as cyclooctatin, sesterfisherol, quiannulatene, trichobrasilenol, asperterpenol, preasperterpenoid, spiroviolene, and mangicol.

Key words density functional theory, computational chemistry, biosynthesis, terpene

1. Introduction

Unraveling the intricate tapestry of natural product biosynthesis is one of the greatest challenges facing modern chemistry. Traditional experimental methods, while invaluable, often fall short in illuminating the labyrinthine pathways of chemical transformations. In response, the scientific vanguard has turned to the power of computational methods to pierce the veil of complexity surrounding biosynthetic reactions.¹⁾ This review will highlight the vanguard of our research, where we are applying the precision of computational chemistry to demystify the biosynthesis of terpene cyclases.

Terpenes, the true giants of natural products, boast a repertoire of over 80000 identified compounds.^{2,3)} At the forefront of exploring their reaction mechanisms, Tantillo *et al.* and their distinguished colleagues have spent some two decades using density functional theory (DFT) calculations to reveal that cation species exhibit a kaleidoscope of reactivities.^{4–7)}

Complementing these efforts, recent years have witnessed the advent of QM/MM studies, driven by luminaries such as Dan Thomas Major and Ruibo Wu, further expanding our understanding of these molecular marvels.^{8–10)}

In our first foray, we explored the structural intricacies of a diterpene known as cyclooctatin,¹¹⁾ a compound meticulously crafted by the organism *Streptomyces melanosporofaciens* MI614-43F2. The hallmark of this molecule is its unique 5/8/5 tricyclic skeleton, a marvel of natural architecture shown in Fig. 1. Our DFT calculations navigated with precision through the labyrinth of an 11-step sequential reaction, a digital choreography that maps the molecule's complex formation.^{12,13)} Crucially, we revealed that the pivotal moment in this biosynthetic dance, the anticipated carbon–carbon rearrangement is orchestrated by the delicate balance between the cyclopropyl carbanyl and homoallyl cations, an interaction now understood to be the linchpin of the entire pathway.

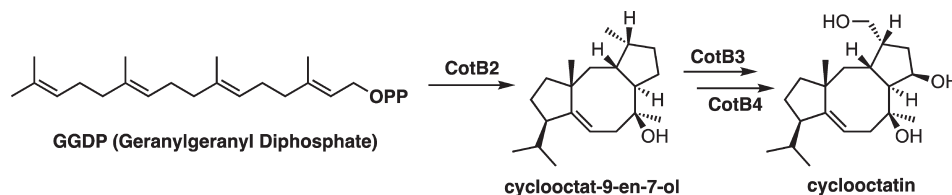


Fig. 1. Biosynthetic Pathway of Cyclooctatin

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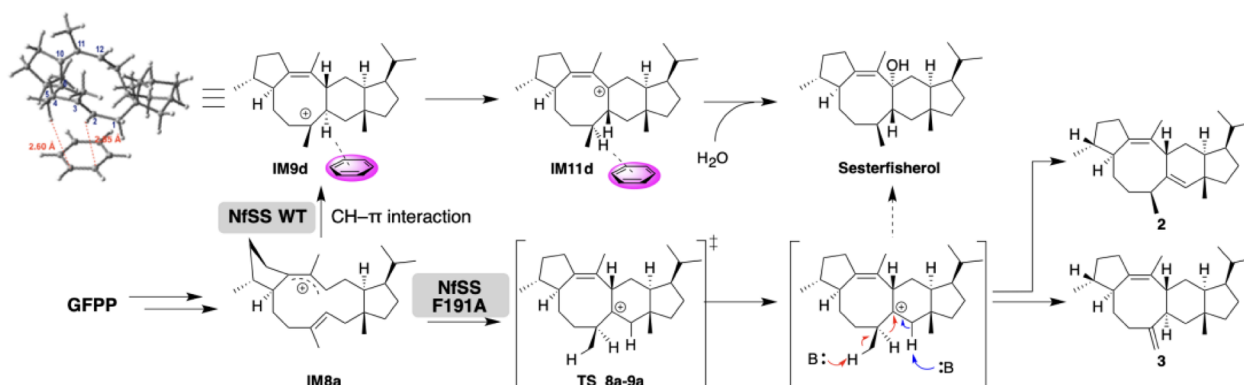


Fig. 2. The Late Stage of the Biosynthetic Pathway of Sesterfisherol

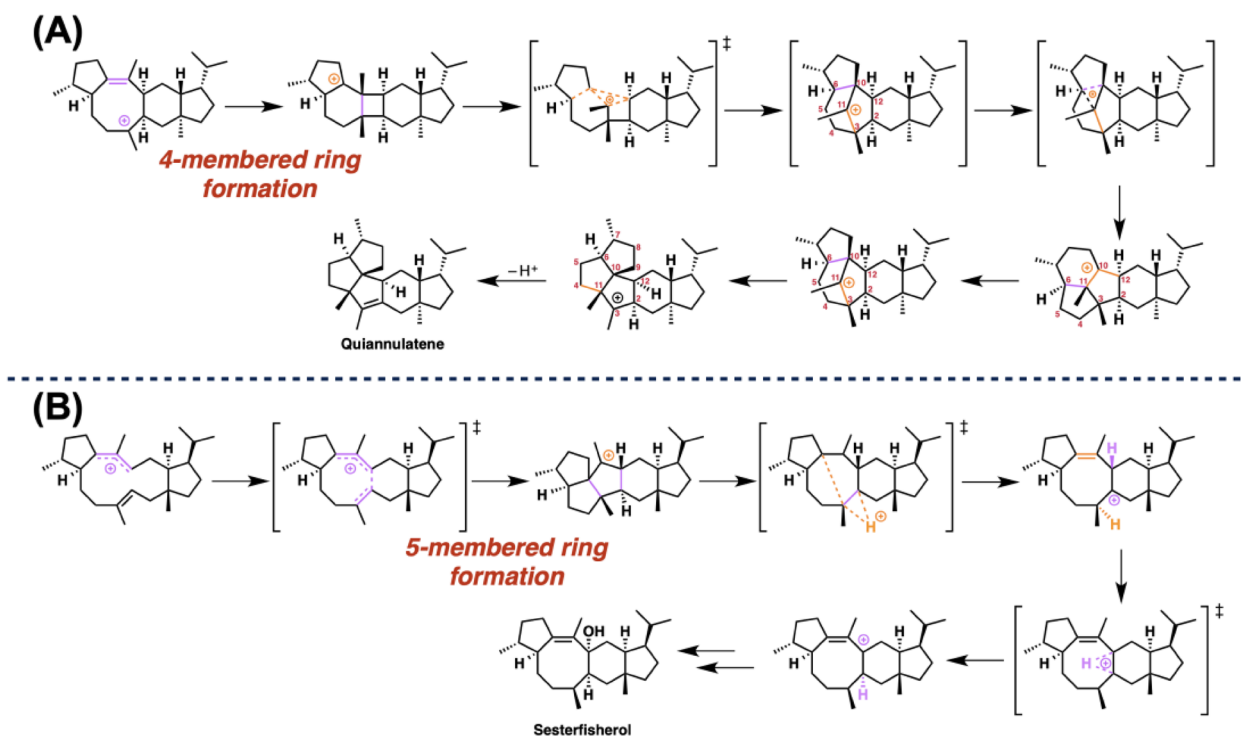


Fig. 3. The Biosynthetic Pathway of (A) Quiannulatene and (B) Sesterfisherol

As we proceeded, we encountered a twist in the story: the biosynthetic pathway revealed a previously unexplored course, marked by 1,2-H and 1,3-H shifts, that deviated from its previously proposed pathway. Rigorous experiments with labeled compounds confirmed our computational predictions as well as their veracity. This investigation not only underscored the power of computational chemistry as an indispensable ally

in biosynthetic research, but also demonstrated its ability to achieve harmonious concordance with empirical science.

Our scientific quest then led us to unravel the biosynthesis of sesterfisherol,¹⁴⁾ an exquisite sesterterpene molecule harvested from the intricate fungal networks of mold. The crux of our theozyme analysis revealed a fascinating molecular tango: the CH- π interaction within the enzyme's active site

Biography

Hajime Sato received his B.S. from the Department of Pharmaceutical Sciences, Tokyo University of Science in 2011, M.S. and Ph.D. from Graduate School of Pharmaceutical Sciences, The University of Tokyo, in 2013 and 2016, respectively. Since 2021, he has been an Assistant Professor at Graduate School of Medicine and Engineering, University of Yamanashi. During 2016–2020, he carried out postdoctoral research in the laboratory of Prof. Kazuki Saito and Prof. Mami Yamazaki at Chiba University. His research interest is the Automation of Chemistry.



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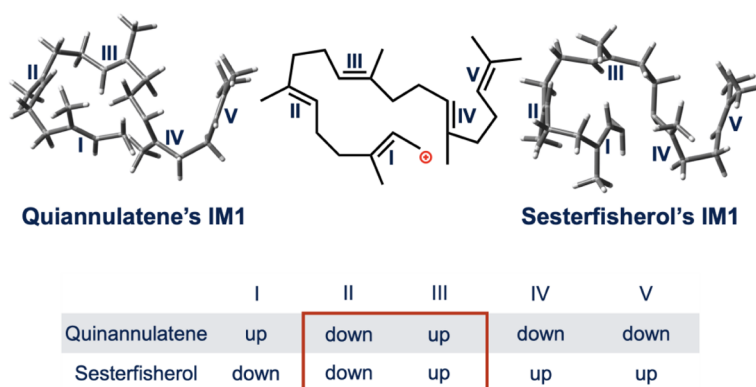


Fig. 4. 3D Representative of the Initial Conformation of Quiannulatene's IM1 and Sesterfisherol's IM1

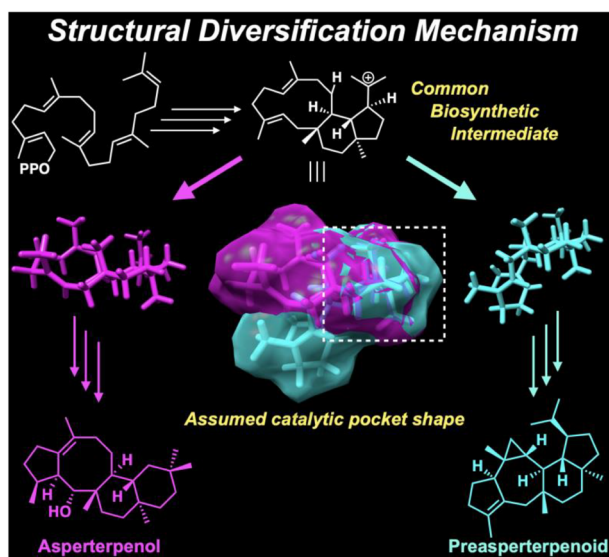


Fig. 5. 3D Representative of Common Intermediates in Asperterpenol and Preasperteronoid Biosynthesis

(Fig. 2), dancing closely with phenylalanine, gracefully side-steps an energetic dead end, guiding the reactants toward the formation of the desired sesterfisherol.¹⁵⁾ This atomic dance was observed to falter when we introduced a mutation at F191, confirming our hypothesis as sesterfisherol synthesis was impeded, lending credence to our computational foresight.

As our investigation deepened, a revelation emerged: in the absence of the central CH- π interaction, a triquinane scaffold was destined to form, an alternative molecular fate. This insight focused our attention on quiannulatene, a ses-terterpene with a triquinane core. Sketched in Fig. 3 is a tale of two fates from a common intermediate: quiannulatene¹⁶⁾ sees the emergence of a four-membered ring nested within an eight-membered one, while sesterfisherol witnesses the birth of a five-membered ring encapsulated by the same octagonal embrace. These molecular narratives underscore the finesse of enzymatic control, creating distinct architectures from a common canvas.

Comparing the three dimensional (3D) structures obtained by DFT calculations, it is clear that these differences in regioselectivity are due to the initial conformation of the substrates^{17,18)} (Fig. 4). Using the orientation of the Me group as a feature to easily represent the difference in initial conformation, it was found that the orientation of the Me group in

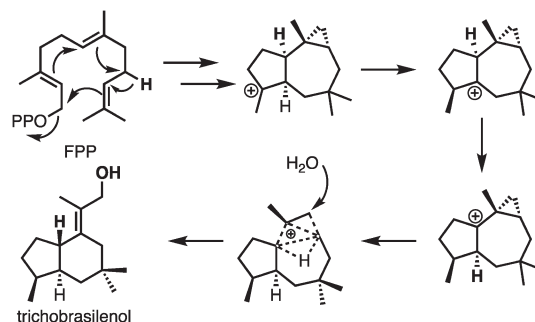


Fig. 6. Proposed Biosynthetic Pathway of Trichobrasilenol

II and III is the same, but the rest is opposite. These studies clearly indicate that terpene cyclases determine the conformation and regioselectivity of the intermediates and ultimately the structure of the product by appropriately fixing the initial conformation of the substrate.

Continuing on our exploratory path, we turned our attention to the biosynthetic divergence of asperterpenol and preasperterpenoid,¹⁹⁾ both descended from a common intermediate. Rigorous DFT calculations allowed us to dissect these pathways, revealing a fascinating dichotomy in structure.^{20,21)} The three-dimensional comparisons, shown in Fig. 5, reveal a tale of two halves: while the right segment of the intermediate scaffold retains its structure, the left segment unfolds into a markedly different conformation. This structural ballet is not merely aesthetic; in the biosynthesis of asperterpenol and preasperterpenoid, it is this common intermediate's conformational poise that controls the chemoselectivity of the cyclization reaction and orchestrates the precise formation of these complex molecular entities.

In addition to analyzing various terpene forming reactions, we also focused on non-classical cation species that occur during terpene cyclization.

Studies of trichobrasilenol²²⁾ biosynthesis focused on its reaction (Fig. 6). The putative reaction mechanism in previous studies predicted that up to six chemical events would occur simultaneously to cause the rearrangement reaction. We decided to analyze the rearrangement reaction in detail. We found that the secondary carbocation-like structure is actually a homoallyl cation and plays an important role.

Embarking on a molecular odyssey, we have charted the transformative journey of IM8b, initially a cyclopropylcarbiny cation²³⁾ (Fig. 7). This entity gracefully transitions to a homoallyl cation labeled TS6b-7a, along with the emergence of

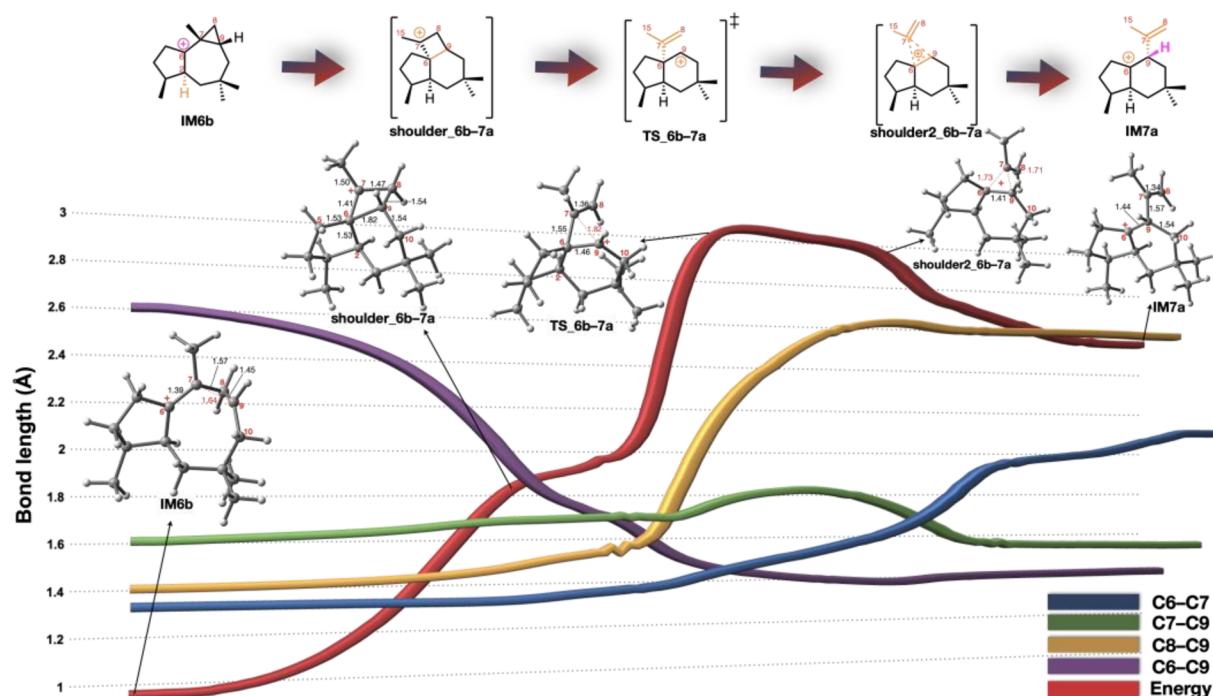


Fig. 7. Rearrangement Reaction in Trichobrasilenol Biosynthesis

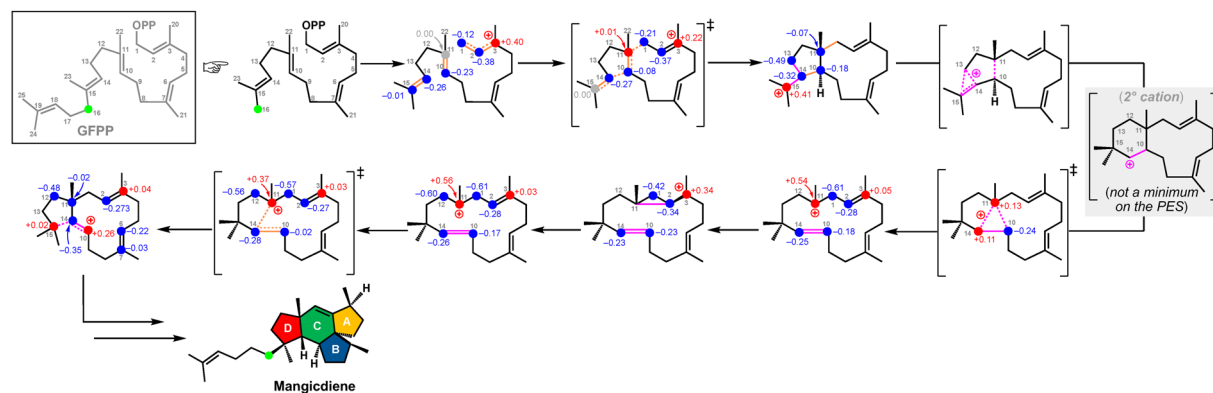


Fig. 8. The Late Stage of the Biosynthetic Pathway of Mangicol

a cyclobutyl cation. This intricate dance of bonds and charges seamlessly orchestrates the formation of the C7–C9 bond while simultaneously regenerating the cyclopropylcarbiny cation and introducing a new cationic state at C9. In a synchronized ballet, the C6–C7 bond is cleaved to form another homoallyl cation, IM7a. What appears to be a complex rearrangement is, in fact, a beautifully orchestrated equilibrium between the cyclopropylcarbiny cation and homoallyl cations a molecular equilibrium of profound simplicity and elegance.

Our exploration extended to the biosynthetic secrets of mangicol (Fig. 8), a masterpiece of natural synthesis.²⁴⁾ Here we encountered a seemingly unlikely scenario: the formation of two consecutive secondary cations a notion that defies conventional chemical wisdom. But our DFT calculations shed light on the mystery, revealing that the formation of a secondary cation paves the way for the cleavage of an adjacent C–C bond, its transformation into a more stable tertiary cation, and the emergence of a double-bond scaffold. This revelation sets the stage for a subsequent ring-closing event, which then gracefully transitions into a ring-expansion reaction.

This series of insightful analyses has revealed a profound truth: secondary carbocations, once thought to be mere chemical phantoms, fleeting and elusive, do in fact exist. These transient entities serve as key transition states that significantly influence the course and outcome of reactions, redefining our understanding of reactive intermediates in the symphony of chemical transformations.^{25–28)}

2. Conclusion

Through the meticulous application of DFT calculations, we successfully unraveled the intricate details of terpene cyclization reactions details that remained obscured by experimental methods alone. Our journey extended beyond terpene cyclization to the complex biosynthetic pathways of polyketides, including the redox reaction in anthocyanins,²⁹⁾ dimerization reaction in phomoidride³⁰⁾ and the anion-type rearrangement reaction in bisorbicillinoids,³¹⁾ as well as plant alkaloids such as the quinolizidine alkaloids.³²⁾ We have also shed light on the oxidation processes central to the synthesis of anditomin,³³⁾ shimalactone,³⁴⁾ lolitrems,³⁵⁾ and setosusin.³⁶⁾

This synergy of computational chemistry with experimental approaches has proven to be a formidable force in unlocking the secrets of natural product biosynthesis. Encouraged by our findings, we are at the forefront of exploration and will continue to use computational methods to decipher the biosynthetic codes of nature's myriad compounds.

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Conflict of Interest The author declares no conflict of interest.

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