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Effect of Isotopically Sensitive Branching on Product Distribution for Pentalenene Synthase: Support for a Mechanism Predicted by Quantum Chemistry

Liansuo Zu,[†,∥] Meimei Xu,[‡,∥] Michael W. Lodewyk,[†,∥] David E. Cane,*[,§] Reuben J. Peters,*[,‡] and Dean J. Tantillo*[,†]

[†]Department of Chemistry, University of California, Davis, California 95616, United States
[‡]Department of Biochemistry, Biophysics & Molecular Biology, Iowa State University, Ames, Iowa 50011, United States
[§]Department of Chemistry, Box H, Brown University, Providence, Rhode Island 02912-9108, United States

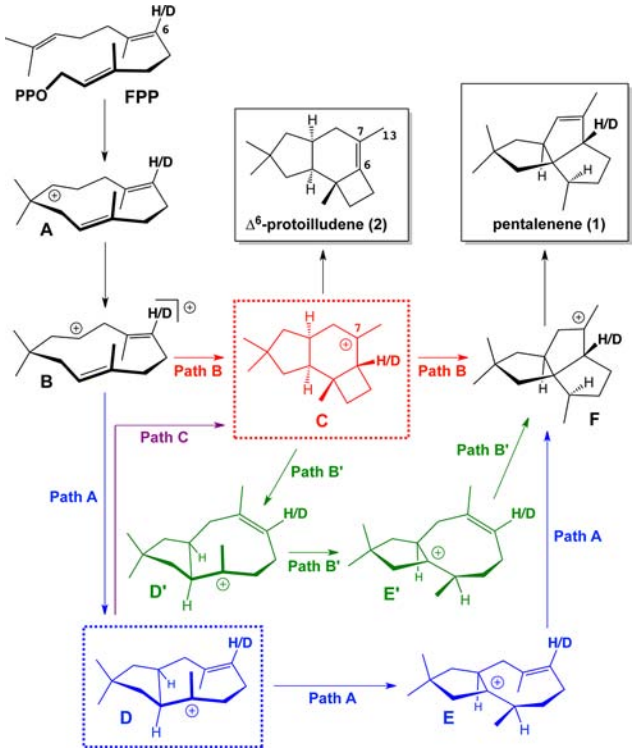
Supporting Information

ABSTRACT: Mechanistic proposals for the carbocation cascade reaction leading to the tricyclic sesquiterpene pentalenene are assessed in light of the results of isotopically sensitive branching experiments with the H309A mutant of pentalenene synthase. These experimental results support a mechanism for pentalenene formation involving a 7-protoilludyl cation whose intermediacy was first predicted using quantum-chemical calculations.

Pentalenene (**1**, Scheme 1) is a tricyclic sesquiterpene[1,2] that is produced in nature from farnesyl diphosphate (FPP) through a cationic cascade reaction promoted by the enzyme pentalenene synthase.[3] The mechanism of this transformation is one of the most highly studied among terpene-forming reactions,[3−5] in part because of the efficient generation of complexity that accompanies conversion of FPP—the universal acyclic, achiral precursor of all sesquiterpenes—into pentalenene, a tricyclic, chiral, stereodense product.

At least two mechanisms have been suggested for the formation of pentalenene from FPP. Path A in Scheme 1 (**A** → **B** → **D** → **E** → **F**) represents the earliest and until recently the most commonly accepted mechanistic proposal, involving conversion of the humulenyl cations **A** and **B** to a secoillud-6-en-3-yl cation (**D**) that then undergoes a 1,2-hydride shift and subsequent cyclization to produce the penultimate intermediate, the pentalenyl cation (**F**).[3,4] The basic details of this mechanism have been supported by a wide range of experiments with stereospecifically labeled FPP and determination of the precise position and stereochemistry of isotopic labeling in the enzymatically derived pentalenene product.[3,4] In 2006, Gutta and Tantillo proposed an alternative cyclization mechanism leading from **B** to **F** based on quantum-chemical calculations [mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G-(d,p) in the absence of the enzyme active site].[2d,5] In this mechanism, the 7-protoilludyl cation (**C**), formed directly from **B**, would be a mandatory intermediate along the pathway to pentalenene (path B in Scheme 1; **A** → **B** → **C** → **F**).[5] Although this mechanism invokes an unexpected intermediate (**C**) followed by an unusual dyotropic rearrangement (**C** → **F**),[6,7] it is completely consistent with all of the reported

Scheme 1. Three Mechanisms Proposed for the Formation of Pentalenene (1) and Δ^6-Protoilludene (2) from (*E*,*E*)-Farnesyl Diphosphate



mechanistic and stereochemical results on the pentalenene synthase reaction.[3,4] Moreover, the predicted intermediacy of protoilludyl cation **C** is also consistent with the previously reported formation of the corresponding deprotonation product, Δ^6-protoilludene (**2**), as a minor (10−13%) coproduct of pentalenene resulting from the cyclization of FPP by the four pentalenene synthase active-site mutants H309A, H309C, H309S, and H309F.[4d] It is also noteworthy that refluxing $\Delta^{7,13}$-protoilludene or either epimer of the 7-protoilludyl

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alcohol in formic acid gives pentalenene in up to 28% yield, consistent with the intermediacy of a species such as cation **C**.[8] Although the enzymatic generation of cation **C** by the pentalenene synthase mutants had previously been thought to result from *diversion* (path C) of the natural cyclization path A,[4d,e] the quantum-mechanical calculations would place the protoilludyl cation **C** directly on the natural cyclization path B. More recently, further quantum-mechanical calculations on other possible conformations of intermediate **C** revealed that **C** can be converted to **F** by an alternative stepwise rearrangement, illustrated as path B′ in Scheme 1 (**A** → **B** → **C** → **D**′ → **E**′ → **F**), in which **D**′ and **E**′ are geometric isomers of **D** and **E**, having *Z* rather than *E* C=C double bonds. In fact, path B′ is predicted to have a barrier of only ∼6 kcal/mol for the conversion of **C** to **F** from the lowest-energy conformer of **C** (at the mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G(d,p) level),[9] which is considerably lower than the barrier of nearly 20 kcal/mol for the direct dyotropic reaction.[5,7]

While these experimental observations and calculations are consistent with a 7-protoilludyl cation intermediate, they do not provide conclusive evidence as to whether such an intermediate is on the direct pathway to pentalenene (paths B and B′) or instead represents a diversion of the pentalenene pathway (path C). The production of both **1** and **2** by H309A pentalenene synthase does, however, provide us with an opportunity to distinguish path A from paths B and B′ using the well-established method of the isotopically sensitive branching experiment.[10,11] A key difference between these two mechanistic scenarios is the point at which each pathway diverges toward pentalenene and Δ^6-protoilludene. In the path A mechanism, the branch point for commitment to the formation of either the natural product pentalenene or the diversion product Δ^6-protoilludene would be cation **D**. In contrast, for both paths B and B′, the branch point is the protoilludyl cation **C** itself. We therefore envisaged that substitution of the C6 proton of FPP by deuterium in [6-^{2}H]FPP should have essentially no effect on the ratio of **1** to **2** if the cyclization mechanism proceeds through intermediate cation **D** via path A, whereas this substitution would result in an increase in the **1**:**2** ratio if either path B or B′ is followed because of a primary deuterium kinetic isotope effect (KIE) on the deprotonation of **C** to give **2**. The KIE suppressing the formation of Δ^6-protoilludene when [6-^{2}H]FPP is used as the substrate if path B or B′ is followed should result in an increased partition of the common cation intermediate **C** toward pentalenene, resulting in a net increase in the ratio of the final products **1** and **2**.

Incubation of FPP with the purified recombinant pentalenene synthase mutant H309A gave a 6.0:1 mixture of **1** (81%) and the coproduct **2** (13.4 ± 0.3%), accompanied by minor quantities (<6%) of germacrene A, detected as the derived Cope rearrangement product *β*-elemene as described previously, consistent with the results of previously reported incubations with H309 mutants.[4d,e] The assays were carried out in triplicate and analyzed by capillary GC−MS (Figure 1, top) with the identity of each product confirmed by comparison of both the electron-impact mass spectrum and retention index with standards in the MassFinder 4.0 database.[12] When [6-^{2}H]FPP was used as the substrate,[13] the distribution of sesquiterpene products was significantly shifted, with the intensity of the protoilludene peak being reduced to only 7.5 ± 0.4% of the total products while the intensity of the pentalenene peak *increased* to 87% (Figure 1, bottom). This nearly 2-fold increase in the **1**:**2** ratio (11.6 vs 6.0) as a result of

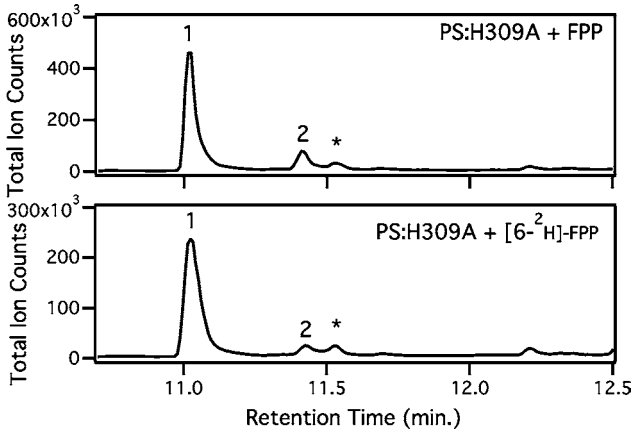

Figure 1. Effect of deuteration at C6 of FPP on the ratio of products generated by H309A pentalenene synthase. GC−MS chromatograms of reaction mixtures using (top) unlabeled FPP and (bottom) [6-^{2}H]FPP are shown. The peaks labeled * are due to *β*-elemene from Cope rearrangement of germacrene A.

isotopically sensitive branching establishes that the protoilludyl cation **C** is a common intermediate in the pathways for formation of **1** and **2**, *as required by either path B or B′ but inconsistent with formation of cation* **C** *as a diversion product of path A to pentalenene* (assuming that **C** and **D** do not rapidly interconvert, i.e., for path A, conversion of **D** to **C** is effectively irreversible).

The observed increase in the **1**:**2** ratio corresponds to a primary KIE of k_H/k_D = 1.9 on the deprotonation of cation **C** to yield **2**, consistent with previously measured k_H/k_D values for deprotonation of tertiary carbocations in terpene synthase-promoted reactions (typically ranging from 2−6).[11] Quantum-chemical calculations using $H_2PO_4^-$ as a model base predicted a k_H/k_D of 1.6−1.8.[14,15] The conversion of **C** to **F**, whether by path B or B′, would be expected to be subject to at most a small normal secondary KIE as C6 changes from sp^3 toward sp^2 hybridization in the transition-state structures for the **C** → **F**[5] and **C** → **D**′ reactions (these assumptions are supported by our quantum-chemical calculations[14]). In contrast, the diversion of cation **D**, formed by the previously postulated path A, to give the protoilludyl cation **C**, would be expected to be subject to only a minor secondary KIE (k_H/k_D slightly less than 1), since C6 would be changing from sp^2 toward sp^3 hybridization. Similarly, conversion of **D** to **E** along path A should have no significant KIE since H6 is not directly involved in this step. Path A for the cyclization of FPP to pentalenene by way of cation **D** is therefore excluded by the observation of a decrease in the proportion of **2** due to isotopically sensitive branching of the common intermediate **C**, whether **C** is further converted to **1** by path B or path B′. The mechanisms proposed on the basis of quantum-chemical calculations on the enzyme-free reaction mechanism are therefore fully consistent with the experimental results described herein. Further experimentation will be necessary to distinguish between the downstream paths B and B′.[16]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional details on computations, including the complete Gaussian citation, and description of enzyme assays and product identification. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

David_Cane@brown.edu; rjpeters@iastate.edu; djtantillo@ucdavis.edu

Author Contributions

[‖]L.Z., M.X., and M.W.L. contributed equally.

Notes

The authors declare no competing financial interest.

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