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The Taxadiene-Forming Carbocation Cascade

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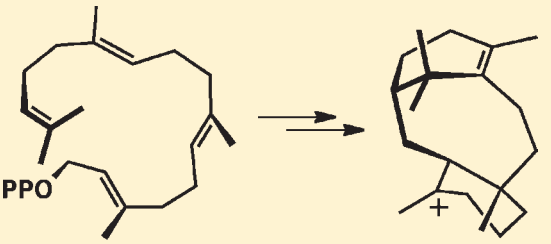
Supporting Information

ABSTRACT: A complete pathway (structures and energies of intermediates and transition state structures connecting them) from geranylgeranyl diphosphate to taxadiene, obtained using quantum chemical calculations, is described. This pathway is fully consistent with previous labeling experiments, despite differing in several subtle ways (in terms of conformations of certain carbocation intermediates and in the concertedness and synchronicity of certain bond-forming events) from previous mechanistic proposals. Also, on the basis of the theoretical results, it is proposed that the 2-fluoro-geranylgeranyl diphosphate substrate analogue in the recently reported X-ray crystal structure of taxadiene synthase is bound in a nonproductive orientation.



INTRODUCTION

The diterpene taxa-4(5),11(12)-diene (Scheme 1; heretofore referred to simply as taxadiene) is a biosynthetic precursor to the polycyclic diterpenoid Taxol (also known as paclitaxel), a natural product that has received substantial attention not only for its complex structure, but also for its anticancer activity.[1] Diterpenes such as taxadiene are derived from the acyclic precursor (*E*,*E*,*E*)-geranylgeranyl diphosphate (GGPP, Scheme 1) in diterpene synthase-promoted multistep rearrangement/cyclization reactions.[2,3] The mechanism generally proposed for formation of taxadiene is shown in Scheme 1.[4] On the basis of the results of stereochemical labeling experiments, it was proposed that the GGPP → **A** → **B** → **C** transformation is likely a concerted process.[5,6] Previous experimental and theoretical studies also led to the suggestion that the **C** → **D** conversion can occur without the intervention of an active site base that deprotonates **C** and then reprotonates the resulting C=C double bond, although this possibility has not been definitively excluded.[7−9] A two-step proton transfer sequence (**C** → **F** → **D**) has also been shown, on the basis of quantum chemical calculations, to be energetically viable.[8,10] Herein we describe the results of quantum chemical calculations on the complete **A** → **E** reaction, delineating a pathway that is consistent with the results of all previous mechanistic experiments of which we are aware.[11] This pathway differs somewhat from previously proposed mechanisms. In addition, the computationally derived structures are used to assess the mechanistic relevance of the recently reported X-ray crystal structure of taxadiene synthase.[12]

METHODS

All calculations were performed with *GAUSSIAN03*.[13] All structures were optimized using the B3LYP/6-31+G(d,p) method.[14] Previous studies have suggested that the B3LYP method performs reasonably well in predicting geometries and reactivity of carbocations, and results using this method have been compared with other density functional theory and non-density functional theory methods.[15,16] We also report mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G(d,p) and mPWB1K/6-31+G(d,p)//B3LYP/6-31+G(d,p) energies to account for known shortcomings of B3LYP (in terms of relative energies; B3LYP tends to overestimate the relative stabilities of acyclic isomers).[15−17] All stationary points were characterized by frequency calculations, and reported energies include zero-point energy corrections (unscaled) from the method used for geometry optimization. Intrinsic reaction coordinate (IRC) calculations were used for further characterization of all transition state structures,[18] and IRC plots are shown in the Supporting Information. In this study, only conformations of intermediates that are productive for the biosynthetic reaction steps of interest were examined. Structural drawings were produced using *Ball & Stick*.[19]

RESULTS AND DISCUSSION

In this study, we endeavored to answer the following three specific questions: (1) Can a GGPP-derived allylic cation with a conformation that is productive for cyclization be a discrete intermediate? (2) Which mechanistic steps shown for the carbocation rearrangement in Scheme 1, if any, are merged into concerted processes? (3) Is the GGPP analogue 2-fluoro-geranylgeranyl diphosphate (FGP), which is present in the recently disclosed crystal structure of taxadiene synthase,[12] truly representative of the actual bound substrate in terms of its conformation?

Question 1. To our delight (and our surprise, given the paucity of isoprenyl diphosphate-derived allylic cations that have been located as minima in previous theoretical studies[16,20,21]) we were able to locate allylic cation **A** as a discrete minimum in a conformation productive for cyclization (Figure 1). An intramolecular cation−π interaction[22] between the primary end of the allylic cation substructure (C1) and the C14=C15 π-bond appears to preorganize

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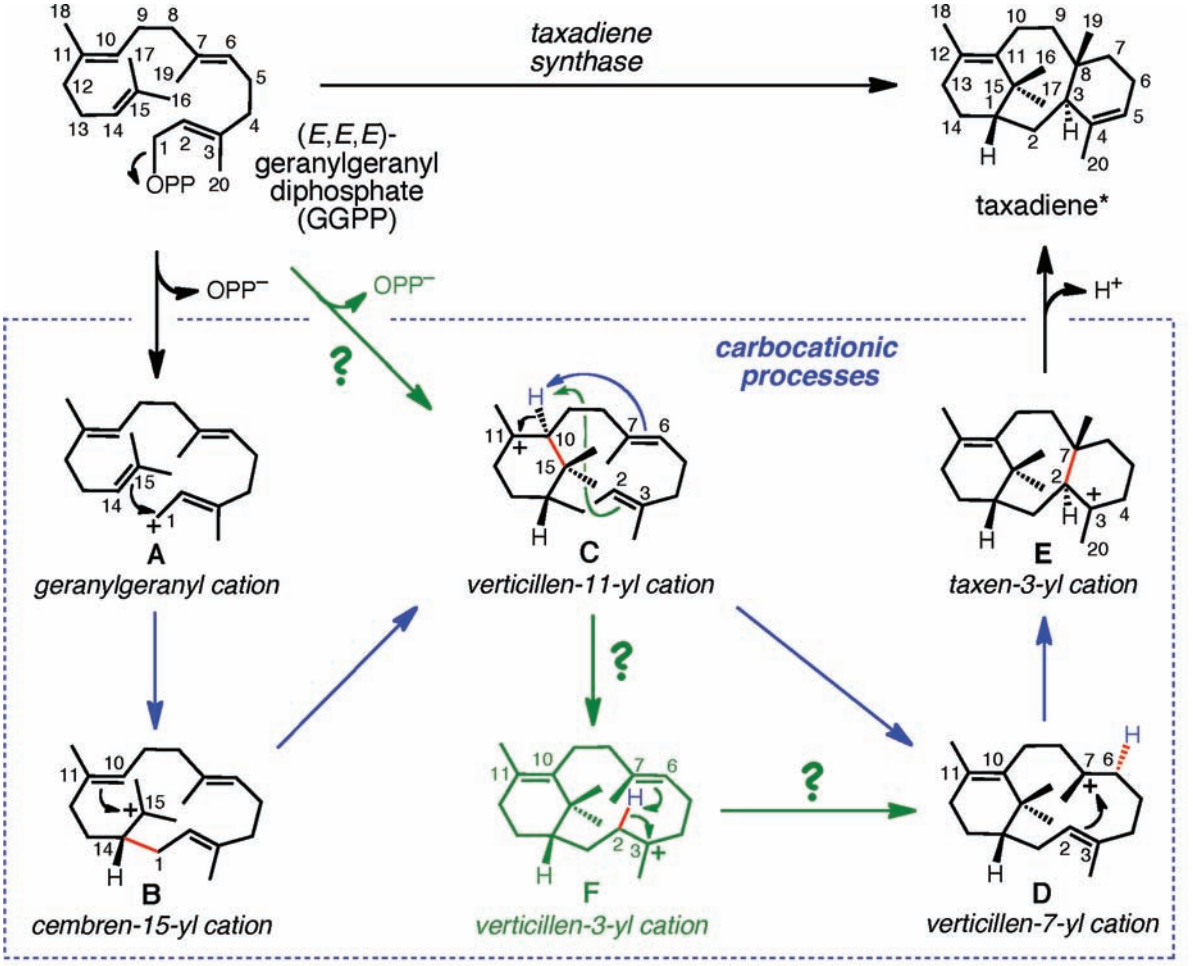
Scheme 1



cation **A** for subsequent cyclization (**A** → **B**); although the π-orbitals of the allylic cation substructure and C14═C15 double bond are not parallel to each other, they do converge. It has been argued previously, on the basis of the results of deuterium labeling experiments that indicate that pyrophosphate loss and cyclization in taxadiene synthase occur with a specific stereochemical outcome (*re* face attack of the C14═C15 π-bond and anti displacement of the pyrophosphate group), that such a carbocation is likely not an intermediate in taxadiene formation, avoided instead *via* a concerted pyrophosphate loss/cyclization process.[5,6] Our results bear on this issue in that the barrier for conversion of **A** to **B** (the cembren-15-yl cation) is predicted to be only 2 kcal/mol at the B3LYP/6-31+G(d,p) level. It is more likely, however, given the known tendency of B3LYP to overestimate the relative stability of acyclic, versus cyclic, isomers,[17a] that the cyclization process is barrierless. This contention is born out by the energies computed with mPW1PW91 and MPWB1K (Figure 2), which indicate that the B3LYP minimum is likely an artifact. Thus, without some means of binding more tightly to cation **A** than to **TS(A-B)**, which would seem to serve no purpose in taxadiene formation, concerted (albeit asynchronous)[16,23] pyrophosphate loss/cyclization seems likely.

Question 2. Clearly questions 1 and 2 are interrelated. Moreover, again on the basis of the results of labeling experiments, it was proposed that not only is carbocation **A** avoided by concerted pyrophosphate loss/cyclization, but also carbocation **B** is likely avoided by coupling these two events with a third: another cyclization that forms a 6-membered ring, leading directly to carbocation **C** (Scheme 1).[5,6] We find, however, that cation **B** is a discrete minimum (Figures 1 and 2). In the structure of **B**, the newly formed C1−C14 σ-bond is significantly elongated (to 1.65 Å) due to hyperconjuation with cationic center C15, but this interaction will be lost as the C15$(CH_3)_2$ group rotates to allow for subsequent cyclization, contributing to the barrier for the **B**-to-**C** reaction. This barrier is predicted to be very small, however, <2.2 kcal/mol (Figure 2). Thus, although the GGPP → **A** → **B** → **C** reaction is not predicted to be fully concerted, the lifetime of intermediate cation **B** is predicted to be very short, allowing cation **C** (the verticillen-11-yl cation) to form without stereochemical scrambling.[24,25] Note that the **B** → **C** reaction is also quite exothermic, more so than the **A** → **B** reaction; in the **A** → **B** reaction, allylic delocalization was lost upon cyclization.[6,26] Note also that the rotation of the C15$(CH_3)_2$ group during the cyclization reaction (which has mostly occurred by the time **TS (B−C)** has been reached, while formation of the C10−C15 bond has not; Scheme 2) allows for a productive conformation of GGPP in which the positions of C16 and C17 differ significantly from their positions in cation **C**; if there is not room in the taxadiene synthase active site for rotation of the C15$(CH_3)_2$ group, the situation could

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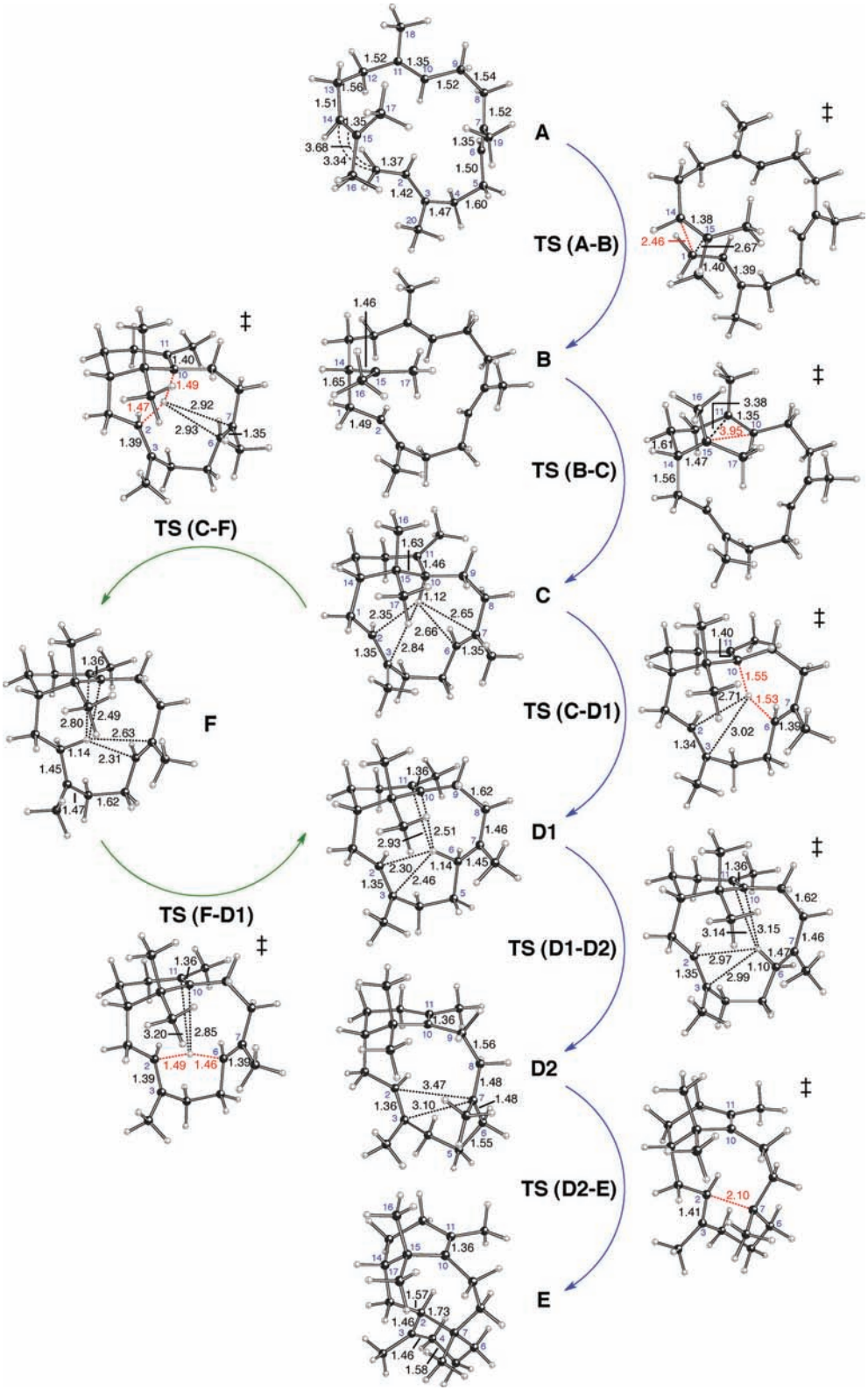

Figure 1. Geometries (B3LYP/6-31+G(d,p), distances in Å) of species involved in the conversion of the geranylgeranyl cation (**A**) to cation **E**.

be different.[27] While one could argue that a restrictive active site could promote the GGPP → **C** reaction through preorganization, the results of our calculations indicate that this is not a necessity. In fact, in that the C15$(CH_3)_2$ group continues to rotate in the same direction as the **A**-to-**B**-to-**C** process proceeds (Scheme 2), this motion is likely to be dynamically enhanced.[28]

Initially, we intended to build on the work reported previously by Gutta and Tantillo in which structures of cations **C**, **D**

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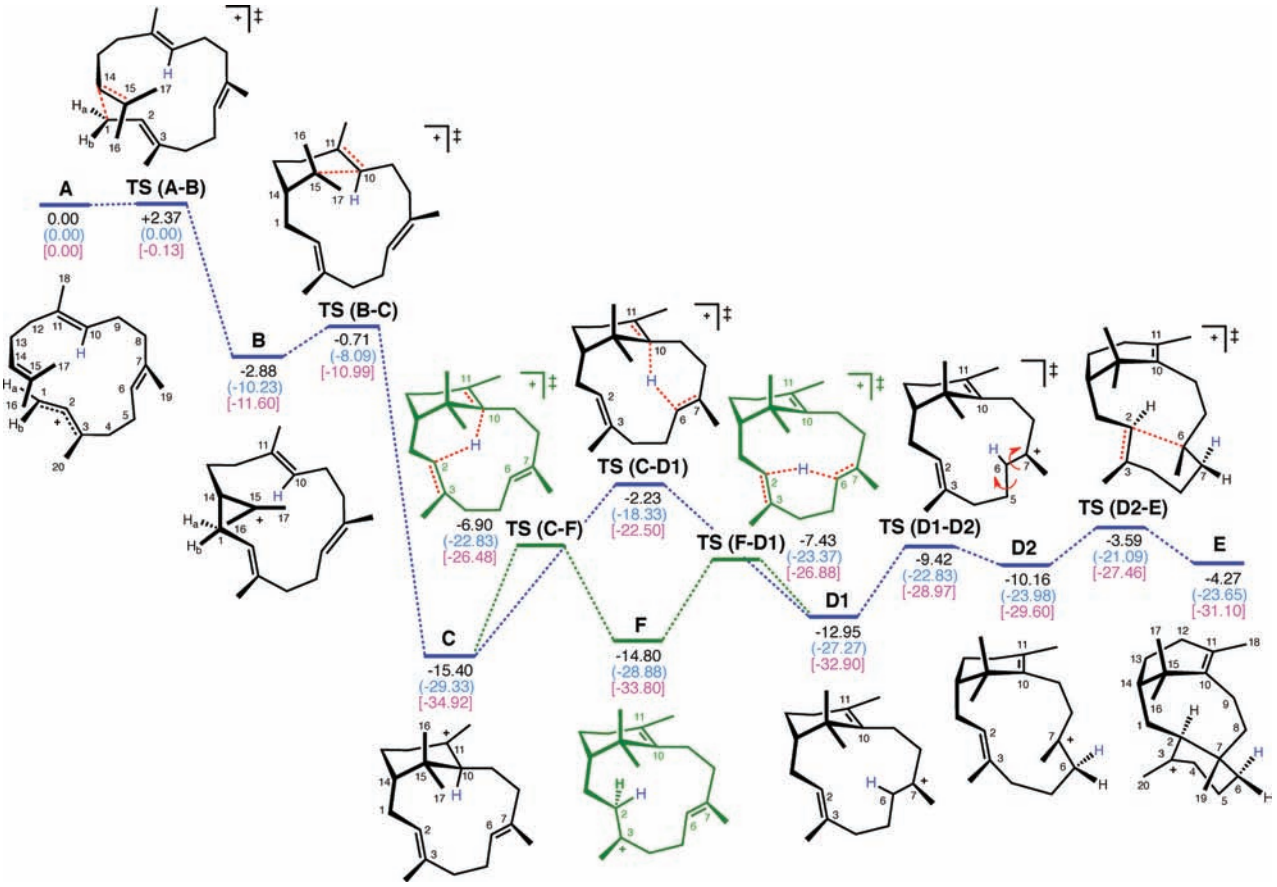
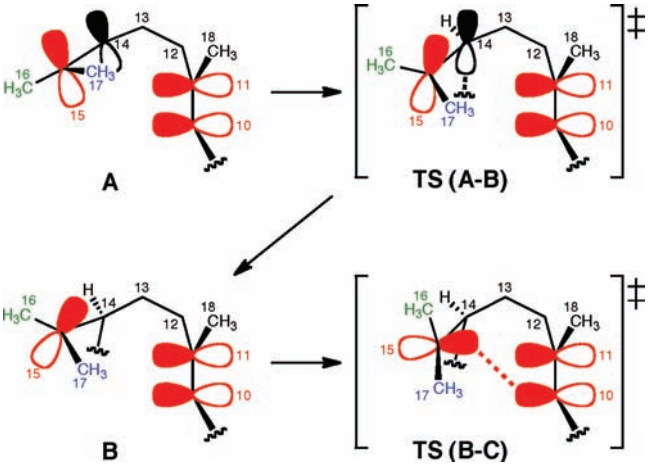


Figure 2. Relative energies [in kcal/mol, relative to the energy of **A**; B3LYP/6-31+G(d,p) in normal text, mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G(d,p) in blue and in parentheses, MPWB1K/6-31+G(d,p)//B3LYP/6-31+G(d,p) in magenta and in brackets; mPW1PW91 results were used in graphing the energies] for the conversion of the geranylgeranyl cation (**A**) to cation **E**. See Figure 1 for computed geometries.

Scheme 2



(the verticillen-7-yl cation), and **F** (the verticillen-3-yl cation) and transition state structures for their interconversion were obtained.[8] However, in extending the taxadiene-forming pathway back toward cation **A**, we realized that the conformer of cation **C** originally examined is not directly connected to the **B**-to-**C** transition state structure described above. We were, however, able to locate an alternative conformer of cation **C** that is directly connected to the relevant conformer of cation **B** *via* a cyclization reaction (*vide supra*) and is also competent in terms of direct intramolecular proton transfer (Figure 1). The new and previously reported conformers of cation **C** differ in three significant ways: (a) the C11−C10−C9−C8 dihedral angle is −78° for the conformer described herein versus −149° for the conformer in the previous report, (b) the C10−C15 bond (elongated to 1.63 Å in the conformer described herein and 1.59 Å in **C** in the conformer in the previous report) and C10−H bond (elongated to 1.12 Å in the conformer described herein and 1.14 Å in the conformer in the previous report) are aligned slightly differently with the formally empty p-orbital at cationic center C11, (c) the hydrogen that will migrate in the **C**-to-**D** and **C**-to-**F** reactions is further away from the recipient carbons (C6 and C2, respectively) in the conformer described herein (2.66 Å and 2.35 Å, respectively) than in the conformer in the previous report (2.46 and 2.20 Å, respectively). The newly located conformer of cation **C** is 5.94 kcal/mol lower in energy than the previously reported conformer, but the predicted barriers for intramolecular proton transfer to convert the conformer of cation **C** described herein to cations **D** and **F** are similar to those for the previously reported conformer, although slightly larger (consistent with the longer distances from the migrating proton to the recipient carbons for the conformer of cation **C** described herein, *vide supra*). Thus, our new calculations still suggest that the two-step route (**C** → **F** → **D**) is energetically more favorable than the one-step route (**C** → **D**) for proton transfer. Consistent with the viability of the **C** → **F** → **D** pathway, previous incubation

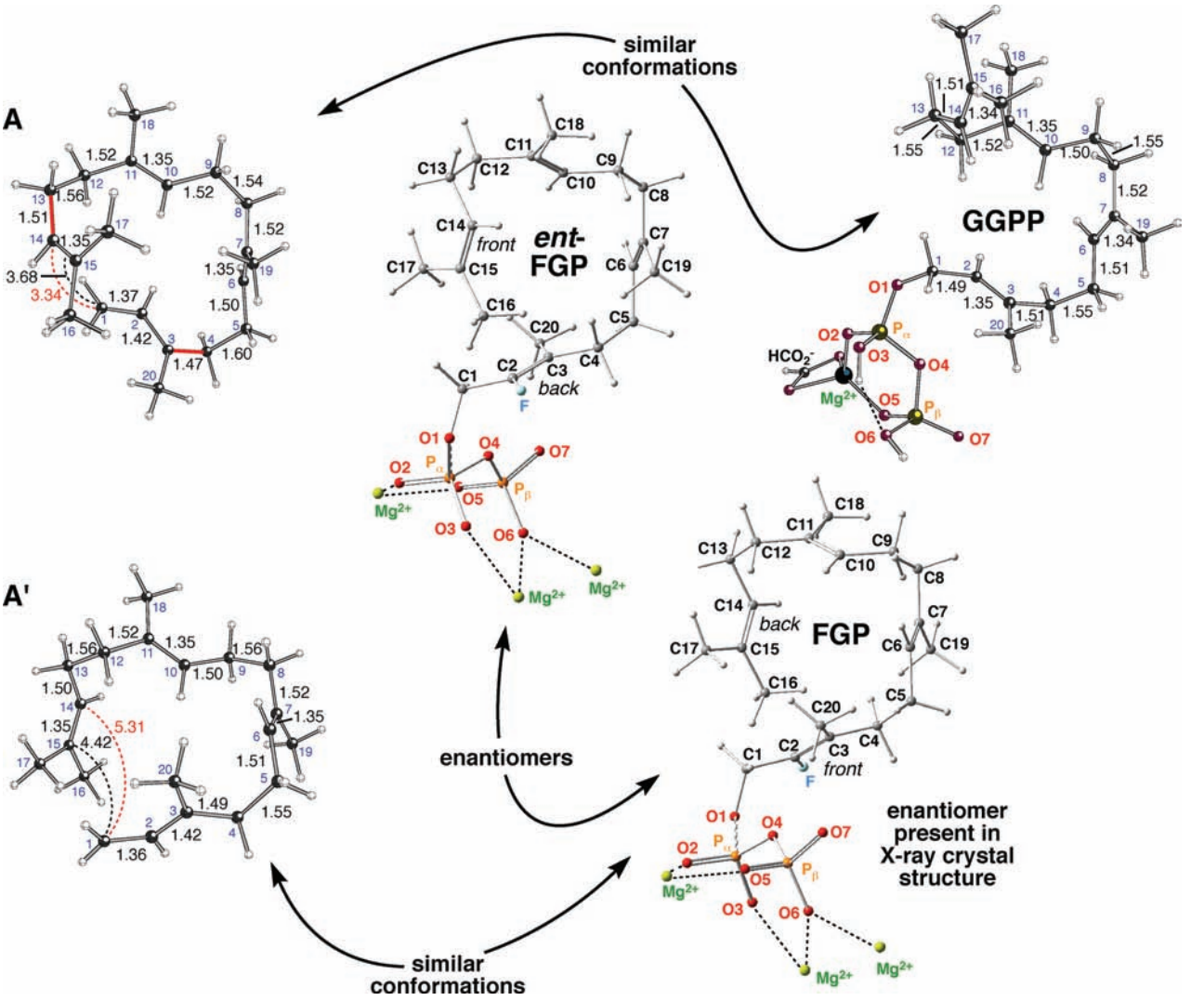


Figure 3. Fully optimized geometries (B3LYP/6-31+G(d,p)) of cations **A** and **A**′ and model of GGPP, along with the structure of FGP from the taxadiene synthase X-ray crystal structure (PDB id: 3P5R)[12] and its mirror image. Selected distances are shown in Å. Note that the experimentally determined C1−C14 distance for FGP is 6.19 Å,[12] and the computed C1−C14 distance for the conformer of GGPP shown is 6.54 Å.

of taxadiene synthase with 6-fluoro-GGPP, which stalls the taxadiene-forming pathway at the verticillenyl stage, led to a significant amount of product derived from deprotonation of the fluoro-analogue of cation **F**.[6,29] Moreover, the C15$(CH_3)_2$ rotation during the **A**-to-**C** process described above, as well as direct formation of a conformer of cation **C** (that is productive for proton transfer) with a chairlike 6-membered ring,[10] stands in contrast to the previous proposal that a conformational change ("ring inversion") of cation **C** occurs before proton transfer;[5,6] our model, in which the first two cyclization events along the taxadiene-forming pathway occur rapidly but "separately", frees one from invoking a conformer of cation **C** with a boat-like 6-membered ring.

The conformation of cation **D** generated upon intramolecular proton transfer (**D1**, Figure 1), however, is not productive with respect to the subsequent cyclization to form cation **E**. A conformational change, as proposed previously by Coates and co-workers,[5] associated with a small barrier (approximately 4 kcal/mol; Figure 2) leads to a productive conformer (**D2**). Cation **D2** is approximately 3 kcal/mol higher in energy than **D1**. This conformational change, which primarily involves rotation about the C6−C7 and C5−C6 bonds, brings the carbocationic center C7 nearer to the C2═C3 π-bond. Cyclization through a chairlike transition state structure produces cation **E**. Note that the newly formed bond in cation **E** is significantly elongated (to 1.73 Å) due to hyperconjugation with new cationic center C3. A small barrier is predicted for this cyclization (Figure 2), which is perhaps less exothermic than expected for a reaction in which a π-bond is traded for a σ-bond; the expected gain in energy is apparently offset by an increase in ring strain upon pinching closed the taxadiene framework (e.g., note that the first-formed 6-membered ring is induced to adopt a boat-like conformation as cation **E** is formed). Deprotonation of the pro-*R* proton at C4, as indicated by previous labeling experiments,[5] will generate taxa-4(5), 11(12)-diene.

Overall, the results of our calculations suggest the following mechanistic picture: GGPP is converted directly to cation **B** via a concerted but asynchronous pyrophosphate loss/cyclization process; cation **B** is a discrete intermediate but is rapidly converted to cation **C**; cation **C** is converted to cation **D** via a two-step route involving cation **F**, although a direct, one-step, intramolecular

proton transfer is also possible; cation **D** undergoes a conformational change before cyclization to cation **E**. Although the calculations reported herein do not include the enzyme active site or the pyrophosphate counterion,[15,30] the mechanistic picture derived from them is entirely consistent with all mechanistic experiments on taxadiene synthase of which we are aware, providing yet another example of a terpene-forming carbocation cascade that does not require enzymatic intervention in terms of barrier-lowering[15,16,20,31−33] (intervention in terms of conformational control is no doubt important, however[10]). Overall, the **A**-to-**E** pathway is quite exothermic (Figure 3), but all of the energetic gain appears to be achieved upon formation of carbocation **C**, the verticillenyl cation. It therefore seems likely that taxadiene-formation is promoted by site-specific deprotonation; i.e., cations **C**−**E** may be in equilibrium until deprotonation occurs.[2,34]

Question 3. Conformational preorganization has long been considered to be a dominant controlling factor in terpene-forming polycyclization reactions.[35] Recent computational studies on terpene-forming carbocation cascades have shown that, in most cases, pathways to major enzymatic products do not involve large conformational changes;[9,15,16,20,31,32,36] more often than not, the product of a given mechanistic step is formed in a conformation that is productive for the next mechanistic step, in many cases allowing two or more steps to be merged into concerted processes.[16,23,32,36] The taxadiene-forming cascade described above fits this mold, involving only one significant conformational change, the **D1**-to-**D2** interconversion (Figures 1 and 2). Thus, it seems reasonable to assume that a key role of taxadiene synthase is to enforce a productive conformation for GGPP. One should ask, then, whether the conformation of the 2-fluorogeranylgeranyl diphosphate (FGP) substrate analogue present in the recently reported X-ray crystal structure of taxadiene synthase is bound in a productive orientation.[12] The authors of the report on the X-ray crystal structure did not imply that it is, noting instead caveats associated with the structure.[12] Our computed structures can be compared directly with FGP bound to taxadiene synthase, allowing for firm conclusions to be drawn about the competence of the conformation adopted by FGP (and by analogy GGPP) in the crystal structure.

The structures of bound FGP and cation **A** are shown in Figure 3 (bottom right and top left, respectively). Note that in **A**, which leads to taxadiene with the absolute configuration found in nature, C15 is in "front" of C1 while in bound FGP, C1 is in "front" of C15. The mirror image of bound FGP is also shown in Figure 3, next to that of cation **A**. Although the relative positions of the C15 and C1 regions of this structure are similar to those of cation **A**, the conformations of the hydrocarbon chains in these two species differ considerably; in particular, the groups around the C3−C4 and C13−C14 bonds (highlighted in red for **A**) are arranged quite differently. The conformer of **A** that most closely resembles the shape of bound FGP, **A′**, is also shown in Figure 3 (**A′** was derived from bound FGP by replacing the appropriate atoms and reoptimizing). Note that cyclization of **A′** would lead to subsequent carbocations in which the configuration of several, but not all, stereogenic centers are inverted with respect to those in the pathway shown in Figures 1 and 2. Thus, cyclization of **A′**, or GGPP in the shape of bound FGP, could not produce taxadiene with the correct configuration (relative and absolute) without substantial conformational changes occurring during the cascade reaction. That FGP is bound in a nonproductive orientation is not entirely surprising, given that some substrate and carbocation analogues in crystal structures of other terpene synthases are also bound in nonproductive orientations,[37] the fact that the N-terminal "active site cap" was absent in the taxadiene synthase X-ray crystal structure,[12] and the observation that the taxadiene synthase active site in the X-ray crystal structure is somewhat voluminous.[12]

We were also able to locate a structure of GGPP in a conformation similar to the productive conformation of **A** (Figure 3, right) using a model containing a diphosphate group, two protons, one Mg^{2+} ion, and one formate (we have previously used such a model in a study of bornyl diphosphate formation).[15a] The hydrocarbon chain in this structure is less coiled than that in **A**, since **A** benefits from the cation−π interaction described above, while GGPP does not. Note also that **A′**, whose conformation reflects that of bound FGP, does not enjoy the same cation−π interaction that does **A**, an interaction that is expressed as σ-bond formation upon cyclization.

CONCLUSIONS

In summary, a complete carbocation cyclization/rearrangement pathway from GGPP to taxadiene was delineated on the basis of the results of quantum chemical calculations. This pathway involves concerted but asynchronous pyrophosphate loss and cyclization to form carbocation **B**, followed by rapid and exothermic conversion of this species into the verticillen-11-yl cation **C**, which can interconvert with cations **D1**/**D2**, **E**, and **F** before deprotonation to form taxadiene. While this pathway is energetically viable, further experiments are necessary to verify its biological relevance; we look forward to these. For example, cembrene isomers may be expected to form from deprotonation of cation **B** as byproducts in mutant taxadiene synthases.[25] In addition, our quantum chemical calculations suggest that the substrate analogue FGP found in the recently reported taxadiene synthase X-ray crystal structure is bound in a nonproductive orientation with respect to taxadiene formation and also reveal which conformations would be productive. Coupled together, the information obtained from X-ray crystallography and the information obtained from our theoretical study provide a firm basis for future modeling (theoretical or experimental) of the taxadiene synthase reaction.

ASSOCIATED CONTENT

Supporting Information. Coordinates and energies for all computed structures, IRC plots, and complete ref 13 (*GAUSSIAN* citation). This material is available free of charge via the Internet at http://pubs.acs.org.

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(26) (a) Coates and co-workers have shown that incubation of 6-fluoro-GGPP with taxadiene synthase led to products derived from deprotonation of the fluoro-analogue of cation **C**, consistent with the involvement of cation **C** in taxadiene formation.[6] (b) Studies on incubation of taxadiene synthase with des-7-methyl GGPP have shown that interception of the taxadiene-forming pathway at the cation **C** stage can lead to phomactatrienes, which appear to result from alternative rearrangements of a des-7-methyl-**C** cation: Chow, S. Y.; Williams, H. J.; Pennington, J. D.; Nanda, S.; Reibenspies, J. H.; Scott, A. I. *Tetrahedron* **2007**, *63*, 6204–6209. (c) The diterpene alcohol verticillol, which was first isolated from the conifer *Sciadopitys verticillata*, could be also derived by water capture of cation **C**: Erdtman, H.; Norin, T.; Sumimoto, M.; Morrison, A. *Tetrahedron Lett.* **1964**, 3879–3886.

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