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Theoretical Studies on Farnesyl Cation Cyclization: Pathways to Pentalenene

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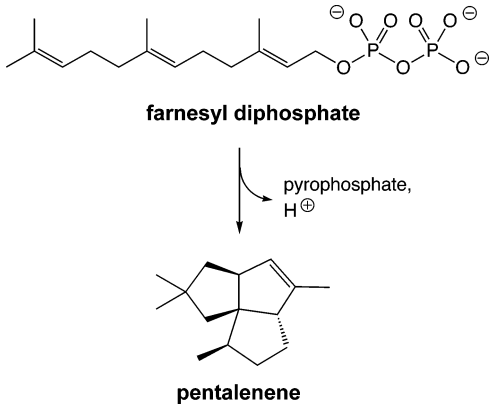
Abstract: In this article, we describe studies, using quantum chemical computations, on possible polycyclization pathways of the farnesyl cation leading to the complex sesquiterpene pentalenene. Two distinct pathways to pentalenene with similar activation barriers are described, each differing from previous mechanistic proposals, and each involving unusual and unexpected intermediates. Direct deprotonation of intermediates on these pathways leads to sesquiterpene byproducts, such as humulene, protoilludene, and asteriscadiene, supporting the notion that a key function of pentalenene synthase, the enzyme that produces pentalenene in Nature, is to regulate the timing and location of proton removal. The implications of the computational results for experimental studies on pentalenene synthase are discussed.

Introduction

Nature constructs a wide range of complex, stereodense molecules from acyclic, achiral precursors. For example, thousands of terpenoid natural products with polycyclic molecular structures are produced from simple derivatives of isoprene oligomers (e.g., farnesyl diphosphate, Scheme 1).[1] Our understanding of the complex mechanisms that occur in the active sites of enzymes that produce polycyclic terpenoid architectures is far from complete, however.[1−3] Herein we describe theoretical studies on the mechanism of one such reaction: the formation of pentalenene[4] from farnesyl diphosphate (Scheme 1). In Nature, this reaction is catalyzed by the enzyme pentalenene synthase.[4−6] By characterizing the inherent (i.e., gas phase) reactivity of the carbocation intermediates formed along the pathway from farnesyl diphosphate to pentalenene, we provide a standard against which the effects of a surrounding environment (e.g., the active site of pentalenene synthase[5]) can be evaluated.

Scheme 1



Formation of pentalenene, which contains three fused five-membered rings and four adjacent stereocenters—one of them quaternary[7]—inside a single enzymatic cavity is an impressive feat. A reasonable mechanism for the formation of pentalenene that is representative of the mechanisms usually proposed is shown in Scheme 2.[1,5,6] In this mechanism, several elementary

(1) Leading references: (a) Fraga, B. M. *Nat. Prod. Rep.* **2005**, *22*, 465−486. (b) Wolf-Rainer, A. *Curr. Med. Chem.* **2001**, *8*, 583−606. (c) Lesberg, C. A.; Caruthers, J. M.; Paschall, C. M.; Christianson, D. W. *Curr. Op. Struct. Biol.* **1998**, *8*, 695−703. (d) Cane, D. E. *Chem. Rev.* **1990**, *90*, 1089−1103. (e) Abe, I.; Rohmer, M.; Prestwich, G. D. *Chem. Rev.* **1993**, *93*, 2189−2206. (f) Dewick, P. M. *Nat. Prod. Rep.* **2002**, *19*, 181−222. (g) For an interesting description of sesquiterpene synthases that produce multiple products, see: Steele, C. L.; Crock, J.; Bohlmann, J.; Croteau, R. *J. Biol. Chem.* **1998**, *273*, 2078−2089.

(2) Recent theoretical studies and leading references: (a) Rajamani, R.; Gao, J. *J. Am. Chem. Soc.* **2003**, *125*, 12768−12781. (b) Nishizawa, M.; Yadav, A.; Imagawa, H.; Sugihara, T. *Tetrahedron Lett.* **2003**, *44*, 3867−3870. (c) Jenson, C.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1997**, *119*, 10846−10854. (d) Gao, D.; Pan, Y.-K.; Byun, K.; Gao, J. *J. Am. Chem. Soc.* **1998**, *120*, 4045−4046. (e) Vrcek, V.; Saunders, M.; Kronja, O. *J. Org. Chem.* **2003**, *68*, 1859−1866. (f) Hess, B. A., Jr.; Smentek, L. *Org. Lett.* **2004**, *6*, 1717−1720.

(3) Wendt, K. U. *Angew. Chem., Int. Ed.* **2005**, *44*, 3966−3971.

(4) Pentalenene has been isolated from *Streptomyces* bacteria and is the precursor of the pentalenolactone antibiotics. See: (a) Seto, H.; Yonehara, H. *J. Antibiot.* **1980**, *33*, 92−93. (b) Cane, D. E.; Sohng, J. K.; Williard, P. G. *J. Org. Chem.* **1992**, *57*, 844−852.

(5) (a) Crystal structure of pentalenene synthase: Lesburg, C. A.; Zhai, G.; Cane, D. E.; Christianson, D. W. *Science* **1997**, *277*, 1820−1824. (b) Note that covalently bound enzyme−substrate complexes are generally not invoked as intermediates in terpenoid synthase catalyzed reactions, and the putative carbocation rearrangements involved are generally formulated as involving *noncovalent* enzyme−substrate interactions (steric and/or electrostatic).

(6) (a) Seemann, M.; Zhai, G.; de Kraker, J.-W.; Paschall, C. M.; Christianson, D. W.; Cane, D. E. *J. Am. Chem. Soc.* **2002**, *124*, 7681−7689. (b) Seemann, M.; Zhai, G.; Umezawa, K.; Cane, D. *J. Am. Chem. Soc.* **1999**, *121*, 591−592. (c) Cane, D. E.; Sohng, J.-K.; Lamberson, C. R.; Rudnicki, S. M.; Wu, Z.; Lloyd, M. D.; Oliver, J. S.; Hubbard, B. R. *Biochemistry* **1994**, *33*, 5846−5857. (d) Cane, D. E.; Oliver, J. S.; Harrison, P. H. M.; Abell, C.; Hubbard, B. R.; Kane, C. T.; Lattman, R. *J. Am. Chem. Soc.* **1990**, *112*, 4513−4524. (e) Harrison, P. H. M.; Oliver, J. S.; Cane, D. E. *J. Am. Chem. Soc.* **1988**, *110*, 5922−5923. (f) Cane, D. E.; Abell, C.; Lattman, R.; Kane, C. T.; Hubbard, B. R.; Harrison, P. H. M. *J. Am. Chem. Soc.* **1988**, *110*, 4081−4082. (g) Cane, D. E.; Abell, C.; Tillman, A. M. *Bioorg. Chem.* **1984**, *12*, 312−328. (h) Cane, D. E.; Tillman, A. M. *J. Am. Chem. Soc.* **1983**, *105*, 122−124. (i) Cane, D. E.; Rossi, T.; Tillman, A. M.; Pachlatko, J. P. *J. Am. Chem. Soc.* **1981**, *103*, 1838−1843.

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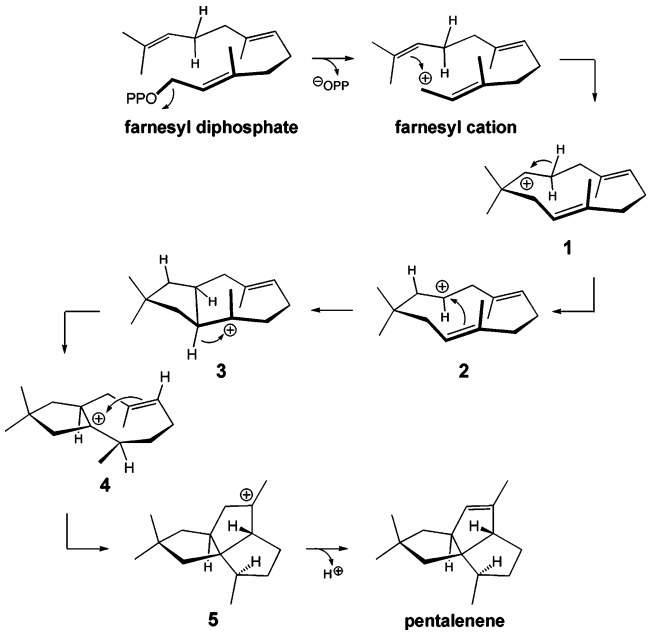
Scheme 2



steps are involved (primarily hydride shifts and alkene attacks on carbocations), and six different carbenium ions are invoked as intermediates (in some proposals fewer intermediates are invoked and/or deprotonation/reprotonation sequences replace hydride shifts).[5b] But does this sort of mechanism adequately represent the reactivity of such complex carbocations? In particular, are all of the steps shown discrete or are some of them more likely to be combined with others into complex concerted processes? Also, does the inherent reactivity of the carbocation intermediates provide any clues as to the role that pentalenene synthase might play in controlling the rate and regio- and stereoselectivity of the pentalenene forming reaction that it promotes? These are the key questions we aim to address herein.

Methods

All calculations were performed with Gaussian03.[8] Geometries were optimized without symmetry constraints using the B3LYP/6-31+G(d,p) method.[9] All structures were characterized by frequency calculations, and reported energies include zero-point energy corrections (unscaled). The B3LYP method has been used previously to describe, for example, nonclassical cations,[10a,b] crystallographically characterized vinyl cations,[10c] transition structures for cationic [1,2] shifts,[2e] and carbocation−alkene cyclization reactions.[2f,10d] The B3LYP method has also been shown to be effective in reproducing the chemical shifts of hydrogens involved in delocalized $[C\cdots H\cdots C]^+$ arrays.[10e,f] Several recent studies have compared the B3LYP and MP2 methods for computing geometries and relative energies of various carbocations.[2e,10g−j] In general, although MP2 tends to favor more delocalized carbocation structures compared to B3LYP, differences between these methods are often small. One of these studies also compared the B3LYP and MP2 methods to CCSD, showing (for the particular cations investigated) that B3LYP and CCSD produced similar results.[10h] Of particular relevance to the study described herein are recent model studies on carbocations closely related to structure **8** in which the B3LYP/6-31+G(d,p) method was shown to produce very similar results to those obtained with the B3LYP/6-311+G(d,p) and MP2/6-31+G(d,p) methods.[11a,b] A recent report, however, suggests that B3LYP may systematically underestimate the reaction energies for carbocation−alkene cyclization reactions and suggests that mPW1PW91 single-point calculations may improve the energetics in such systems.[10d] Consequently, we include mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G(d,p) energies in Figures 1 and 3 for comparison; these energies include unscaled zero-point energy corrections from B3LYP/6-31+G(d,p) frequency calculations. As suggested in ref 10d, mPW1PW91 does in fact predict more exothermic cyclization reactions than does B3LYP for the cases we have explored, consistently lowering the relative energy of species that contain more rings. The qualitative conclusions of our study are not affected by the differences in the two sets of energetics, however. Intrinsic reaction coordinate (IRC) calculations[12] were also used to verify the identity of transition structures. For some structures in Figures 1 and 3, additional conformers were also located; all of these were very close in energy to those shown. Structural drawings were produced using *Ball & Stick*.[13]

Results and Discussion

Pathways to Pentalenene. First, consider the putative mechanism shown in Scheme 2. Fully optimized intermediates and transition state structures along the pathway from farnesyl cation to **5** are shown in Figure 1. No appropriately folded farnesyl cation structures, such as the one depicted in Scheme 2, could be found; attempts to optimize the geometries of such structures consistently led to **1** (the humulyl cation). Thus, if the enzyme enforces the correct fold (and induces the pyrophosphate group to leave), cation **1** could be formed directly without the intermediacy of a discrete farnesyl cation.[14] Note that one of the C−C bonds β to the cationic carbon is elongated considerably (to 1.67 Å) due to hyperconjugation. Cation **1** is approximately 15 kcal/mol higher in energy than unfolded, fully extended farnesyl cation, **6** (distances shown in Å). For the remainder of our mechanistic discussion, we use

(7) Recent informative reviews on the challenges associated with constructing quaternary all-carbon stereocenters: (a) Peterson, E. A.; Overman, L. E. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 11943−11948. (b) Douglas, C. J.; Overman, L. E. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 5363−5367. (c) Denissova, I.; Barriault, L. *Tetrahedron* **2003**, *59*, 10105−10146.

(8) Frisch, M. J.; et al. *Gaussian03*, revision B.04; Gaussian, Inc.: Pittsburgh, PA, 2003 (full reference in Supporting Information).

(9) (a) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648−5652. (b) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 1372−1377. (c) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785−789. (d) Stephens, P. J.; Devlin, F. J.; Chabalowski, C. F.; Frisch, M. J. *J. Phys. Chem.* **1994**, *98*, 11623−11627. (e) The value of diffuse functions in density functional based calculations was recently discussed in: Lynch, B. J.; Zhao, Y.; Truhlar, D. G. *J. Phys. Chem. A* **2003**, *107*, 1384−1388.

(10) Recent reports on the application of the B3LYP method to carbocations (with leading references to others) include: (a) Smith, W. B. *J. Org. Chem.* **2001**, *66*, 376−380. (b) Schreiner, P. R.; Schleyer, P. v. R.; Schaefer, H. F., III. *J. Org. Chem.* **1997**, *62*, 4216−4228. (c) Müller, T.; Juhasz, M.; Reed, C. A. *Angew. Chem., Int. Ed.* **2004**, *43*, 1543−1546. (d) Matsuda, S. P. T.; Wilson, W. K.; Xiong, Q. *Org. Biomol. Chem.* **2006**, *4*, 530−543. (e) Ponec, R.; Yuzhakov, G.; Tantillo, D. J. *J. Org. Chem.* **2004**, *69*, 2992−2996. (f) DuPre, D. B. *J. Phys. Chem. A* **2005**, *109*, 622−628. (g) Vrcek, I. V.; Vrcek, V.; Siehl, H.-U. *J. Phys. Chem. A* **2002**, *106*, 1604−1611. (h) Farcasiu, D.; Lukinskas, P.; Pamidighantam, S. V. *J. Phys. Chem. A* **2002**, *106*, 11672−11675. (i) Fuchs, J.-F.; Mareda, J. *J. Mol. Struct. THEOCHEM* **2005**, *718*, 93−104. (j) Siebert, M. R.; Tantillo, D. J. *J. Org. Chem.* **2006**, *71*, 645−654. (k) See also ref 2.

(11) (a) Gutta, P.; Tantillo, D. J. *Angew. Chem., Int. Ed.* **2005**, *44*, 2719−2723. (b) Ponec, R.; Bultinck, P.; Gutta, P.; Tantillo, D. J. *J. Phys. Chem. A* **2006**, *110*, 3785−3789. (c) With zero-point energies included, the transition structure preceding **8** is actually 0.07 kcal/mol lower in energy than **8** with B3LYP/6-31+G(d,p) and 0.81 kcal/mol higher than **8** with mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G(d,p), and the transition structure following **8** is 0.98 kcal/mol higher in energy than **8** with B3LYP/6-31+G(d,p) and 2.00 kcal/mol higher than **8** with mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G(d,p).

(12) (a) Gonzalez, C.; Schlegel, H. B. *J. Phys. Chem.* **1990**, *94*, 5523−5527. (b) Fukui, K. *Acc. Chem. Res.* **1981**, *14*, 363−368. (c) IRC plots are available in the Supporting Information.

(13) Müller, N.; Falk, A. *Ball & Stick V.3.7.6*, molecular graphics application for MacOS computers, Johannes Kepler University, Linz, 2000.

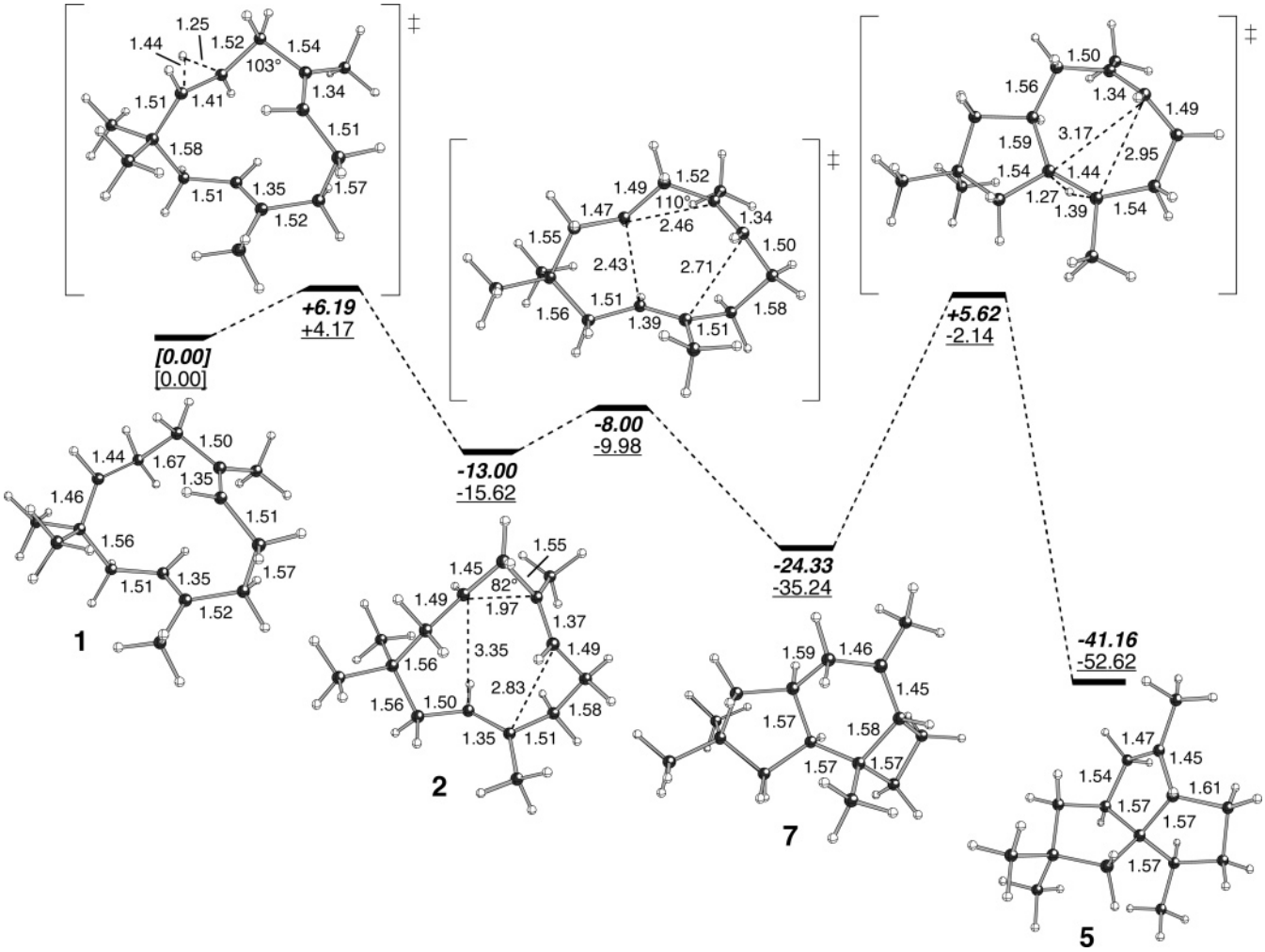
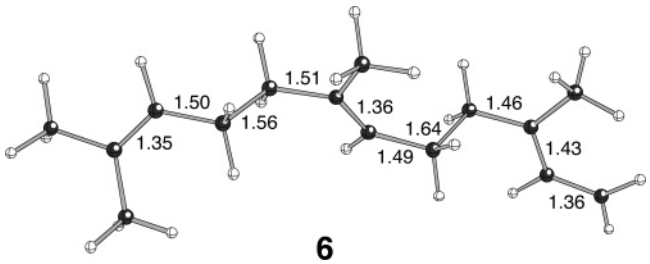


Figure 1. Geometries and relative energies (B3LYP/6-31+G(d,p) in bold italics, distances in Å, relative energies in kcal/mol) for structures involved in the conversion of **1** to **5**. Underlined energies are from mPW1PW91/6-31+G(d,p) single points on the B3LYP/6-31+G(d,p) optimized geometries and include B3LYP/6-31+G(d,p) zero-point energy corrections (see Methods section for details).

cation **1** as our reference point, setting its relative energy to 0.00 kcal/mol.



The first step in the proposed mechanism (Scheme 2) after the formation of cation **1** involves a [1,2] hydrogen shift.[15] The transition structure for this sigmatropic rearrangement (Figure 1) is typical of [1,2] hydrogen shift transition structures, resembling a nonclassical carbocation with a 3-center 2-electron delocalized bonding array.[16,17] The barrier for conversion of **1** to **2** is estimated to be 4−6 kcal/mol.[16b] Cation **2** can be thought of as a very highly hyperconjugated cyclopropylcarbinyl cation[18] or an intramolecular cation−π complex[19] between a π-bond and

(14) (a) The enzymatic cyclization is thought to be initiated by ionization of the diphosphate group aided by Mg^{2+}; see, for example, ref 6. Kinetic studies on related sesquiterpene synthases have suggested that this ionization may be the slow chemical step in such reactions; see: Cane, D. E.; Chiu, H.-T.; Liang, P.-H.; Anderson, K. S. *Biochemistry* **1997**, *36*, 8332−8339 and Mathis, J. R.; Back, K.; Starks, C.; Noel, J.; Poulter, C. D.; Chappell, J. *Biochemistry* **1997**, *36*, 8340−834. (b) Based on recent experimental (isotope effect) studies, it has been suggested that a discrete farnesyl cation may be avoided in a related cyclization reaction of farnesyl diphosphate: the enzyme catalyzed formation of premnaspirodiene via the germacradienyl cation. See: Schenk, D. J.; Starks, C. M.; Rising Manna, K.; Chappell, J.; Noel, J. P.; Coates, R. M. *Arch. Biochem. Biophys.*, in press.

(15) A competing [1,2] methyl shift leading to other products is also possible. This reaction and others that may follow it will be described in detail in a separate account.

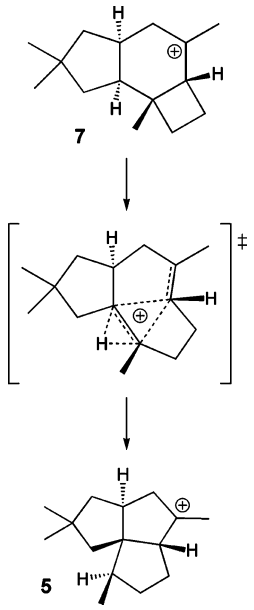
(16) (a) Cationic [1,2] hydrogen or alkyl shifts generally have very low activation barriers, and in some cases, bridged hypercoordinate structures are actually minima rather than transition structures (so-called nonclassical ions). For leading references, see ref 17. (b) A small amount of ring strain in the **1**-to-**2** transition structure likely contributes to the barrier of 6 kcal/mol that we observe in this case.

(17) Leading references on nonclassical cations: (a) Issue 12 of *Acc. Chem. Res.* **1983**, *16*. (b) Brown, H. C. (with comments by Schleyer, P. v. R.) *The Nonclassical Ion Problem*; Plenum: New York, 1977. For leading references on proposed nonclassical structures in terpenoid biosynthesis, see: (c) Wessjohann, L. A.; Brandt, W. *Chem. Rev.* **2003**, *103*, 1625−1647. (d) Giner, J.-L.; Buzek, P.; Schleyer, P. v. R. *J. Am. Chem. Soc.* **1995**, *117*, 12871−12872. (e) Erickson, H. K.; Poulter, C. D. *J. Am. Chem. Soc.* **2003**, *125*, 6886−6888. (f) He, X.; Cane, D. E. *J. Am. Chem. Soc.* **2004**, *126*, 2678−2679. (g) Dewar, M. J. S.; Ruiz, J. M. *Tetrahedron* **1987**, *43*, 2661−2674. For leading references to older proposals by Djerassi, Arigoni, Ruzicka, Eschenmoser, and others, see: (h) Giner, J.-L. *Chem. Res.* **1993**, *93*, 1735−1752.

(18) A thorough review on cyclopropylcarbinyl cations: Olah, G. A.; Reddy, V. P.; Prakash, G. K. S. *Chem. Rev.* **1992**, *92*, 69−95.

(19) Reviews on cation−π interactions include: (a) Ma, J. C.; Dougherty, D. A. *Chem. Rev.* **1997**, *97*, 1303−1324. (b) Gokel, G. W. *Chem. Commun.* **2003**, 2847−2852.

Scheme 3



a homoallylic cationic site; note the small C−C−C angle of 82° and the C···C distance of 1.97 Å.

It is generally proposed that **2** then cyclizes to form **3** (the secoilludyl cation) en route to pentalenene. We were unable to find a minimum corresponding to **3**, but we did locate a transition structure leading directly from **2** to **7** (Figure 1). Production of cation **7** involves the concerted formation of two new σ-bonds along which new five-, six-, and four-membered rings are fused.[20] The barrier for this reaction is quite low (5−6 kcal/mol from **2**).

Is this path then a dead end as far as pentalenene formation is concerned, or could the pentalenene forming reaction actually involve **7** instead of **3**? Interestingly, we found a transition structure that connects **7** to **5** directly (Figure 1 and Scheme 3). At least formally, this reaction is a type 1 dyotropic rearrangement,[21] involving the simultaneous migration of two groups past each other (here a hydrogen and an alkyl group). IRC calculations on the transition structure for this rearrangement indicated, though, that ring-opening and subsequent cyclization precede and follow the hydrogen shift, respectively; in other words, although this is a concerted process, the various bond breaking and forming events involved are not synchronous.[22] In fact, the transition structure resembles a transition structure for a [1,2] hydrogen shift in proximity to and perhaps stabilized by an

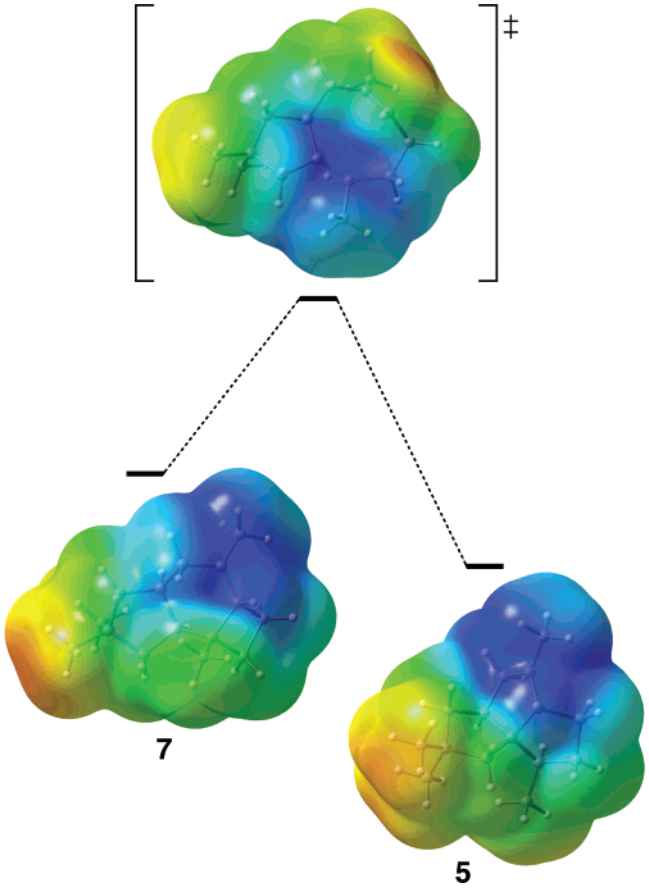


Figure 2. Electrostatic potential surfaces for **7**, **5**, and the transition structure that connects them (red is least positive and blue is most positive; the range is +0.07 to +0.15 au; structures are displayed in the same orientations as shown in Figure 1).

alkene through an intramolecular cation−π interaction.[19,23] Although this step has a high barrier from **7** (approximately 30 kcal/mol), the transition structure is actually lower in energy than that for formation of **2**. It is unclear, however, how much energy would be dissipated along an enzyme promoted path to **7**; in other words, how big of a barrier would realistically be faced in converting **7** to **5**. Consequently, selective stabilization of the transition structure connecting **7** to **5** may be a key role of the enzyme if this mechanism is followed.

How might such selective transition state stabilization arise? The structures of **7**, **5**, and the transition structure that connects them are quite different with respect to their distribution of charge (Figure 2); while much of the positive charge in **7** and **5** resides in the vicinity of the tertiary sp^2 carbon (at the top of these species as drawn in Figures 1 and 2), the positive charge shifts in the transition structure to the other side of the molecule (to the site of the hydrogen shift). This immediately suggests a

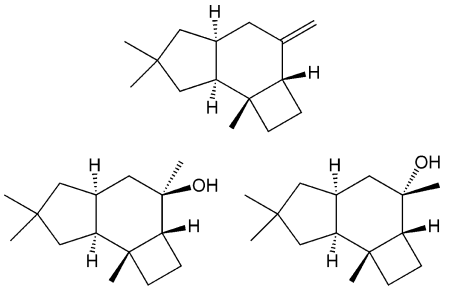
(20) This reaction can be viewed as an orbital symmetry forbidden all-suprafacial 4-electron (2+2+1) pericyclic reaction (if we think of the reactant as a simple secondary cation we have two C=C π-bonds and an empty p-orbital interacting, but if we think of the reactant as a cyclopropylcarbinyl cation we have a C−C σ-bond, an empty p-orbital, and a C=C π-bond interacting). However, the asynchronicity of bond formation (formation of the five-membered ring leads formation of the four-membered ring) and the relatively long partial C−C bonds in the transition structure suggest that this reaction may actually belong to the class of "apparent violations" of the orbital symmetry rules for which significantly strong overlap of *all* of the cyclically disposed orbital fragments does not occur at any point along the reaction coordinate. For a related example in a neutral system, see: Kless, A.; Nendel, M.; Wilsey, S.; Houk, K. N. *J. Am. Chem. Soc.* **1999**, *121*, 4524−4525 (addition/correction: *J. Am. Chem. Soc.* **1999**, *121*, 7278).

(21) (a) Reetz, M. T. *Angew. Chem., Int. Ed. Engl.* **1972**, *11*, 129−130. (b) Reetz, M. T. *Angew. Chem., Int. Ed. Engl.* **1972**, *11*, 130−131. (c) Reetz, M. T. *Tetrahedron* **1973**, *29*, 2189−2194. (d) Reetz, M. T. *Adv. Organomet. Chem.* **1977**, *16*, 33−65. (e) For an example of a different sort of dyotropic rearrangement in a carbocationic system, see ref 2e.

(22) We cannot definitively rule out the presence of a shallow minimum on one or the other side of the transition structure. The reaction coordinate mapped out by our IRC calculations[12] flattens out in the vicinity of structures resembling **3** and **4**, but optimization of the final point on the IRC in each direction leads to **7** and **5**, respectively. Thus it seems clear that if any minima that flank the transition structure do exist, the barriers for their conversion to **7** and **5** are very small, at least in the absence of an enzyme active site.

(23) The advantage to a cationic transition structure of having a nearby π-system was explored decades ago with CNDO/2 and INDO molecular orbital calculations in the context of very simple models for squalene cyclization. See: Gleiter, R.; Müllen, K. *Helv. Chim. Acta* **1974**, *57*, 823−831. This report predates the rise to prominence of cation−π interactions, although electrostatic effects were not discussed explicitly in this early report.

Chart 1



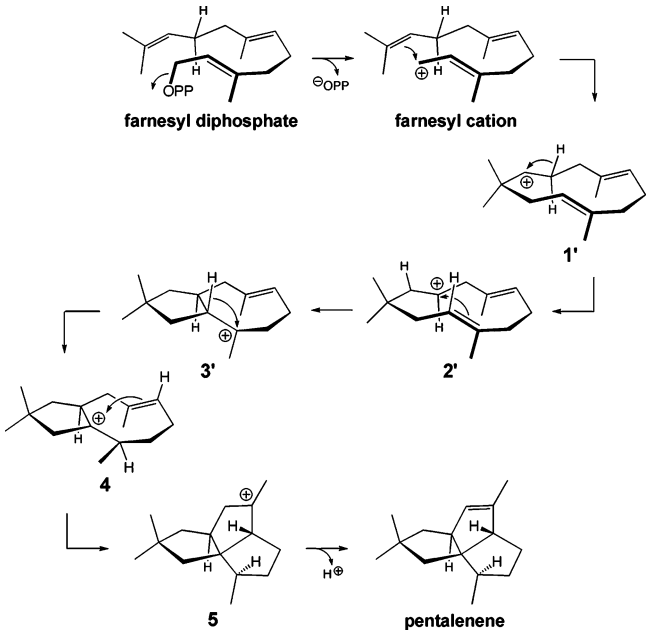
possible catalyst architecture for selective stabilization of this transition structure: a surrounding active site with a cation-stabilizing group or groups near the site of the hydrogen shift. A primary goal of our future computational studies will be to determine if any such groups in pentalenene synthase are appropriately oriented to provide such selective transition state stabilization.[24]

To the best of our knowledge, inclusion of **7** on the enzyme catalyzed pathway from farnesyl diphosphate to pentalenene has not been suggested previously. This structure has been suggested as a player, however, in an alternative reaction of **3** that leads to other sesquiterpene products (see below).[1,6a,b] Interestingly, precursors to cation **7** (Chart 1) have been observed to produce some pentalenene when refluxed in formic acid, although a multistep mechanism for the transformation of **7** into **5** was suggested in this case rather than a direct dyotropic rearrangement.[25] While a mechanism for pentalenene synthase involving the formation of a 5/6/4 ring system that rearranges to a 5/5/5 architecture is far from obvious, it does seem energetically plausible.

The pathway shown in Scheme 2 connects farnesyl diphosphate and **5**, which leads to pentalenene upon deprotonation, and has an estimated overall barrier from cation **1** of only 4−6 kcal/mol. There is another possible path to pentalenene, however, involving a different conformer of the humulyl cation, **1′**, in which one isoprene unit has been "flipped over" (Scheme 4). This mechanism is analogous to the one shown in Scheme 2, except for the stereochemistry of the putative intermediates: **1′** and **2′** are conformers of **1** and **2**, while **3′** is a diastereomer of **3**; structures **4** and **5** are the same in both proposed mechanisms.[26]

We located a minimum corresponding to **1′**, which, like **1**, shows evidence of considerable hyperconjugation (Figure 3). This structure is approximately 4 kcal/mol lower in energy than

Scheme 4



conformer **1** (Figure 1). A transition structure that converts **1′** into **2′** was located and characterized by IRC calculations (Figure 3). Again this transition structure looks like a typical transition structure for a [1,2] hydrogen shift.[16] Cation **2′**, like **2**, resembles a highly hyperconjugated cyclopropylcarbinyl cation,[18] although the C−C−C angle of 76° and the C···C distance of 1.84 Å in **2′** are both smaller than those in **2**.

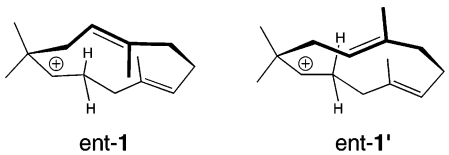
Cation **2′** is connected to cation **3′** via a transition structure that resembles the transition structure for the conversion of **2** to **7** (Figure 1). The barrier for the **2′** to **3′** rearrangement is approximately 6−8 kcal/mol, comparable to the 5−6 kcal/mol barrier predicted for the **2** to **7** rearrangement. Cation **3′** can be described as a strongly hyperconjugated diastereomer of **7** (note that the "internal" C−C bond of the four-membered ring in **3′** is much longer than the corresponding bond in **7**) or just as a diastereomer of **3** that benefits from transannular cation−π stabilization.[19]

To convert **3′** to pentalenene, another hydrogen shift must occur (Scheme 4). When looking for the transition structure for this hydrogen shift and for its presumed product, **4**, we located a particularly unusual structure, **8** (Figure 3). In this structure, a proton is sandwiched between two alkenes, seemingly trapped as it tries to migrate. We have discussed the unusual electronic structures of **8** and related species elsewhere.[11a,b] Although **8** is a minimum, the transition structures for its formation from **3′** and its conversion to **5** are extremely close to it in energy.[11c] Consequently, it is perhaps more reasonable to think of the relatively flat region of the energy surface near **8** as describing

(24) Based on the crystal structure of pentalenene synthase,[5] it appears that Asn219 could be in the vicinity of the cyclic delocalized C−H−C substructure of the transition structure connecting **7** and **5**, on the face of the molecule opposite to the migrating hydrogen. It is possible that the partially negatively charged carbonyl of this group contributes to selective stabilization of the transition structure through a favorable electrostatic interaction. A similar conjecture based on putative intermediates in this reaction is discussed in ref 5, although subsequent work indicates that this residue is likely more significant for Mg^{2+}/pyrophosphate binding.[6c,d,14] Interestingly, when this residue is mutated to the nonpolar Ala or Leu, the enzyme becomes inactive, but when this residue is mutated to Asp, it retains significant activity.[6a]

(25) (a) Ohfune, Y.; Shirahama, H.; Matsumoto, T. *Tetrahedron Lett.* **1976**, 2869−2872 and Misumi, S.; Ohtsuka, T.; Ohfune, Y.; Sugita, K.; Shirahama, H.; Matsumoto, T. *Tetrahedron Lett.* **1979**, 31−34. The maximum yield of pentalenene reported was 28%. This sort of transformation has also been used to produce pentalenene in the laboratory for use in biochemical studies.[6] (b) A related cyclization (using $BF_3{\cdot}OEt_2$) on a bicyclic diene precursor of cation **4** (and therefore, we think, of **7**) has also been reported to produce pentalenene (in 38% yield); see: Pattenden, G.; Teague, S. J. *Tetrahedron* **1987**, *43*, 5637−5652.

(26) Two other conformers of **1**, differing in the spatial disposition of their isoprene units, are also possible. However, these two conformers (below) are actually enantiomers of **1** and **1′**. Although these conformers could, in principle, lead to pentalenene with the absolute stereochemistry shown in Scheme 1, such pathways would not be very direct and are not considered further herein.



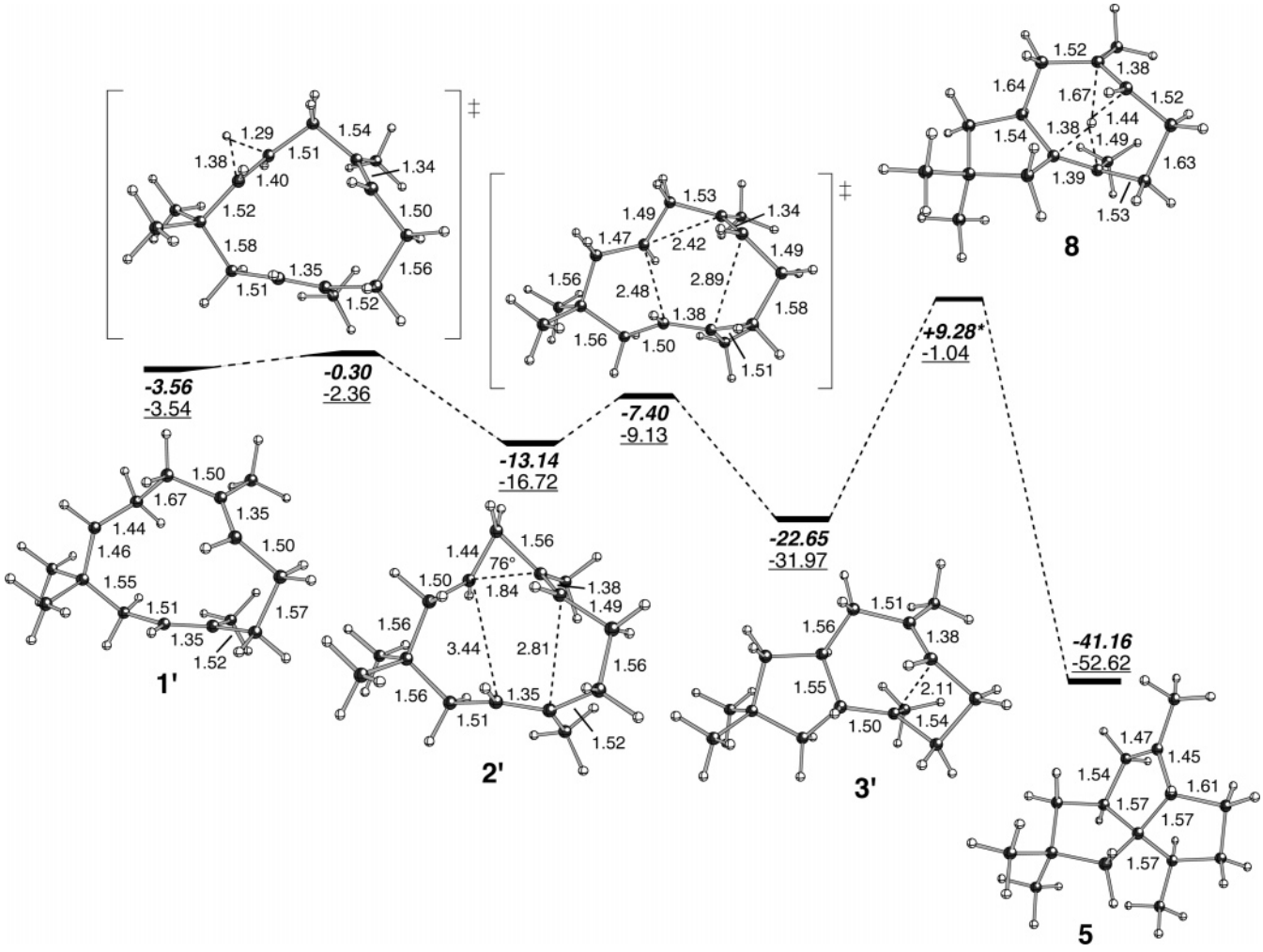
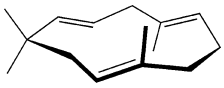


Figure 3. Geometries and relative energies (B3LYP/6-31+G(d,p) in bold italics, distances in Å, energies in kcal/mol relative to that of **1**) for structures involved in the conversion of **1′** to **5**. (*Note that structure **8** is actually on a fairly flat plateau; see text for details.) Underlined energies are from mPW1PW91/6-31+G(d,p) single points on the B3LYP/6-31+G(d,p) optimized geometries and include B3LYP/6-31+G(d,p) zero-point energy corrections (see Methods section for details).

the transition state for the **3′** to **5** reaction, the barrier for this reaction being approximately 31−33 kcal/mol from **2′**. Like the transition structure for the **7** to **5** rearrangement, structure **8** also resembles a [1,2] hydrogen shift transition structure interacting with a nearby C═C double bond, but in this case, the double bond interacts with the bridging H rather than the two carbons between which it bridges. If this pathway is followed in pentalenene synthase, then a key function of the enzyme might be to selectively stabilize **8**. In any case, the intermediacy of such an unusual cation in terpenoid cyclization processes is unprecedented.

Premature Deprotonation. The two pathways described above lead to pentalenene and have low overall barriers relative to **1**/**1′**. However, are there alternative low energy pathways branching out from the intermediates involved in the pathways to pentalenene that can lead instead to other sesquiterpene products? It is important to characterize such diversions since they represent pathways that pentalenene synthase must somehow suppress. We restrict our discussion here to potential byproducts arising from deprotonation of intermediates on the **1**/**1′** to **5** pathways, but studies on the many other possible diversions involving more complicated rearrangements are in progress and will be reported in due course.

Direct deprotonation of **1**, **1′**, **2**, or **2′** can lead to humulene (**9**), which has at times been proposed as an intermediate in the farnesyl diphosphate-to-pentalenene reaction.[6] Isomers of **9** with conjugated diene substructures or cyclopropane rings could also be formed by deprotonation of **2**/**2′** at other positions.

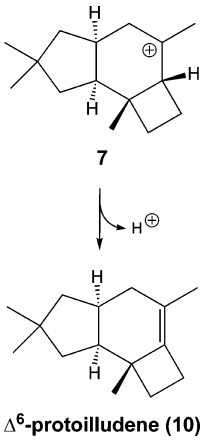


humulene (9)

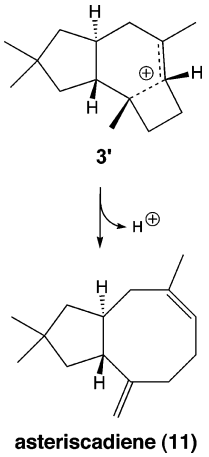
Cations **7** (Figure 1) and **3′** (Figure 3) can also lead to undesired byproducts. For example, deprotonation of **7** could lead directly to Δ^6-protoilludene[27] (**10**, Scheme 5) or isomers with C═C double bonds in other locations. **10** has actually been observed as a product of some pentalenene synthase mutants, in particular, those in which the active site residue His309 is altered;[6a,b,28] this hints that His309 could contribute to selective stabilization of the transition structure connecting **7** and **5** (see above).[24] The orientations of bound farnesyl diphosphate and pentalenene proposed based on the crystal structure of pental-

(27) Isolation of protoilludene: Nozoe, S.; Robayashi, H.; Urano, S.; Furukawa, J. *Tetrahedron Lett.* **1977**, *16*, 1381−1384.

Scheme 5



Scheme 6



enene synthase[5] appear to allow His309 to approach the top left of **7** (as drawn in Figure 1), on the front face.

Deprotonation of **3′** at one of its methyl groups could lead directly to asterisca-3(15),6-diene (**11**, Scheme 6).[29] Deprotonation at other positions, coupled with full closure of the four-membered ring, would lead to isomers of **10**.

Finally, intermediate **8**, if actually formed despite its relatively high energy, could be deprotonated at various positions, leading to a variety of other sesquiterpene byproducts. In short, it is clear that pentalenene synthase must navigate around undesirable avenues for deprotonation.

Overall Implications. We have outlined two possible mechanisms for pentalenene formation (Figure 4). Both are quite exothermic from **1**/**1′** (overall, two π-bonds are exchanged for two new σ-bonds), both have relatively low overall activation barriers, and both differ considerably from previous mechanistic proposals in that they involve formation of 5/6/4 polycycles followed by rearrangement via unusual species that resemble [1,2] hydrogen shift transition structures interacting with nearby alkenes. In both cases, the geometric constraints imposed by the ring system have important effects; consider, for example, the intramolecular cation$-\pi$ interactions described above that are tied to the concertedness of the dyotropic rearrangement in the top mechanism of Figure 4 and the metastability of structure **8** in the bottom mechanism.

Unfortunately, these two mechanisms are indistinguishable based on the starting positions of the hydrogens in **1**/**1′** and their ultimate positions in **5**, and without including the enzyme environment in our models, we cannot know which, if either, of these mechanisms is actually utilized by pentalenene synthase. Deprotonation/reprotonation reactions could also replace some or all of the concerted hydrogen shifts shown in Figures 1 and 3,[6] but the transition structures for these processes appear to be low enough in energy without enzymatic participation that this would not be a necessity.

Much of the catalytic power of pentalenene synthase is likely to be employed in facilitating the departure of the pyrophosphate leaving group[14]—the barriers for rearrangement of the resulting farnesyl cation are, as we've shown above, inherently small—but this does not explain how the selectivity of the reaction is controlled. Although it is clear that farnesyl diphosphate must be folded correctly in order to form pentalenene,[6,30] correct folding does not necessarily ensure that pentalenene alone will be formed with high fidelity and efficiency.[6a] The presence of low energy diversions occurring after the initial humulyl cation forming cyclization step suggests that further enzymatic intervention is necessary to discourage the formation of alternative sesquiterpenes. This intervention could come in two forms: selective *stabilization* of desirable intermediates and transition structures (most likely through oriented noncovalent interactions)[1,5,6] or selective *destabilization* of undesirable intermediates and transition structures (through unfavorable electrostatic and/or steric interactions and/or by excluding basic groups from areas where deprotonation would lead to byproducts).

On the basis of existing evidence, we favor the top mechanism in Figure 4 (or a closely related pathway), although we cannot definitively rule out the bottom mechanism. First, the reported products of pentalenene mutations correspond to predicted diversions off of the top pathway—in terms of both structure and stereochemistry.[6a,b] It is of course dangerous to make arguments about the mechanism utilized by the wild-type enzyme based on byproducts that arise upon mutation of active site residues,[31] but we do not know of any observations of byproducts of mutant pentalenene synthases that have structures inconsistent with the top mechanism (e.g., **11**). We also note that the orientation of farnesyl diphosphate suggested based on the crystal structure of pentalenene synthase corresponds to that which is necessary to form **1** rather than **1′**,[5] although a binding orientation corresponding to **1′** does not appear to be definitively ruled out.[6] The conformation of cation **1** also mirrors that preferred for free humulene,[32] although **1** itself is several kcal/mol higher in energy than **1′**. This issue could in principle be settled if a crystal structure could be obtained using an unreactive analogue[33] of **1**/**1′** (e.g., the $C^+ \rightarrow NH^+$ analogue **12**). Use of an unreactive analogue of **7** could also be illuminating as to the viability of **7** in the wild-type and mutant enzyme active sites, as would experiments using the structures in Chart 1 or closely related structures as potential substrates.

(28) Segura, M. J. R.; Jackson, B. E.; Matsuda, S. P. T. *Nat. Prod. Rep.* **2003**, *20*, 304−317.
(29) (a) Mehta, G.; Umarye, J. D. *Tetrahedron Lett.* **2001**, *42*, 8101−8104. (b) Fricke, C.; Hardt, I. H.; König, W. A.; Joulain, D.; Zygadlo, J. A.; Guzman, C. A. *J. Nat. Prod.* **1999**, *62*, 694−696.
(30) Deligeorgopoulou, A.; Allemann, R. K. *Biochemistry* **2003**, *42*, 7741−7747.
(31) See, for example: Wendt, K. U. *Angew. Chem., Int. Ed.* **2005**, *44*, 3966−3971. Christianson, Cane, and co-workers have referred to such byproducts as "derailment products". See, for example, refs 5, 6.
(32) See, for example: Shirahama, H.; Osawa, E.; Matsumoto, T. *Tetrahedron Lett.* **1978**, 1987−1990. See also, ref 6.

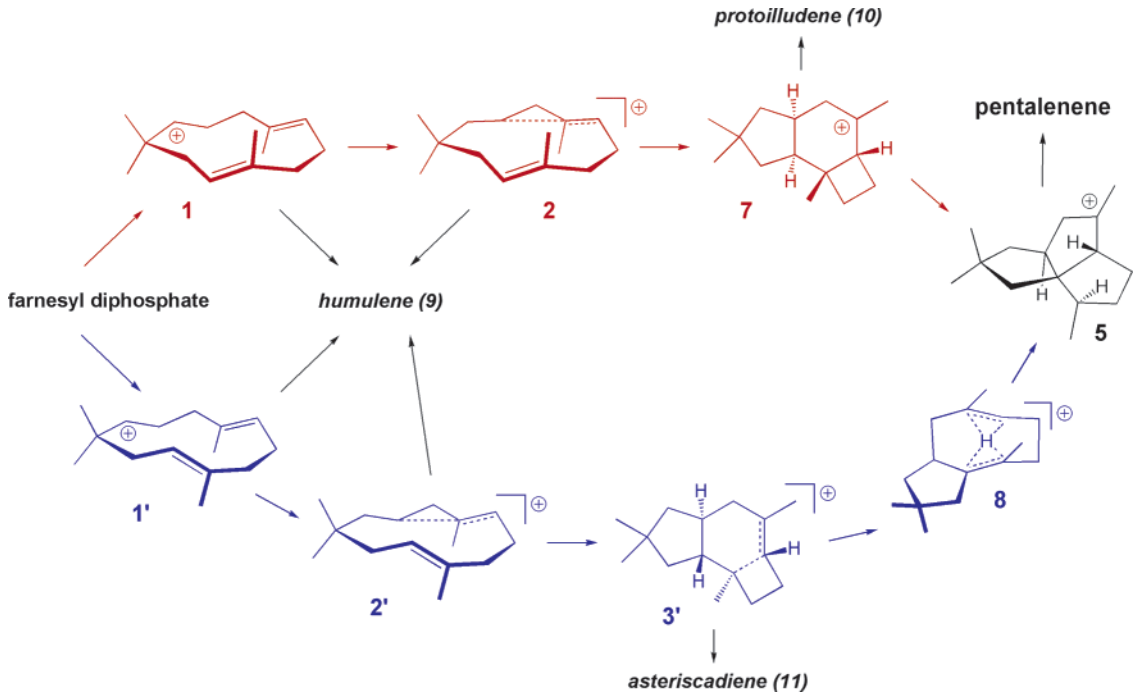
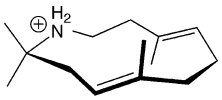


Figure 4. Two pathways to pentalenene (red and blue). Pathways to potential products of premature deprotonation that are known natural products are also shown.

Automated docking, molecular dynamics, and/or "theozyme"[34] studies will also be useful in settling some of the key mechanistic issues, and we are moving in these directions.



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Conclusions

Our quantum chemical studies on the mechanism of pentalenene formation from farnesyl diphosphate have revealed that the structures and inherent reactivities of the carbocations involved in this rearrangement are likely to be different than generally proposed. Unexpected intermediates such as **7** appear to be viable, as do unusual elementary steps such as the formal cycloaddition reaction that produces **7** and the dyotropic reaction that converts **7** to **5**. If either of our proposed mechanisms is utilized in the pentalenene synthase promoted reaction, the enzyme is likely to be involved in the following ways (in addition to facilitating the departure of the pyrophosphate group): (a) preorganization of the reactant conformation so as to favor production of one or the other of **1** and **1′**, (b) selective stabilization of the **7**-to-**5** transition structure or structures such as **8**, and (c) careful placement of basic functionalities such that the timing and location of deprotonation is stringently controlled. We hope that the mechanistic pictures we have presented herein will help to focus future experiments, both computational and biochemical, on this fascinating enzyme and will, in particular, aid in the interpretation and design of experiments involving active site mutants.

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Supporting Information Available: Coordinates and energies for all computed structures, along with the full Gaussian citation and stereoviews of structures in Figures 1 and 3. This material is available free of charge via the Internet at http://pubs.acs.org.

JA058031N

(33) For recent examples of this strategy and leading references, see: (a) Vedula, L. S.; Rynkiewics, M. J.; Pyun, H.-J.; Coates, R. M.; Cane, D. E.; Christianson, D. W. *Biochemistry* **2005**, *44*, 6153−6163. (b) Vedula, L. S.; Cane, D. E.; Christianson, D. W. *Biochemistry* **2005**, *44*, 12719−12727. (c) Whittington, D. A.; Wise, M. L.; Urbansky, M.; Coates, R. M.; Croteau, R. B.; Christianson, D. W. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15375−15380. Caution is necessary in such experiments, however; as noted in these reports, the binding conformations of such analogues do not always mirror the productive conformations of cationic intermediates.

(34) The modeling of enzyme−transition structure interactions using quantum mechanical calculations on small models of residues present in enzyme active sites has been referred to as the "theozyme" approach. For leading references, see: (a) Tantillo, D. J.; Chen, J.; Houk, K. N. *Curr. Opin. Chem. Biol.* **1998**, *2*, 743−750. (b) Tantillo, D. J.; Houk, K. N. Theozymes and Catalyst Design. In *Stimulating Concepts in Chemistry*; Wiley-VCH: Weinheim, Germany, 2000; pp 79−88. (c) Na, J.; Houk, K. N.; Shevlin, C. G.; Janda, K. D.; Lerner, R. A. *J. Am. Chem. Soc.* **1993**, *115*, 8453−8454. (d) Müller, C.; Wang, L.-H.; Zipse, H. Enzymes, Abzymes, Chemzymes—Theozymes? In *Transition State Modeling for Catalysis*; Truhlar, D. G., Morokuma, K., Eds.; ACS Symposium Series 721; American Chemical Society: Washington, DC, 1999; pp 61−73.