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Which Is More Likely in Trichodiene Biosynthesis: Hydride or Proton Transfer?

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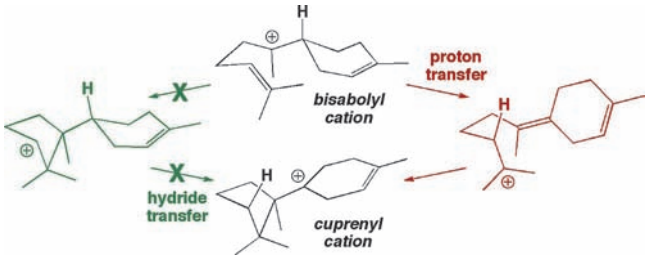
ABSTRACT

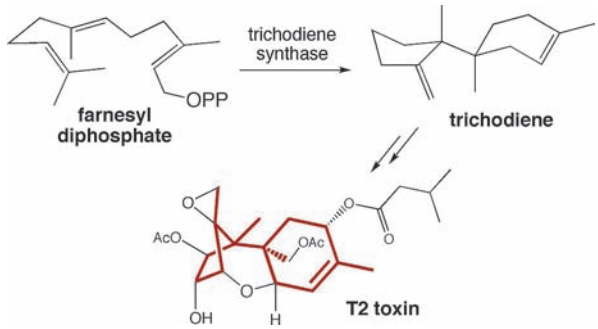


The mechanisms proposed for enzyme-catalyzed formation of the sesquiterpene natural product trichodiene consistently include a step involving a 1,4-hydride transfer. Using quantum chemical methods (B3LYP/6-31+G(d,p) and mPW1PW91/6-31+G(d,p)), we discovered two alternative pathways for transformation of the intermediate bisabolyl cation to the cuprenyl cation, one of which—a proton-transfer pathway—appears to be much more energetically favorable (by more than 10 kcal/mol) than the hydride transfer pathways usually proposed.

Trichodiene is a sesquiterpene natural product that is produced in fungi through the enzyme-catalyzed cyclization of farnesyl diphosphate (Scheme 1).[1] This hydrocarbon is the biosynthetic precursor of various antibiotics and toxins. For example, tricothecene mycotoxins such as T2 toxin (Scheme 1, trichodiene skeleton highlighted in red) disrupt protein synthesis in proliferating tissues and can cause various unpleasant medical conditions in humans ranging from skin irritation to death, depending on the nature of exposure.[2] Such compounds have also been implicated as biological warfare agents.[1,2]

The mechanisms generally proposed for enzyme-catalyzed trichodiene formation are exemplified by the pathways shown in black and green in Scheme 2.[1] In this mechanistic framework, initial isomerization of farnesyl diphosphate to (*R*)-nerolidyl diphosphate prepares the substrate for cyclohexene formation, which could occur either via formation

Scheme 1



(1) For leading references on trichodiene biosynthesis, including crystal structures of trichodiene synthase, see: (a) Vedula, L. S.; Cane, D. E.; Christianson, D. W. *Biochemistry* **2005**, *44*, 12719−12727. (b) Rynkiewicz, M. J.; Cane, D. E.; Christianson, D. W. *Biochemistry* **2002**, *41*, 1732−1741. (c) Rynkiewicz, M. J.; Cane, D. E.; Christianson, D. W. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 13543−13548. (d) Cane, D. E. *Pure. Appl. Chem.* **1989**, *61*, 493−496.

(2) Wannemacher, R. W., Jr.; Wiener, S. L. Trichothecene Mycotoxins. In *Medical Aspects of Chemical and Biological Warfare*; Sidell, F. R., Takafuji, E. T., Franz, D. R., Eds.; Office of the Surgeon General, Walter Reed Army Medical Center: Washington, DC, 1997; Chapter 34.

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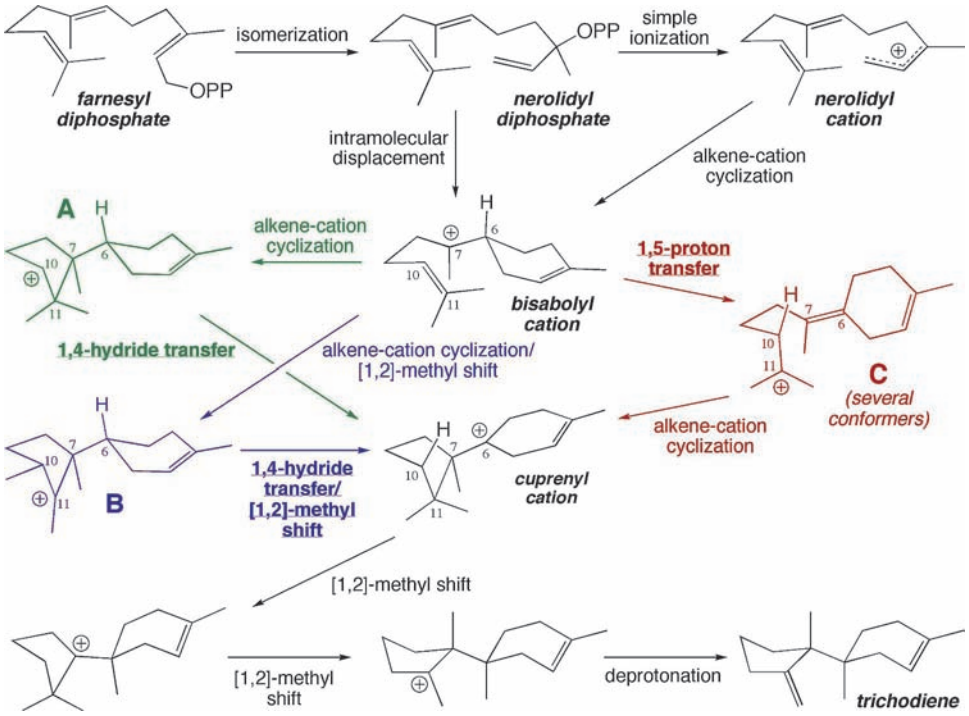
Scheme 2



of an allylic cation or via direct displacement of the pyrophosphate group by the central C=C π-bond of nerolidyl diphosphate. The resulting (*R*)-bisabolyl cation then rearranges to trichodiene by cyclization to cation **A**, hydride transfer (between carbons 6 and 10), two methyl shifts, and deprotonation. In this scheme, four carbocation intermediates between the bisabolyl cation and trichodiene are invoked, but it is still unclear which of these are actually formed as intermediates (consequently, in most mechanistic schemes, some of these structures are not shown explicitly).[1] In the course of our theoretical investigations on mechanisms for sesquiterpene formation,[3] however, we came across two alternative pathways for transformation of the bisabolyl cation to the cuprenyl cation (Scheme 2, blue and red), one of which appears to be considerably more favorable energetically than the hydride transfer pathways generally proposed.

The cyclization reaction leading to **A** (Scheme 2, green) would convert a tertiary to a secondary cation, but one could postulate that the associated loss of local stability could be counterbalanced by the conversion of a π-bond to a new σ-bond. Nonetheless, we were unable to locate a minimum corresponding to cation **A** using B3LYP calculations and instead consistently found a minimum corresponding to tertiary cation **B** (Scheme 2, blue).[4,5] We were able to locate a transition-state structure whose geometry looked as expected for formation of cation **A** (Figure 1, top left), but subsequent calculations indicated that this transition structure actually led to **B** via asynchronous *but concerted* cyclization and methyl shifting (between carbons 10 and 11) events. Another transition structure was located that connects **B** directly to the cuprenyl cation through asynchronous but concerted methyl shifting (back in the opposite direction) and hydride transfer events (Figure 1, top right). This

(3) This report is Part 2 in our "Theoretical Studies on Farnesyl Cation Cyclization" series; for Part 1, see: Gutta, P.; Tantillo, D. J. *J. Am. Chem. Soc.* **2006**, *128*, 6172−6179. For our related work on terpene-forming reactions, see: Bojin, M. D.; Tantillo, D. J. *J. Phys. Chem. A* **2006**, *110*, 4810−4816. Gutta, P.; Tantillo, D. J. *Angew. Chem., Int. Ed.* **2005**, *44*, 2719−2723. Ponec, R.; Bultinck, P.; Gutta, P.; Tantillo, D. J. *J. Phys. Chem. A* **2006**, *110*, 3785−3789. Ho, G. A.; Nouri, D. H.; Tantillo, D. J. *J. Org. Chem.* **2005**, *70*, 5139−5143.

(4) (a) All calculations were performed with: Frisch, M. J. et al. *Gaussian03*; Gaussian, Inc.: Pittsburgh, PA, 2003 (full reference in Supporting Information). Geometries were optimized using the B3LYP/6-31+G(d,p) method (Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648−5652. Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 1372−1377. Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785−789. Stephens, P. J.; Devlin, F. J.; Chabalowski, C. F.; Frisch, M. J. *J. Phys. Chem.* **1994**, *98*, 11623−11627). All structures were characterized by frequency calculations, and reported energies include zero-point energy corrections (unscaled). On the basis of a recent report that suggests that B3LYP may systematically underestimate the reaction energies for carbocation−alkene cyclization reactions and therefore suggests that mPW1PW91 single-point calculations may improve the energetics in such systems (Matsuda, S. P. T.; Wilson, W. K.; Xiong, Q. *Org. Biomol. Chem.* **2006**, *4*, 530−543), we also include mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G(d,p) energies for comparison; these energies include unscaled zero-point energy corrections from B3LYP/6-31+G(d,p) frequency calculations. Intrinsic reaction coordinate (IRC) calculations (Gonzalez, C.; Schlegel, H. B. *J. Phys. Chem.* **1990**, *94*, 5523−5527. Fukui, K. *Acc. Chem. Res.* **1981**, *14*, 363−368) were used to verify the identity of transition structures. Structural drawings were produced using Ball & Stick (Müller, N.; Falk, A. *Ball & Stick V.3.7.6*, molecular graphics application for MacOS computers; Johannes Kepler University: Linz, 2000). (b) Although the enzyme active site is not included in these calculations, we believe that the large energetic preference for one of the two pathways discussed herein is unlikely to be eliminated or counteracted by the enzyme. To test this contention, we are now pursuing additional calculations aimed at elucidating the role of specific substrate−active site and substrate−pyrophosphate interactions.

(5) Several conformations, close in energy, for each intermediate and transition structure were located. In this report, only one representative series of conformers is described. Other conformers and structures following the cuprenyl cation will be described in detail in a subsequent report.

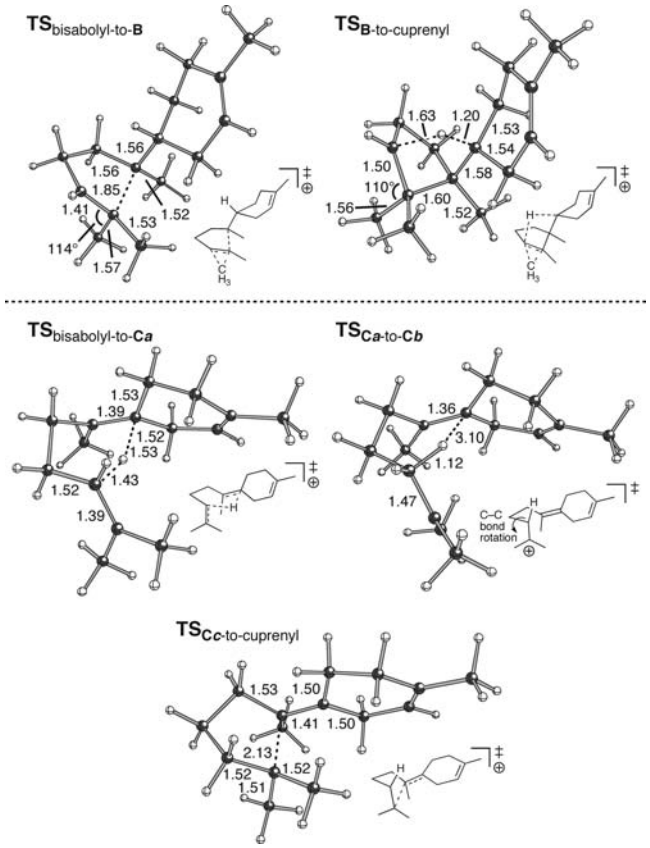


Figure 1. Transition-state structures[4,5] involved in the bisabolyl−cuprenyl cation interconversions shown in Scheme 2. Selected distances are shown in angstroms. Only transition structures whose relative energies are highlighted in bold in Table 1 are shown. See Supporting Information for geometries of other structures.

"temporary methyl shift" (i.e., the methyl group returns to exactly its original position) allows the system to avoid the formation of a secondary cation. The energies of the transition structures leading to and away from **B** are similar (Table 1).[6]

The second alternative pathway connecting the bisabolyl and cuprenyl cations involves a proton transfer (from carbon 6 to the *si* face of carbon 10), rather than a hydride transfer, to form one conformer of cation **C** (Scheme 2, red; transition structure in Figure 1). This sort of intramolecular proton transfer with a C=C π-bond acting as a base has been proposed previously in mechanisms for taxadiene[7] and abietadiene[8] biosynthesis. Although such a proton transfer could occur through a deprotonation/reprotonation sequence

Table 1. Relative Energies (kcal/mol) for Structures Involved in Interconverting Bisabolyl and Cuprenyl Cations[5]

	B3LYP/6-31+G(d,p)	mPW1PW91/6-31+G(d,p)// B3LYP/6-31+G(d,p)
bisabolyl[a]	[0.0]	[0.0]
$TS_{bisabolyl\text{-}to\text{-}B}$	**+25.9**	***+20.3***
B	−3.1	−9.6
$TS_{B\text{-}to\text{-}cuprenyl}$	***+27.1***	**+18.2**
$TS_{bisabolyl\text{-}to\text{-}C_a}$	**+8.6**	**+5.4**
$\mathbf{C_a}$	+4.5	+3.5
$TS_{C_a\text{-}to\text{-}C_b}$	***+9.1***	***+7.8***
$\mathbf{C_b}$	+8.8	+7.3
$TS_{C_b\text{-}to\text{-}C_c}$	+9.1	+7.6
$\mathbf{C_c}$	+1.7	−2.0
$TS_{C_c\text{-}to\text{-}cuprenyl}$	**+2.2**	**−2.7**
cuprenyl[a]	+0.4	−6.6

[a] Slightly different conformers of the bisabolyl and cuprenyl cations are involved in the two pathways, but only the energies for the lower-energy conformers are shown here; see Supporting Information for details.

involving a residue (or residues) in the enzyme active site, the barrier for the intramolecular process (Table 1) is low enough that this is certainly not a necessity. The initially formed conformer of **C** ($\mathbf{C_a}$) must then proceed through two other closely related conformers ($\mathbf{C_b}$ and $\mathbf{C_c}$; see Supporting Information for details) before ring closure to the cuprenyl cation. In fact, the highest-energy transition structure on this pathway is actually that for the interconversion of $\mathbf{C_a}$ and $\mathbf{C_b}$ (Figure 1), and once the proton transfer and conformational equilibration has occurred, the subsequent cyclization event is predicted to occur with little to no barrier (Table 1).[9] Our calculations indicate that the overall barrier for the bisabolyl−$\mathbf{C_a}$−$\mathbf{C_b}$−$\mathbf{C_c}$−cuprenyl pathway is more than 10 kcal/mol lower than that for the bisabolyl−**B**−cuprenyl pathway (Table 1; the highest-energy transition structures for each pathway are italicized).[4b]

Thus, although both hydride transfer and proton transfer mechanisms are consistent with published experimental observations (e.g., tritium labeling of the hydrogen that is drawn explicitly in Scheme 2),[1,10] our quantum chemical calculations strongly suggest that there is an inherent tendency in this system for the proton-transfer pathway. Considerable enzymatic intervention would be necessary to overcome this preference. Interestingly, although natural products that could be derived from direct deprotonation of **A** or **B** have not yet been reported, the isolation of iso-γ-bisabolene (below) was recently described.[11] Although it was proposed that this sesquiterpene is derived from a cuprenyl cation, a reasonable alternative is that it arises via simple deprotonation of **C**, if **C** exists in the relevant enzyme active site despite its predicted inclination to cyclize to the cuprenyl

(6) Some structures along IRCs from the bisabolyl cation to **B** and from **B** to the cuprenyl cation resemble hypothetical cation **A**. Such structures are generally 20 or more kcal/mol higher in energy than **B**. See Supporting Information for details.

(7) See, for example: Williams, D. C.; Carroll, B. J.; Jin, Q.; Rithner, C. D.; Lenger, S. R.; Floss, H. G.; Coates, R. M.; Williams, R. M.; Croteau, R. *Chem. Biol.* **2000**, *7*, 969−977. Density functional calculations also support the energetic feasibility of intramolecular proton transfer in this system: Gutta, P.; Tantillo, D. J., unpublished.

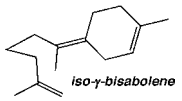
(8) For leading references, see: Ravn, M. M.; Peters, R. J.; Coates, R. M.; Croteau, R. *J. Am. Chem. Soc.* **2002**, *124*, 6998−7006.

(9) Note that, if not for the conformational contortions of cations **C**, the bisabolyl-**C**-cuprenyl conversion could be described as a concerted (but very asynchronous) process (which, if truly pericyclic would be orbital symmetry forbidden with the stereochemistry described).

(10) Arigoni, D.; Cane, D. E.; Müller, B.; Tamm, C. *Helv. Chim. Acta* **1973**, *56*, 2946−2949.

(11) Cool, L. G. *Phytochemistry* **2005**, *66*, 249−260.

cation (the enzyme that produces iso-γ-bisabolene has not yet been characterized).



In summary, we propose that an intramolecular proton transfer (rather than a hydride transfer) likely occurs during the trichodiene synthase reaction. To our knowledge, this is the first time that such a mechanism has been proposed for trichodiene synthase. In ongoing studies, we are exploring the remainder of the trichodiene formation mechanism, including diversions to byproducts, as well as the generality of "temporary alkyl shifts" and intramolecular proton transfers in biosynthesis.

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

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