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A Promiscuous Proton in Taxadiene Biosynthesis?

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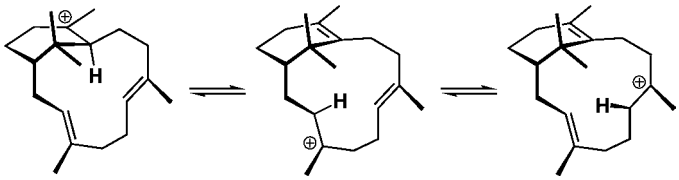
ABSTRACT



Herein we describe quantum chemical calculations on a two-step proton-transfer pathway that interconverts key intermediates in the biosynthesis of taxa-4,11-diene, a precursor of Taxol, that provides an energetically more favorable alternative to the usually proposed direct proton-transfer pathway. In effect, the bicyclic diterpene skeleton involved in this rearrangement provides a cage of three π-bonds that surrounds a locally mobile proton.

Taxa-4,11-diene is the biosynthetic precursor of Taxol (Scheme 1).[1] Like other diterpenoids, taxa-4,11-diene is produced in Nature from geranylgeranyl diphosphate (**GGPP**, Scheme 1).[2] It is generally proposed that conversion of **GGPP** to diterpenes such as taxa-4,11-diene is initiated by enzyme-catalyzed loss of the pyrophosphate group, followed by various cyclizations and rearrangements of the resulting carbocation in the enzyme active site.[1] Herein we describe quantum chemical calculations[3] on an unusual sequence of proton transfers that may occur in the taxadiene synthase-catalyzed production of taxa-4,11-diene.

The carbocation rearrangement leading to taxa-4,11-diene has been of interest for quite some time.[1,4] The type of mechanism generally proposed for this transformation is shown in Scheme 1. As drawn, this mechanism involves the initial production of an allylic carbocation (**1**), followed by four tertiary carbocations (**2**−**5**). The production of carbocations **3** and **5** is accompanied in each case by the conversion of a π-bond to a σ-bond as new rings are formed. Reasonable, and sometimes proposed, variations on this mechanism involve concerted cyclization and departure of the pyrophosphate group to avoid carbocation **1** (and perhaps even **2**),[5] and a deprotonation/reprotonation sequence to replace the intramolecular proton transfer that interconverts carbocations **3** and **4**.[1,4] However, labeling studies have indicated that this proton transfer is either intramolecular or the proton that is transferred is not exchanged with any other protons (from the enzyme or solvent).[4a,b,6] The viability of intramolecular proton transfer is the focus of this report.

(1) (a) Koepp, A. E.; Hezari, M.; Zajicek, J.; Vogel, B. S.; LaFever, R. E.; Lewis, N. G.; Croteau, R. *J. Biol. Chem.* **1995**, *270*, 8686−8690. (b) Hezari, M.; Lewis, N. G.; Croteau, R. *Arch. Biochem. Biophys.* **1995**, *322*, 437−444. (c) Jin, Y. H.; Williams, D. C.; Croteau, R.; Coates, R. M. *J. Am. Chem. Soc.* **2005**, *127*, 7834−7842. (d) Jin, Q. W.; Williams, D. C.; Hezari, M.; Croteau, R.; Coates, R. M. *J. Org. Chem.* **2005**, *70*, 4667−4675.

(2) Hanson, J. R. *Nat. Prod. Rep.* **2006**, 875−885 and references therein.

(3) All calculations were performed with *Gaussian03* (Frisch, M. J.; et al., Gaussian, Inc.: Pittsburgh, PA, 2003). Geometries were optimized using the B3LYP/6-31+G(d,p) method; see the Supporting Information for details, complete references, and comparisons with mPW1PW91 calculations. The range for electrostatic potential surfaces (Scheme 2) is +0.07 (red) to +0.13 au (blue); the front of each surface has been clipped to expose the interior.

(4) (a) Lin, X.; Hezari, M.; Koepp, A. E.; Floss, H. G.; Croteau, R. *Biochemistry* **1996**, *35*, 2968−2977. (b) Williams, C. D.; Carroll, B. J.; Jin, Q.; Rithner, C. D.; Lenger, S. R.; Floss, G. H.; Coates, R. M.; Williams, R. M.; Croteau, R. *Chem. Biol.* **2000**, *7*, 969−977. (c) Chow, S. Y.; Williams, H. J.; Huang, Q.; Nanda, S.; Scott, A. I. *J. Org. Chem.* **2005**, *70*, 9997−10003.

(5) (a) Similar proposals have been advanced for other terpene synthases. See, for example: Schenk, D. J.; Starks, C. M.; Rising Manna, K.; Chappell, J.; Noel, J. P.; Coates, R. M. *Arch. Biochem. Biophys.* **2006**, *448*, 31−44. (b) Labeling experiments (see ref 1c) have shown that the stereochemical course of the taxadiene-forming rearrangement is indeed consistent with direct conversion of **GGPP** to **3** without the intermediacy of **1** or **2**.

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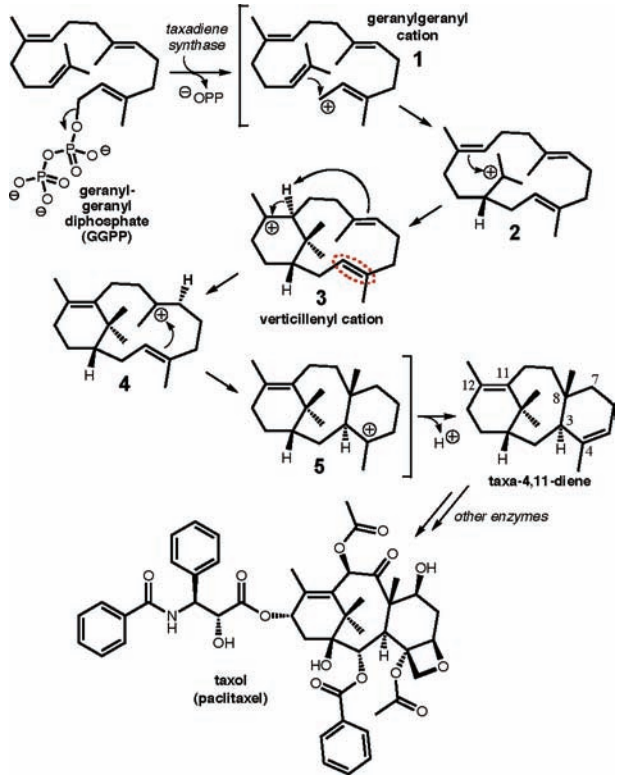
Scheme 1



We attempt to address the following questions related to the **3**-to-**4** reaction using quantum chemical methods,[3] and thereby provide a description of this system's inherent reactivity against which the effects of the surrounding enzyme can later be assessed: (1) Can a simple transition state structure for the **3**-to-**4** reaction be located, and if so, is the proton transfer associated with a small or large activation barrier (the latter would suggest that this step may require enzymatic intervention)? (2) Alternatively, is a minimum with a bridging proton possible for this system?[7] (3) Is the other nearby double bond (highlighted for **3** in Scheme 1) somehow involved in this process?

First, we attempted to locate a stationary point with a bridging proton to ascertain if such a structure is a transition state structure for the **3**-to-**4** reaction or is instead a minimum (a so-called "proton sandwich"[7]). The structure that we located, which has a proton that is nearly symmetrically bridging, is a transition state structure for the direct conversion of **3** to **4** ($\mathbf{TS}_{\mathbf{3-to-4}}$, Figure 1). The computed barrier associated with this proton transfer, in the **3**-to-**4** direction, is 11.3 kcal/mol (Scheme 2).[3,8,9] Structures **3** and **4** are similar in energy, **4** being only 2.2 kcal/mol higher in energy than **3**.[3,9] Note that in **3** and **4** the proton that will migrate is on

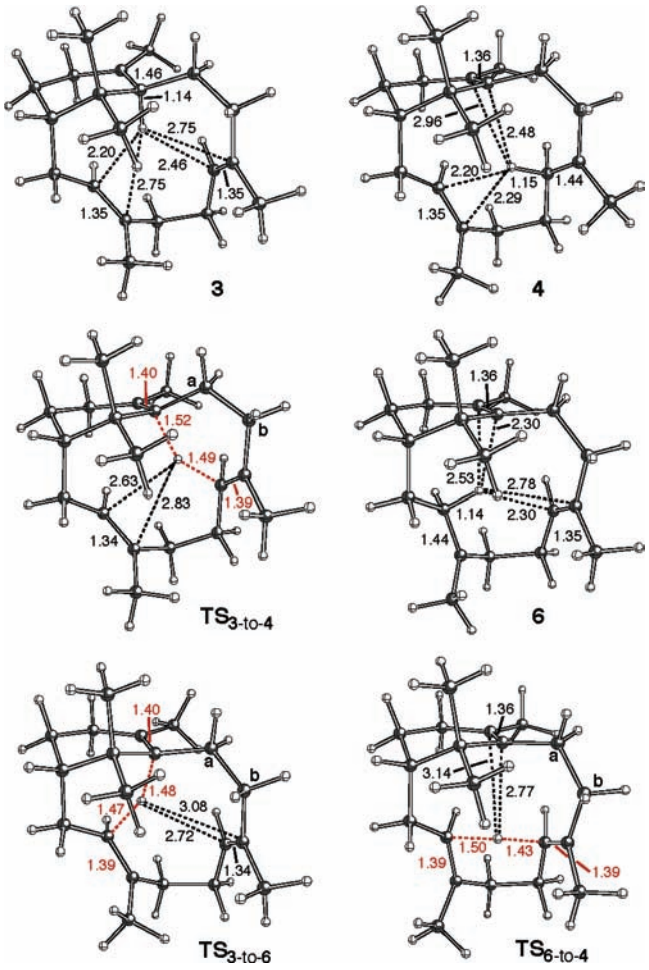


Figure 1. Computed geometries (B3LYP/6-31+G(d,p), selected distances in Å) of carbocations **3**, **4**, and **6**, and the transition state structures for their interconversion.[3] See Scheme 2 for relative energies (in kcal/mol).

the "inside" of the 12-membered ring and the corresponding C−H bonds are elongated slightly to 1.14 and 1.15 Å, respectively (most likely due primarily to hyperconjugation, although intramolecular cation−π interactions may also contribute).

A closer look at structure **3** reveals that while the hydrogen that will migrate in the **3**-to-**4** reaction is 2.46 Å away from the recipient carbon of the appropriate π-bond (the 7,8-bond; taxane numbering), the distance to the other π-bond (the 3,4-bond, highlighted in Scheme 1) is only 2.20 Å. This suggests that an alternative proton transfer to this π-bond (to the *re*

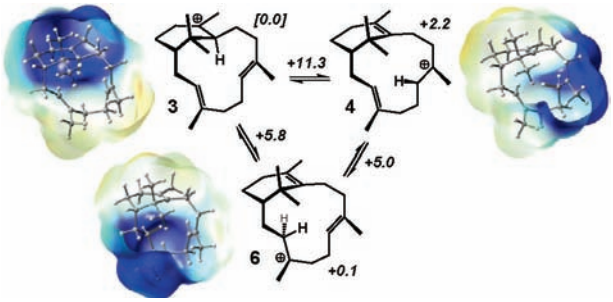
(6) Similar intramolecular proton transfers have been proposed to occur in the biosynthesis of trichodiene (Hong, Y. J.; Tantillo, D. J. *Org. Lett.* **2006**, *8*, 4601−4604) and abietadiene (Ravn, M. M.; Peters, R. J.; Coates, R. M.; Croteau, R. *J. Am. Chem. Soc.* **2002**, *124*, 6998−7006).

(7) We have previously found minima with bridging protons in our studies on the biosynthesis of the sesquiterpene pentalenene: (a) Gutta, P.; Tantillo, D. J. *J. Am. Chem. Soc.* **2006**, *128*, 6172−6179. (b) Gutta, P.; Tantillo, D. J. *Angew. Chem., Int. Ed.* **2005**, *44*, 2719−2723.

(8) In ref 4b, semiempirical (AM1) calculations on **3** and **4** were described, but no computed transition structures were reported. This report did, however, suggest that direct proton transfer to form **4** was geometrically feasible.

(9) HF/6-31G(d) calculations on **3**, **4**, and **6** (but not the transition structures between them) were described previously; their relative energies spanned a range of <4 kcal/mol. See: Tokiwano, T.; Endo, T.; Tsukagoshi, T.; Goto, H.; Fukushi, E.; Oikawa, H. *Org. Biomol. Chem.* **2005**, *3*, 2713−2722. Although the **3** to **6** rearrangement was mentioned in this paper, it was not proposed as a route to taxa-4,11-diene (i.e., that **6** could go on to **4**). A similar proton transfer was also proposed as a route to a fluoroverticellitriene from 6-fluoro-**GGPP**; see ref 1c.

Scheme 2



face of C3), leading to **6**, is also feasible (Scheme 2). If such a proton transfer occurs readily, it could be followed by another proton transfer that then leads to **4**.

Structure **6** is higher in energy than **3** by only 0.1 kcal/mol (Scheme 2).[3,9] The transition state structures that connect **3** to **6** and **6** to **4** (Figure 1) are only 5.8 and 5.0 kcal/mol higher in energy, respectively, than **3**, indicating that the **3**-to-**6**-to-**4** pathway is actually substantially more favorable energetically than the direct **3**-to-**4** pathway (whose transition structure is 11.3 kcal/mol higher in energy than **3**). The higher energy of $\mathbf{TS_{3-to-4}}$ compared to that of $\mathbf{TS_{3-to-6}}$ and $\mathbf{TS_{6-to-4}}$ is likely the result of many small differences in strain in various portions of these structures; for example, the $C-C_a-C_b-C$ dihedral angle in $\mathbf{TS_{3-to-4}}$ is 30°, while those in $\mathbf{TS_{3-to-6}}$ and $\mathbf{TS_{6-to-4}}$ are 57° and 38°, respectively (see Figure 1).

Although no minimum with a bridging proton was found,[7] our calculations indicate that movement of the proton between the π-bonds of the surrounding carbon framework (i.e., interconversion of **3**, **4**, and **6**) is facile and certainly would not require enzymatic intervention on energetic grounds.[8,10] Here we have a mobile proton in a cage of three π-bonds.

If **6** is an intermediate in the taxadiene synthase reaction, it is possible that byproducts derived from it might be observed, for example, in future studies involving mutation of active site residues.[11,12] Several natural products that could arise from **6** via (often somewhat circuitous) diversions away from the taxadiene-forming pathway have been described.[13−15] In addition, structures resembling **6**, which has a considerably different charge distribution than **3** or **4** (Scheme 2[3]), might be competent inhibitors of taxadiene synthase and/or might be useful for crystallographic studies.[16]

To our knowledge, the **3**-to-**6**-to-**4** detour for the formation of taxa-4,11-diene has not been proposed previously.[9] Nonetheless, our calculations indicate that it is the inherently preferred pathway from **3** to **4**.[17] If and how the enzyme active site might manipulate the relative energies of **3**, **4**, **6**, and the transition structures connecting them (their different charge distributions provide an avenue for selective stabilization via noncovalent interactions) remains an open question. This issue,[18] the energetics of the remaining mechanistic steps in Scheme 1, and possible diversions from intermediate **6**[12,13] are currently under study.

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

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(10) Tunneling may also contribute in these reactions. For related examples and leading references, see: Nagel, Z. D.; Klinman, J. P. *Chem. Rev.* **2006**, *106*, 3095−3118.

(11) Similar studies have proven useful in probing mechanisms for formation of other terpenes. For a review, see: Segura, M. J. R.; Jackson, B. E.; Matsuda, S. P. T. *Nat. Prod. Rep.* **2003**, *20*, 304−317.

(12) See the Supporting Information and ref 9 for structures of possible byproducts resulting from diversions from **6**.

(13) See, for example, the following structures in the references indicated: structure **74** in Hanson, J. R. *Nat. Prod. Rep.* **2002**, *19*, 125−132, structure **97** in Hanson, J. R. *Nat. Prod. Rep.* **2000**, *17*, 165−174, structure **118** in Hanson, J. R. *Nat. Prod. Rep.* **1996**, *13*, 59−71, structure **166** in Hanson, J. R. *Nat. Prod. Rep.* **1994**, *11*, 265−277, and structure **126** in Hanson, J. R. *Nat. Prod. Rep.* **1998**, *15*, 93−106. See also refs 1c and 9 for leading references on verticillatrienes that might also be formed from **6**.

(14) Note that proton transfers of the **4**-to-**6** variety, in which other (diastereotopic) protons are transferred, also provide mechanisms of *E*/*Z* isomerization of the C═C double bonds in **3**, **5**, and **6**.

(15) Several conformers of **3** (less than 3.5 kcal/mol away from the energy of **3**) were also examined. In all of the structures located, the "lower left" C_3═C_4 double bond is closer to the migrating hydrogen than is the "righthand" C_7═C_8 double bond. The conformer of **3** discussed explicitly in the text is the one that leads directly to the experimentally observed diastereomer of taxadiene. Details on the other conformers can be found in the Supporting Information. These structures are similar to those described previously at a lower level of theory,[9] although some differences in the conformational landscape for **3** are observed at the higher level of theory used herein.

(16) Recent crystallographic studies of terpene synthase complexes with carbocation analogues include: (a) Vedula, L. S.; Cane, D. E.; Christianson, D. W. *Biochemistry* **2005**, *44*, 12719−12727. (b) 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.

(17) This is another example of a case where the "simplest" or "least-motion" mechanism is not necessarily the lowest energy pathway. For an interesting discussion of such issues, see: Hoffmann, R.; Minkin, V. I.; Carpenter, B. K. *HYLE−Int. J. Phil. Chem.* **1997**, *3*, 3−28 (reprinted from: *Bull. Soc. Chim. Fr.* **1996**, *133*, 117−130).

(18) We are pursuing additional calculations aimed at probing specific substrate−active site and substrate−pyrophosphate interactions.