ORGANIC LETTERS

2011
Vol. 13, No. 6
1294–1297

How Many Secondary Carbocations Are Involved in the Biosynthesis of Avermitilol?

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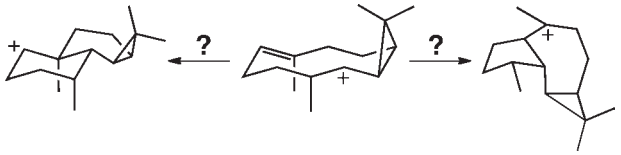
Received December 20, 2010

ABSTRACT



Quantum chemical calculations were used to assess the viability of proposed secondary carbocations as intermediates in the biosynthesis of avermitilol. One, a cyclopropylcarbinyl cation, was found to be a true minimum, while another, a simple secondary cation, was found to exist only as part of a transition structure for water capture.

Secondary carbocations have been proposed as intermediates in the biosynthesis of many terpene natural products.[1] On the basis of quantum chemical calculations, however, the formation of many of these species has been shown to be inherently unfavorable. Although some secondary carbocations are found to be true minima on potential energy surfaces for sesquiterpene-forming carbocation rearrangements,[2] many more are not and instead appear to be transition-state structures (or occur at other points along reaction coordinates, if at all) for rearrangements where the steps in which secondary cations were expected to form and react are combined (usually asynchronously) into concerted processes.[3] In several cases, two secondary carbocations that were proposed to form sequentially can be avoided via so-called "triple shifts".[4] It has also been shown, however, that some (though definitely not all) secondary carbocations that otherwise would not be potential energy surface minima can actually exist as minima if they participate in specifically oriented intermolecular interactions that enhance particular stereoelectronic interactions within the carbocation framework.[4,5] Clearly, whether or not a secondary carbocation will exist as a discrete intermediate is challenging to predict, but the guiding principles are becoming ever more clear.[3−5]

Set against this backdrop, we embarked on a study of avermitilol (**4**, Scheme 1).[6] This sesquiterpene alcohol, isolated

(1) (a) Reviews: Davis, E. M.; Croteau, R. *Top. Curr. Chem.* **2000**, *209*, 53–95. Christianson, D. W. *Chem. Rev.* **2006**, *106*, 3412–3442. Giner, J. L. *Chem. Rev.* **1993**, *93*, 1735–1752. Cane, D. E. *Chem. Rev.* **1990**, *90*, 1089–1103. (b) Selected examples of proposed secondary carbocations: Vedula, L. S.; Zhao, Y.; Coates, R. M.; Koyama, T.; Cane, D. E.; Christianson, D. W. *Arch. Biochem. Biophys.* **2007**, *466*, 260–266. Croteau, R. B.; Wheeler, C. J.; Cane, D. E.; Ebert, R.; Ha, H. J. *Biochemistry* **1987**, *26*, 5383–5389. Ravn, M. M.; Peters, R. J.; Coates, R. M.; Croteau, R. *J. Am. Chem. Soc.* **2002**, *124*, 6998–7006. Roy, A.; Roberts, F. G.; Wilderman, P. R.; Zhou, K.; Peters, R. J.; Coates, R. M. *J. Am. Chem. Soc.* **2007**, *129*, 12453–12460. Lesburg, C. A.; Zhai, G.; Cane, D. E.; Christianson, D. W. *Science* **1997**, *277*, 1820–1824. Little, D. B.; Croteau, R. B. *Arch. Biochem. Biophys.* **2002**, *402*, 120–135. Chou, W. K. W.; Fanizza, I.; Uchiyama, T.; Komatsu, M.; Ikeda, H.; Cane, D. E. *J. Am. Chem. Soc.* **2010**, *132*, 8850–8851.

(2) (a) Gutta, P.; Tantillo, D. J. *J. Am. Chem. Soc.* **2006**, *128*, 6172–6179. (b) Wang, S. C.; Tantillo, D. J. *Org. Lett.* **2008**, *10*, 4827–4830. (c) Lodewyk, M. W.; Gutta, P.; Tantillo, D. J. *J. Org. Chem.* **2008**, *73*, 6570–6579. (d) Hong, Y. J.; Tantillo, D. J. *Chem. Sci.* **2010**, *1*, 609–614.

(3) For leading references on concerted but asynchronous carbocation rearrangements relevant to terpene biosynthesis, see: (a) Tantillo, D. J. *Chem. Soc. Rev.* **2010**, *39*, 2847–2854. (b) Tantillo, D. J. *J. Phys. Org. Chem.* **2008**, *21*, 561–570. (c) Hong, Y. J.; Tantillo, D. J. *Org. Biomol. Chem.* **2010**, *8*, 4589–4600. (d) A seminal report: Hess, B. A., Jr. *J. Am. Chem. Soc.* **2002**, *124*, 10286–10297. Note, however, that most studies to date have not included full-sized enzyme models; for those that have, see: (e) Weitman, M.; Major, D. T. *J. Am. Chem. Soc.* **2010**, *132*, 6349–6360. (f) Rajamani, R.; Gao, J. *J. Am. Chem. Soc.* **2003**, *125*, 12768–12781. (g) Allemann, R. K.; Young, N. J.; Ma, S.; Truhlar, D. G.; Gao, J. *J. Am. Chem. Soc.* **2007**, *129*, 13008–13013.

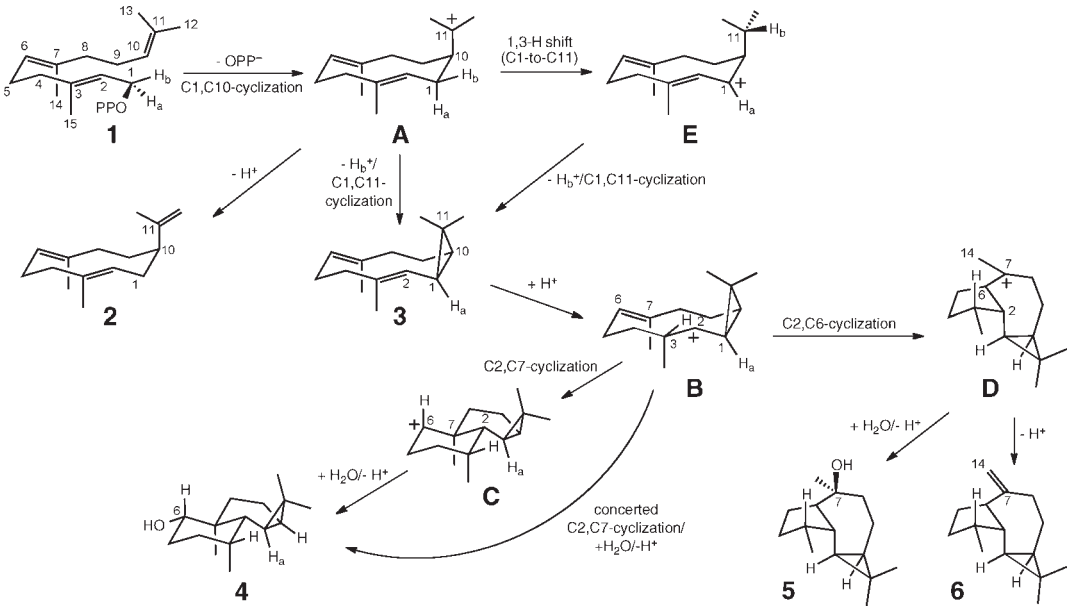
(4) Hong, Y. J.; Tantillo, D. J. *J. Am. Chem. Soc.* **2010**, *132*, 5375–5386.

(5) (a) Hong, Y. J.; Tantillo, D. J. *J. Org. Chem.* **2007**, *72*, 8877–8881. (b) Hong, Y. J.; Tantillo, D. J. *J. Am. Chem. Soc.* **2009**, *131*, 7999–8015.

(6) Theoretical Studies on Farnesyl Cation Cyclization. 8. For part 7, see ref 2d. For reviews with leading references to theoretical work (by us and others) on closely related systems, see ref 3a,3b.

10.1021/ol103079v
Published on Web 02/15/2011

Scheme 1. Proposed Mechanisms for Formation of **2**−**6**



recently by Cane and co-workers,[7] looks as though it should arise via a rearrangement involving the sequential formation of two secondary carbocations (**B** and **C**, Scheme 1).[8] We were curious as to whether or not these proposed intermediates are likely to be true minima or instead will occur elsewhere along a reaction coordinate in which C2−C7 bond formation, C6−O bond formation, and perhaps even protonation to form **B**, are combined into a concerted process. At first glance, we felt that carbocation **B** could be a true intermediate if the geometric constraints associated with its bicyclic framework would allow the carbocation center (C2) to enjoy the expected stabilization associated with being adjacent to a cyclopropane ring (i.e., **B** is a cyclopropylcarbinyl cation).[9] Conversely, carbocation **C** does not look to be predisposed to enjoy any special stabilization, and consequently, we suspected that this carbocation likely would not exist as a minimum in the absence of C5−H···X interactions with active site functionality[4,5,10] or perhaps direct donation to C6 by the lone pair of an active site water molecule.[4,5b] These proposals were tested herein using quantum chemical calculations.[11] The reactivity of putative carbocation **B** was also further explored by examining the formation of viridiflorol (**5**) and *allo*-aromadendrene (**6**).[12]

Although we had no reason to question the reported structure of **4**, we felt it would be prudent to confirm that ^{1}H and ^{13}C NMR chemical shifts predicted using our

(7) Chou, W. K. W.; Fanizza, I.; Uchiyama, T.; Komatsu, M.; Ikeda, H.; Cane, D. E. *J. Am. Chem. Soc.* **2010**, *132*, 8850–8851.

(8) Note that the reactions that involve these two putative secondary cations are different than those associated with the "triple shifts" mentioned above.[4]

(9) Leading references: Nouri, D. H.; Tantillo, D. J. *J. Org. Chem.* **2006**, *71*, 3686–3695. Olah, G. A.; Prakash, G. K. S.; Rawdah, T. N. *J. Org. Chem.* **1980**, *45*, 965–969. Hong, Y. J.; Tantillo, D. J. *Org. Biomol. Chem.* **2009**, *7*, 4101–4109. Friedrich, E. C. In *The Chemistry of the Cyclopropyl Group*; Rappoport, Z., Ed.; Wiley: New York, 1987. Boche, G.; Walborsky, H. M In *Cyclopropane Derived Reactive Intermediates*; Patai, S., Rappoport, Z., Eds.; Wiley: Chichester, 1990; Chapter 3.

(10) Bojin, M. D.; Tantillo, D. J. *J. Phys. Chem. A* **2006**, *110*, 4810–4816.

(11) All calculations were performed with GAUSSIAN03 (Frisch, M. J. et al. *Gaussian03, revision D.01*, Gaussian, Inc.: Pittsburgh, PA, 2003, full reference in the Supporting Information). Structures 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). Previous studies have suggested that the B3LYP method performs well in predicting geometries and reactivity of carbocations, and results using this method have been compared with other DFT and non-DFT methods (e.g., ref 3c,3e). We also report mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G(d,p) energies, which compensate for a well-known tendency of B3LYP to underestimate the stability of cyclic structures compared to acyclic isomers (e.g., ref 3c and: Matsuda, S. P. T.; Wilson, W. K.; Xiong, Q. *Org. Biomol. Chem.* **2006**, *4*, 530−543). Stationary points were characterized by frequency calculations and reported energies include B3LYP/6-31+G(d,p) zero-point energy corrections. Intrinsic reaction coordinate (IRC) calculations were used for further characterization of all transition-state structures (Gonzalez, C.; Schlegel, H. B. *J. Phys. Chem.* **1990**, *94*, 5523–5527. Fukui, K. *Acc. Chem. Res.* **1981**, *14*, 363−368) and IRC plots are included in the Supporting Information. Structural drawings were produced using *Ball & Stick* (Müller, N.; Falk, A.; Gsaller, G. *Ball & Stick V.4.0a12, Molecular Graphics Application for MacOS Computers*, Johannes Kepler University, Linz, 2004). Atom numbering indicated in the structures in this report is based on that of **1** (Scheme 1).

(12) (a) Garms, S; Köllner, T. G.; Boland, W. *J. Org. Chem.* **2010**, *75*, 5590–5600. (b) Bombarda, I.; Raharivelomanana, P.; Ramanoelina, P. A. R.; Faure, R.; Bianchini, J.-P.; Gaydou, E. M. *Anal. Chim. Acta* **2001**, *447*, 113–123.

(13) (a) Selected reviews: Mulder, F. A. A.; Filatov, M. *Chem. Soc. Rev.* **2010**, *39*, 578–590. Petrovic, A. G.; Navarro-Vázquez, A.; Alonso-Gómez, J. L. *Curr. Org. Chem.* **2010**, *14*, 1612–1628. Barone, V.; Improta, R.; Rega, N. *Acc. Chem. Res.* **2008**, *41*, 605–616. Bifulco, G.; Dambruoso, P.; Gomez-Paloma, L.; Riccio, R. *Chem. Rev.* **2007**, *107*, 3744–3779. Bagno, A.; Saielli, G. *Theor. Chem. Acc.* **2007**, *117*, 603–619. Helgaker, T.; Jaszuński, M.; Ruud, K. *Chem. Rev.* **1999**, *99*, 293–352. Casabianca, L. B.; de Dios, A. C. *J. Chem. Phys.* **2008**, *128*, 052201−1–052201−10. (b) A recent application: Smith, S. G.; Goodman, J. M. *J. Am. Chem. Soc.* **2010**, *132*, 12946–12959. (c) See also: http://cheshirenmr.info.

(14) We also computed chemical shifts for **5**. See the Supporting Information for details.

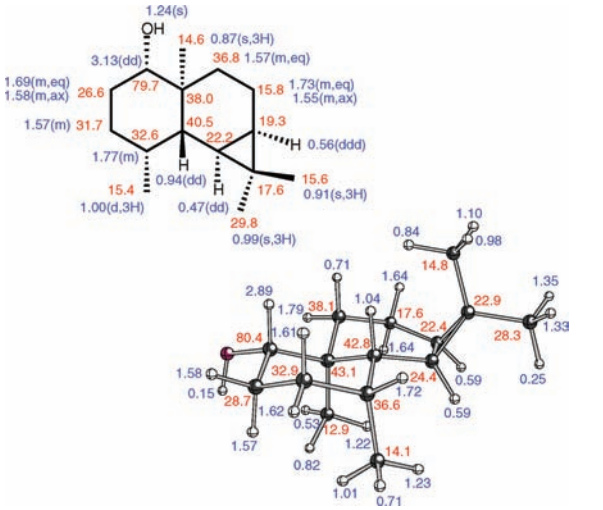


Figure 1. Computed (bottom: CPCM($CHCl_3$,UAKS)-B3LYP/6-311+G(2d,p)//B3LYP/6-31+G(d,p), scaled[13c]) and experimentally determined[7] (top: in $CDCl_3$) ^1H (blue) and ^{13}C (red) NMR chemical shifts (ppm) for **4**.

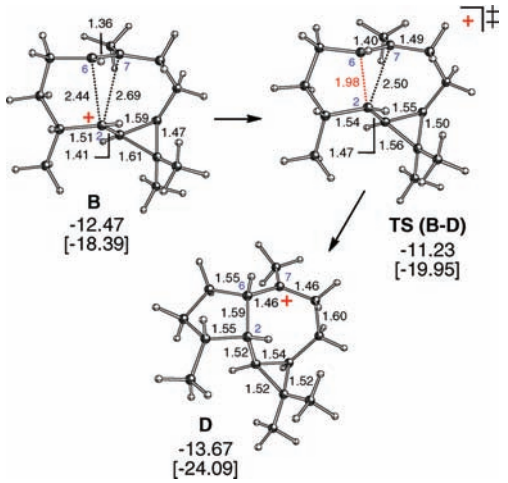


Figure 2. Conversion of **B** to **D**. Computed distances (Å) and energies (kcal/mol, relative to that of **A**) are shown (B3LYP/6-31+G(d,p) in normal text and mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G(d,p) in brackets).

quantum chemical methods were consistent with experimentally determined values. The use of computed chemical shifts to confirm and reassign the structures of complex organic molecules is becoming ever more common,[13] and structure **4** with a polar functional group embedded in an otherwise nonpolar framework sporting a cyclopropane ring and two quaternary carbons provides a good test of this methodology.[14] Computed[13c] and experimentally determined[7] chemical shifts for **4** are shown in Figure 1. Overall, the computed shifts closely match the experimental shifts. Mean absolute deviations of 0.08 and 2.29 ppm for ^1H and ^{13}C, respectively, and maximum deviations of 0.24 and 5.31 ppm for ^1H and ^{13}C, respectively, are observed. Note that the largest deviations are observed for ^{13}C shifts of quarternary carbons, but even these are only approximately 5 ppm, not a large deviation for ^{13}C shifts.[13]

Sesquiterpenes **4**−**6** are all derived, putatively, from secondary carbocation **B**, so we focused first on the inherent stability and reactivity of this proposed intermediate (i.e., without enzyme present).[15] The first issue to be addressed is whether or not **B** even exists as a minimum. Indeed it does, although just barely (Figure 2). The computed structure of **B** shows the geometric features expected for stabilized cyclopropylcarbinyl cations: elongated C−C bonds in the cyclopropane ring involving the carbon adjacent to the carbocation center (here, both are approximately 1.6 Å long) and a shortened C−C bond opposite to the carbon adjacent to the carbocation center (here, 1.47 Å long).[3a,9] The carbocation center in **B** is also close in space to the C=C π-bond on the other side of the 10-membered ring in which it resides. This carbocation-π interaction[3a,5a,16] likely provides some internal stabilization to **B**, but also to transition state structures for cyclization, in which this "noncovalent" interaction progresses toward full covalency. Consequently, conversion of **B** to **D**, for example, is essentially barrierless (Figure 2).[17] Thus, we would consider carbocation **B** to be metastable at best. Deprotonation of **D** (which is only slightly lower in energy than **B**, despite being a tertiary carbocation and possessing one additional σ-bond) would lead to *allo*-aromadendrene (**6**).

Not surprisingly, putative secondary carbocation **C**, which is not expected to enjoy any sort of special stabilization, was not located as a minimum. Optimization of structures resembling **C** led instead to **B**.

Having examined **B**−**D** in isolation, we proceeded to consider their interactions and reactions with water. Conversion of a **B**·H_2O complex to protonated **4** is shown in Figure 3. The **B**·H_2O complex is held together by weak C−H···O interactions[4,5,10] and does not correspond to a productive arrangement for water attack, but there is no significant barrier for moving the water molecule near to C6 (and an appropriate orientation would likely be enforced in the active site of an enzyme that produces **4**). Concerted C2,C7-cyclization and attack of water on C6 via **TS (B-4)** leads directly to protonated **4** and is accompanied by a low barrier (Figure 3). Here, the transition-state

(15) Note that **4** is predicted to be 23 kcal/mol lower in energy than **3** + H_2O (B3LYP/6-31+G(d,p)). We also examined the interconversion of **A**, **B**, **E**, **2**, and **3** and attempted (without success) to find a transition-state structure for concerted protonation of **3**/cyclization/water capture. See the Supporting Information for details.

(16) Jenson, C.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1997**, *119*, 10846–10854. Miklis, P. C.; Ditchfield, R.; Spencer, T. A. *J. Am. Chem. Soc.* **1998**, *120*, 10482–10489. Antonello, F.; Graziella, R.; Gabriele, R.; Felice, G.; Maurizio, S. *Chem.—Eur. J.* **2003**, *9*, 2072–2078. Dietmar, H. *Angew. Chem., Int. Ed.* **2002**, *41*, 3208–3210.

(17) (a) At the B3LYP/6-31+G(d,p) level, the barrier is approximately 1 kcal/mol, and at the mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G(d,p) level, there is no barrier. (b) See the Supporting Information for a discussion of the alternative tertiary cation that could be formed by protonation of the C2=C3 π-bond of **3**.

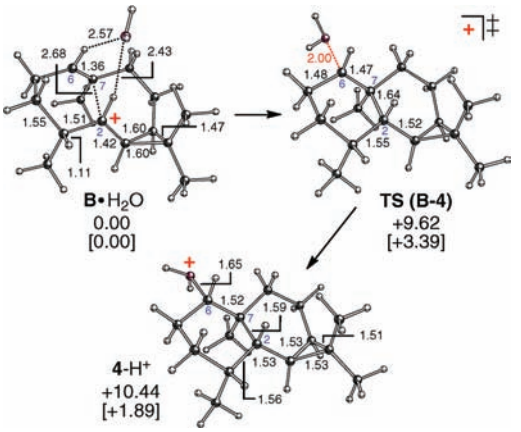


Figure 3. Formation of **4**. Computed distances (Å) and energies (kcal/mol, relative to **B**·H_2O) are shown (B3LYP/6-31+G(d,p) in normal text and mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G(d,p) in brackets). The B3LYP/6-31+G(d,p) energy of **TS (B-4)** is lower than that of **4**-H^+ due to zero-point energy corrections.

structure resembles a water complex of carbocation **C** and thus joins the growing list of transition-state structures that resemble proposed secondary carbocation intermediates.[2c,4,5b] Note that although cyclization and water attack are concerted, they occur asynchronously,[3a-d] with cyclization largely preceding attack. Note also that the reaction is predicted to be readily reversible and would need to be driven forward by deprotonation to form **4**.

Conversion of **D**·H_2O complexes to protonated **5** was also examined, but we were unable to locate a **5**-H^+ structure. Attempts led instead back to **D**·H_2O complexes. Inclusion of a second water molecule, however, changed the situation. As shown in Figure 4, the conversion of **D**·H_2O·H_2O to **5**-H^+·H_2O is predicted to occur with a very small barrier. In an enzyme active site (not modeled explicitly here), two water molecules could be present or the role of the second water molecule could be played by a different active site hydrogen bond acceptor.[18] Note that, like the reaction to form **4**-H^+, formation of **5**-H^+ is predicted to be reversible.

(18) We also examined the potential of formate (a small model of an active site aspartate) as a hydrogen bond acceptor in this reaction. See the Supporting Information for details.

(19) Unlike previous cases where two sequential secondary carbocations have been predicted to be avoided via triple-shift reactions,[4] only one of the putative secondary carbocations in the pathway to avermitilol is predicted to be avoided, perhaps due in part to the different nature of the events that lead to the formation and subsequent reaction of the first carbocation in this case—alkene protonation followed by cation−alkene cyclization, rather than an alkyl shift followed by a hydride shift as in the systems that participate in triple shifts.

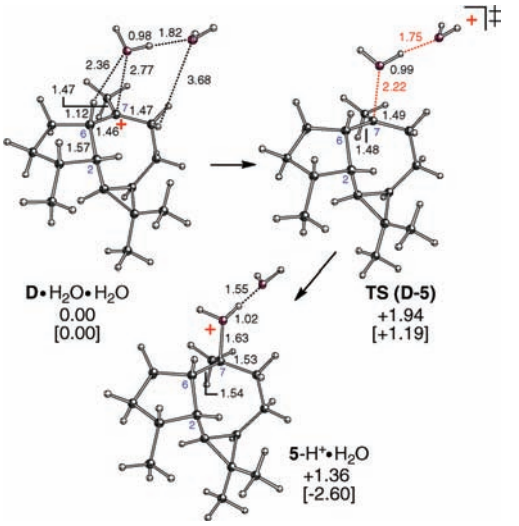


Figure 4. Formation of **5**. Computed distances (Å) and energies (kcal/mol, relative to **D**·H_2O·H_2O) are shown (B3LYP/6-31+G(d,p) in normal text and mPW1PW91/6-31+G(d,p)//B3LYP/6-31+G(d,p) in brackets).

Our calculations thus suggest that secondary carbocation **B** can exist as a minimum in the active site of an enzyme that produces avermitilol. Nonetheless, its conversion to avermitilol or to tertiary carbocation **D** (which precedes viridiflorol and *allo*-aromadendrene) is predicted to be facile. The former process is predicted to involve a concerted cyclization/water capture reaction that avoids the formation of putative secondary carbocation intermediate **C**.[19] Although the conversion of **B** to **D** is predicted to be rapid, it is also predicted to be reversible, suggesting that an enzyme could preferentially form **4** if it does not possess a nucleophilic (activated) water "in front" of C7 to trap **D** (or a base to deprotonate it) and instead possesses a nucleophilic water "behind" C6 to trap **B** (Figure 3).

Acknowledgment. We gratefully acknowledge UC Davis and the National Science Foundation (CHE-0449845, CHE-0957416, and CHE-030089 with the Pittsburgh Supercomputer Center) for support and Prof. David Cane (Brown) for bringing the avermitilol problem to our attention.

Supporting Information Available. Coordinates and energies for all computed structures, additional details on computations not discussed at length in the text and full Gaussian citation. This material is available free of charge via the Internet at http://pubs.acs.org.