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# Concerted Nature of AB Ring Formation in the Enzymatic Cyclization of Squalene to Hopenes

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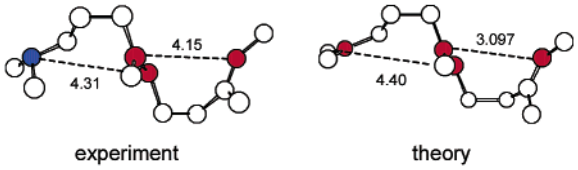
## ABSTRACT




**The cyclization of the A–B rings of squalene to hopene is studied computationally (DFT). A transition structure is found for a concerted, asynchronous pathway for the formation of chair-chair decalin carbocation. The computationally derived conformer leading to this asynchronous transition structure is remarkably similar to the analogous region of 2-azasqualene encapsulated by squalene-hopene cyclase recently reported by Schulz. A concerted A–B ring closure is likely to occur in the cyclization of squalene to hopene.**

The enzymatic conversions of squalene oxide and squalene to steroids and hopanoids are two of the most remarkable biological reactions. It has been established that these cyclization reactions involve the interaction of carbocations with double bonds that are present in protonated squalene oxide and squalene. The cyclizations, which give rise to tetra- and pentacyclic systems, are often referred to as a "cascade" of reactions; however, details of the mechanism are still uncertain.

A central problem is whether the ring formations occur in a concerted or stepwise fashion. Although numerous biomimetic and enzymatic studies have been reported, there is still no definitive answer to this question.[1] Results of computational studies[2−8] suggest, though again not definitively, the possibility that these reactions might be concerted. To provide a deeper insight into this problem and to understand, in particular, the formation of the A and B rings in the squalene-hopene conversion, we performed density functional calculations on a model system. The system embodies the main structural features of the C1−C12 carbons of squalene (see Figure 1).

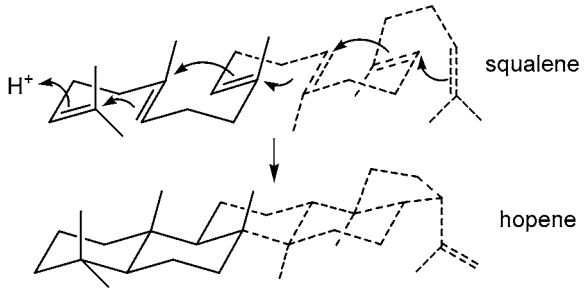



**Figure 1.** Conversion of squalene to hopene. The model system studied is shown with solid lines, with the balance of the squalene and hopene structures shown in dotted lines.

It is known that in the conversion of squalene oxide to lanosterol the B ring must be formed in a boat conformation.

(1) For a recent review see: Wendt, K. U.; Schulz, G. E.; Corey, E. J.; Liu, D. R. *Angew. Chem., Int. Ed.* **2000**, *39*, 2812.
(2) Jenson, C.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1997**, *119*, 10846.
(3) Gao, D.; Pan, Y.-K.; Byun, K.; Gao, J. *J. Am. Chem. Soc.* **1998**, *120*, 4045.
(4) Hess, B. A., Jr. *J. Am. Chem. Soc.* **2002**, *125*, 10268.
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(6) Hess, B. A., Jr. *Collect. Czech. Chem. Commun.* **2003**, *68*, 202.
(7) Hess, B. A., Jr. *Eur. J. Org. Chem.* In press.
(8) Hess, B. A., Jr. *Collect. Czech. Chem. Commun.* **2004**, *69*, 261.

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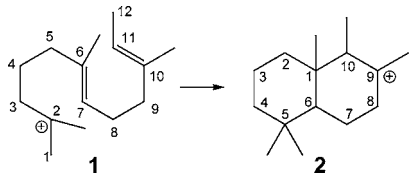
On the other hand, the B ring in the cyclization of squalene to hopene is formed in the more stable chair conformation (see Figure 1). There is computational evidence for the suggestion that the enzymatic protonation of squalene oxide and formation of ring A are probably concerted (as a result of anchimeric assistance of the double bond in ring opening of the epoxide).[2] This is also a possibility in the case of the enzymatic protonation of squalene.[8] However, no experiments or computations have directly addressed the concerted nature of the closure of the A and B rings in either the steroidal or hopanoid cyclizations. In the present paper for the first time this problem is directly addressed by means of results of numerical calculations. While this work was in progress, Schulz reported the crystal structure of squalene-hopene cyclase cocrystallized with 2-azasqualene.[9] It is shown that his results in conjunction with ours provide support for the concerted formation of the A and B rings in the squalene cyclization.

The model system chosen was (6*E*,10*E*)-2,6,10-trimethyl-6,10-dodecadien-2-yl cation (**1**) and its doubly cyclized product, 1,5,5,9,10-pentamethyl-9-bicyclo-[4.4.0]decyl cation (**2**). It is generally believed that a major role of the enzyme



is to position the squalene chain in a conformation that facilitates the cascade of cyclizations and also leads to the observed stereochemistry of the formed triterpenes. Hence it is important to find a conformer of **1**, which would position the double bonds in such a way that they might assist anchimerically in the cyclization and lead to the proper stereochemistry in the ring closures. A conformational study of carbocation **1** yielded such a conformer, **3**, which is shown in Figure 2.[10−13] This conformation was not found randomly but rather results from two previous conformational studies performed on model systems crucial for the formation of rings A[8] and B[7] in the squalene-hopene cyclization were used. As a starting point in the search for the conformation

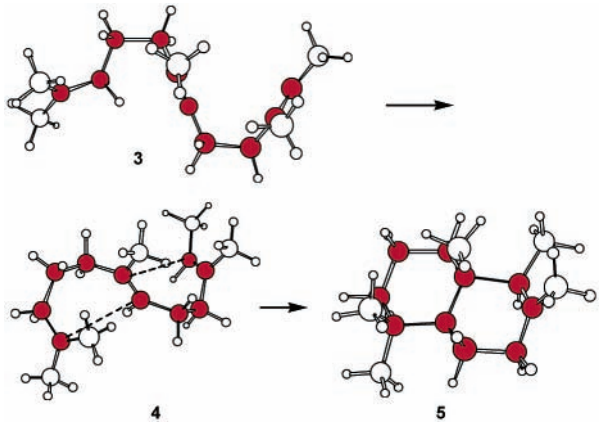
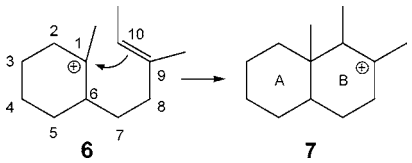


**Figure 2.** Structures of the starting conformer (**3**), transition structure (**4**), and bicyclic product (**5**).

of **1**, which would yield the correct stereochemistry of the A ring, the C2−C7 segment of the previously found conformer of (6*E*)-2,6-dimethyloct-6-en-2-yl cation was used.[8] This particular conformer had been found to form the chair conformation of the tetramethylcyclohexyl carbocation in a concerted ring closure. The choice of the placement of the C6−C11 in the starting structure for the conformational search turns out to be critical, because their placement determines whether a chair-chair or a chair-boat bicyclic conformer would be formed. In the squalene-hopene cyclization it is the chair-chair conformer that is formed. Our previous study on the model system for the conversion of 1-methyl-2-((3*E*)-3-methyl-3-pentenyl)-1-cyclohexyl cation (**6**) to 1,2,3-trimethyl-3-bicyclodecyl cation (**7**)[7] was helpful



in finding the proper conformational starting point for the remaining carbons in **1**. It had been found that the conformation about the C8−C9 bond in **6** is critical in determining whether a chair-chair or chair-boat bicyclic system is formed. In addition it was found that for the chair-chair formation of **6** only two of three possible, expected staggered conformers about the C7−C8 existed. All attempts to find the third staggered conformer simply led to formation of the chair-chair bicyclic system of **7**. To "prejudice" the model system here toward concerted A−B ring formation, the conformation about the C7−C8 bond (this corresponds to the C8−C9 bond in **1**) was chosen for the starting point of the conformational search for **5**. This conformation corresponds to the elusive third staggered conformation mentioned above. It was expected that such a conformation about the C8−C9 bond in **3** exists, because there is no significant positive charge at C7. Indeed this was found to be the case as evidenced by the stable conformer **3**.

(9) Reinert, D. J.; Balliano, G.; Schulz, G. E. *Chem. Biol.* **2004**, *11*, 121−126.

(10) Calculations were performed with the DFT method using *Gaussian 98W*; Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A., Gaussian, Inc., Pittsburgh, PA, 1998. Becke's three-parameter hybrid method[11] with the Lee−Yang−Parr correlation function[12] and the 6-31G* basis set[13] were employed.

(11) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.

(12) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.

(13) Hariharan, P. C.; Pople, J. A. *Theor. Chim. Acta* **1973**, *28*, 213.

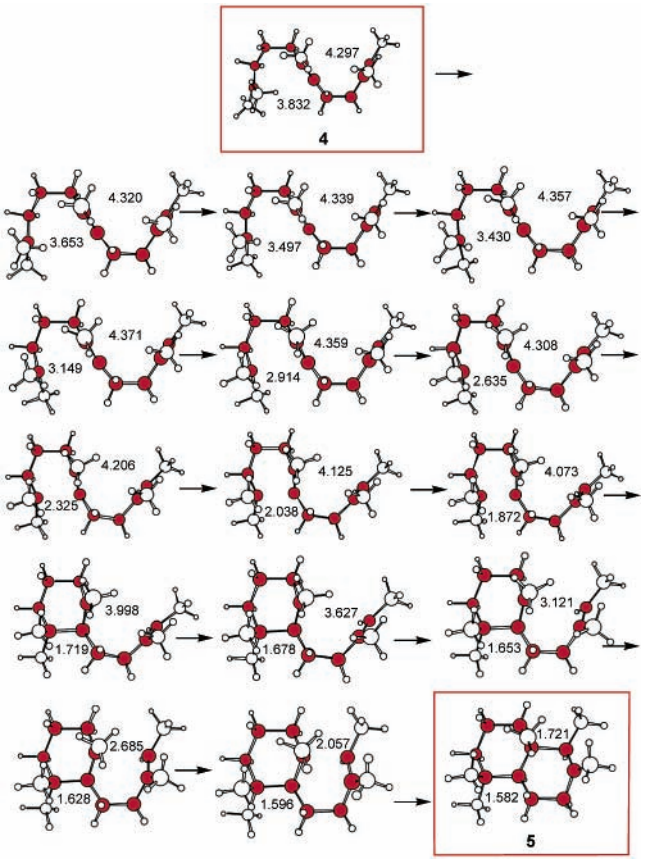



**Figure 3.** The IRC pathway leading from transition structure **4** to product (**5**). Structures shown of points along the IRC pathway from **4** to **5** are at 2 kcal/mol intervals (lower in energy from **4**). Distances are given for each structure in Ångstroms for the two ring-forming bonds.

A search for a transition structure that would lead to cyclization of the A ring yielded **4**. The distance between C2 and C7 in **4** is significantly reduced from that in **1** (3.832 vs 4.400 Å). The geometry of the ring A forming carbons (C2−C7) is very similar to that found previously for the formation of ring A by itself.[8] As was found there, rotation about the C3−C4 bond also occurs here, indicative that **4** is the transition structure for the A ring closure. The calculated energy of **4** is only 4.6 kcal/mol higher than that of **3**. An intrinsic reaction coordinate (IRC) calculation showed that **4** was linked directly to conformer **3** without the intervention of any intermediates.

The question at this point was whether **4** would lead to a carbocation intermediate in which only ring A had been formed or directly to the bicyclic carbocation **7** in which both rings A and B were formed (that is, concerted formation of rings A and B). To resolve this question an IRC calculation was carried out following the reaction pathway downhill in the direction opposite from that toward **3**. The results of these calculations are shown in Figure 3. The pathway towards product is very long,[14] but it eventually led to the bicyclic product **5** without the intervention of any intermediates; hence the pathway is concerted.

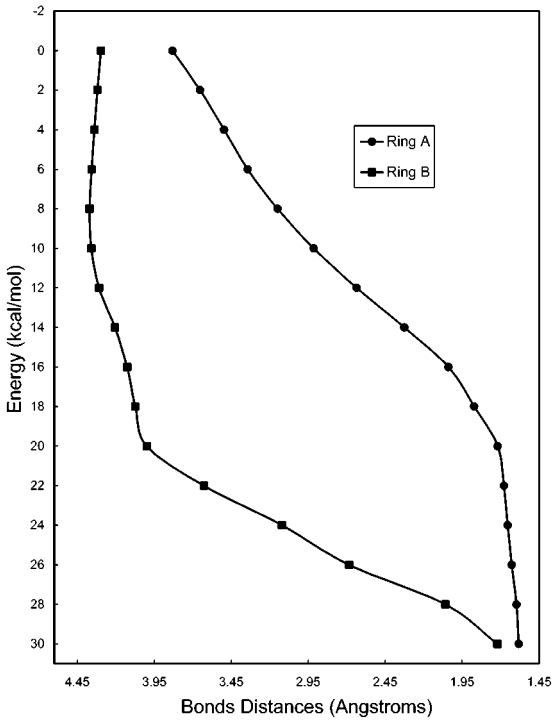



**Figure 4.** A plot of the C2−C7 and C6−C11 bond distances vs the energy relative to that of transition structure **4**. Points correspond to those structures shown in Figure 3.

To analyze this double ring closure in more detail, a plot was made of the distances between the two ring-forming bonds at 2 kcal/mol intervals (Figure 4). It is clearly seen that closure of ring A is not initially accompanied by closure of ring B. In fact in the early stages of the closure of ring A the C6−C11 distance actually increases slightly. It is not until ring A is almost closed (C2−C7 distance of ~1.7 Å) that there is significant closure of ring B. It is also seen from Figure 4 that the initial drop in energy of the system is due primarily to closure of ring A. When the overall decrease in energy reaches about 20 kcal/mol, a dramatic change in the course of the reaction occurs. The rate of decrease of the C2−C7 bond distance (forming ring A) practically stops, while the rate of decrease of the C6−C11 bond distance (forming ring B) increases significantly. It is apparent that at this point the charge on C7 is built up to the point that it allows significant interaction with the C10−C11 double bond. Hence the remaining decrease in energy is due primarily to formation of ring B.[15] Since no intermediate along the pathway was found, the closure of rings A and B is concerted. This concerted process, as seen clearly from

(14) Over 500 points were necessary to reach the bicyclic product (**5**). In places where the pathway was very flat (small change in energy for large change in structure) the IRC often failed and frequencies had to be recomputed and the IRC continued. Many attempts were also made to find intermediates along the pathway, particularly in the flat regions. All failed.

(15) A referee suggested that this surface be checked by computing single point MP2/6-31G* energies at the DFT geometries shown in Figure 3. This was done, and a plot of these data (Figure S1 in Supporting Information) was found to be very similar to the DFT plot in Figure 4.

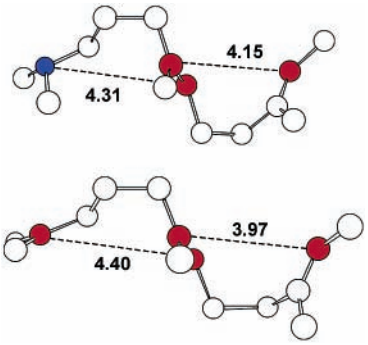



**Figure 5.** X-ray structure of 2-azasqualene encapsulated in SHC (upper) and the DFT structure of **3** (lower). The nitrogen is shown as a blue atom and the carbons that are involved in the cyclizations are shown in red. Distances are shown in Ångstroms.

Figure 3, is best described as a domino effect that is indeed concerted but not synchronous.

The overall energy change for the conversion of **3** to **5**, without the inclusion of zero-point energy (ZPE), is 25.5 and 19.9 kcal/mol with inclusion of ZPE. This is in line with what one expects for converting two $\pi$ bonds into two $\sigma$ bonds, especially taking into account that the bicyclic, chair-chair product (**5**) has significant steric strain that is caused by the five methyl substituents.

Since all of the calculations presented here are performed in the gas phase in the absence of any solvent or surrounding enzyme, it is possible that the enzymatically catalyzed conversion of squalene to hopene might certainly be quite different in nature. However Schulz's recent report[9] of the X-ray crystal structure of 2-azasqualene encapsulated in SHC provides an excellent opportunity to compare our gas-phase results with those obtained from experiment. It is apparent from his X-ray data and molecular modeling calculations that one of the main tasks of the enzyme is to "hold" the squalene molecule in the proper conformation for the cyclization reactions to occur. Not only does the enzyme hold the double bonds in the proper proximity for interaction with the developing carbocations during the course of the cyclization, but it also arranges the squalene chain in a conformation that will produce the proper stereochemistry for the cyclization. This raises the question whether the conformation of our model system (**3**) is the same as that which exists in the enzyme-bound squalene. In Figure 5 the structure of 2-azasqualene (taken from Schulz's X-ray data) is compared with that of **3**. The squalene in Schulz's X-ray structure has a nitrogen replacing C2 and does not have the C2−C3 double bond. However, his modeling calculations showed that substitution by the nitrogen in squalene has apparently little effect on the overall structure of squalene in the enzyme cavity. The remarkable similarity between Schulz's X-ray structure and our calculated results (see Figure 5) certainly provides justification for treating the chosen model system with DFT calculations. It also suggests, given the concerted nature of our calculated results obtained for the cyclization of the A and B rings, that the enzymatic cyclization of these rings is also a concerted process. It may be concluded that the enzyme positions squalene in the observed conformer in order for a concerted cyclization of the A and B rings to take place. It is important to note that positioning of the segment of the squalene chain by the enzyme that gives rise to the B ring corresponds to the staggered conformer of **6**, which we showed does not exist. During the course of the enzymatically controlled formation of ring A a positive charge begins to develop on C6. The carbons that will become involved in the formation of ring B are seen to be judiciously placed such that the C10−C11 double bond interacts with the developing positive charge, therefore allowing formation of ring B in concert with ring A.

Finally it should be noted that these results (the concerted nature of the formation of rings A and B in the squalene cyclization) are in direct contrast to the results of recent modeling calculations reported by Rajamani and Gao.[16] They found that the formation of ring A leads to a carbocation *intermediate* and then to a second carbocation intermediate (the A−B bicyclic carbocation). At this point it is difficult to say what the reason is for this disagreement. Perhaps the gas-phase calculations reported here are not appropriate for an enzyme-catalyzed reaction, the AM1 calculations used by Gao are not appropriate for these cyclization reactions, or the initial conformation of squalene chosen by Gao differs from that found in the enzyme encapsulated squalene. We note that Schulz found with his molecular dynamics calculations[9] a definite tetracyclic carbocation intermediate, whereas Gao found that the C−E rings are formed in a concerted fashion.[17]

**Acknowledgment.** We would like to thank Professor Schulz for providing us with the X-ray structure of squalene-SHC.

**Supporting Information Available:** Energies and geometries for **3**−**5** and Figure S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) Poralla, K. *Chem Biol.* **2004**, *11*, 14.