J|A|C|S
COMMUNICATIONS

Published on Web 08/10/2002

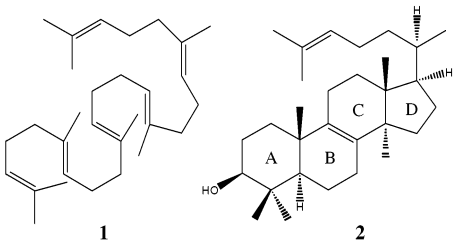
Concomitant C-Ring Expansion and D-Ring Formation in Lanosterol Biosynthesis from Squalene without Violation of Markovnikov's Rule

B. Andes Hess, Jr.*

Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37235

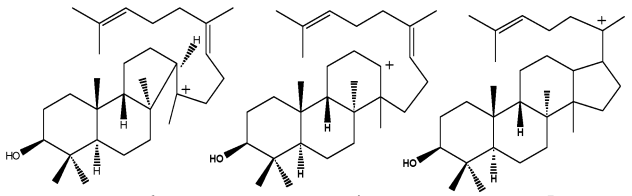
Received May 9, 2002

The remarkable conversion of squalene (**1**) to lanosterol (**2**) has fascinated chemists for half a century. While there has been much



1 **2**

speculation on the mechanism of this conversion, there have been relatively few papers which have provided definitive evidence on the course of the formation of the four rings of the steroid system from the acyclic squalene. Initially it was thought that the formation of the four rings (A−D) might proceed in a concerted fashion, once squalene was epoxidized and subsequently protonated.[1] However, van Tamelen, on the basis of the results of a variety of experiments,[2] proposed that a "series of conformationally rigid, partially cyclized carbocationic intermediates"[3] were involved. In the mid-1990s Corey and co-workers published convincing evidence that discrete carbocation intermediates were involved in the ring closures.[4] In particular they showed that formation of ring C involves initially the formation of a cyclopentylcarbinyl carbocation intermediate **3** (the Markovnikov-favored ring closure) which must subsequently undergo a ring expansion (anti-Markovnikov) to form the six-membered C ring in **4**. It was suggested that the D ring was then formed by ring closure of this secondary carbocation to give 17β-protosterol carbocation **5**. This aspect was first noted in the classic



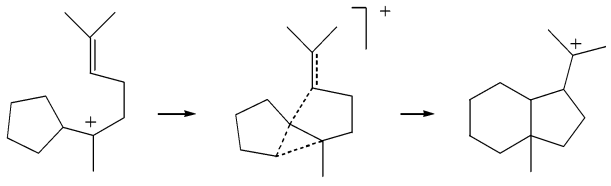
3 **4** **5**

paper of van Tamelen in 1966 dealing with the biomimetic cyclization of squalene 2,3-oxide.[2a] He concluded that "direct enzymatic control is obviously necessary for the prevention of the purely chemical tendency for five-membered ring C formation, and for emergence of the biologically required six-membered ring." Other biomimetic studies have helped to clarify the carbocation nature of the cyclization of squalene oxide, in particular those by Nishizawa[5] and Johnson.[6] More recently studies by Corey have shown that the formation of ring A involves a concerted protonation of the epoxide and ring A closure in both enzymatic and biomimetic processes.[7]

The presence of discrete carbocation intermediates was given further support by two theoretical papers. Jenson and Jorgensen[8] suggested that rings A and B likely are formed in concert, but they proposed that ring C is formed via a cyclopentylcarbinyl−cyclohexyl carbocation rearrangement (anti-Markovnikov) in agreement with Corey's finding. An additional theoretical study[9] on the formation of rings A and B supported Jenson and Jorgensen's conclusions.

However, there still remains the question of how does the C-ring cyclization process overcome the energy barrier required to expand the tertiary cyclopentyl carbocation to the less stable secondary cyclohexyl carbocation. On the basis of calculations on a model system, Jensen and Jorgensen found the secondary cyclohexyl carbocation to be 12 kcal/mol higher in energy than the tertiary dimethylcyclopentylcarbinyl carbocation. This value was reduced to 10 kcal/mol when solvent effects were taken into account. They suggested, on the basis of force-field calculations, that this barrier might be further lowered by "selective placement of nucleophilic groups from the protein backbone or side chains including the indole ring of tryptophans."

It occurred to us that there might be another explanation of how the six-membered C and five-membered D rings are formed, that is, the double bond between carbon atoms 18 and 19 of squalene might be involved anchimerically in the cyclopentylcarbinyl−cyclohexyl ring expansion. This would mean the concerted ring expansion of ring C and formation of ring D, in which **3** would be converted directly to **5**, without the intervention of intermediate **4**. To test this hypothesis we undertook density functional calculations[10−13] on the model system **6**, a cyclopentylcarbinyl carbocation with the appropriate side chain and methyl groups to mimic the essential parts of the structure of **3** involved in the ring expansion. The proposed mechanism would involve the rearrangement of the tertiary carbocation **6**, through transition structure **7(ts)**, to the bicyclo[4.3.0]nonyl tertiary carbocation **8**. Such a mechanism would avoid the intermediacy of the less stable secondary cyclohexyl carbocation **4** as well as the violation of Markovnikov's rule.[14]



6 **7(ts)** **8**

A transition structure was located which confirmed our hypothesis (see Figure 1). From the figure it is seen that the ring expansion

* To whom correspondence should be addressed. E-mail: hessba@ctrvax.vanderbilt.edu.

10.1021/ja026850r CCC: $22.00 **© 2002 American Chemical Society**

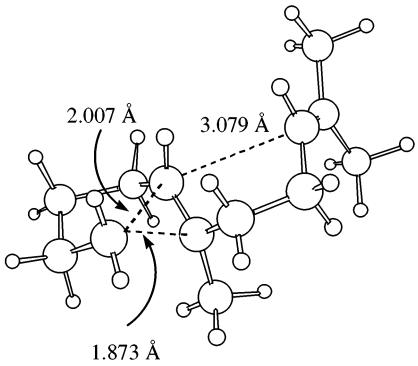


Figure 1. The DFT structure of transition structure **7(ts)**.

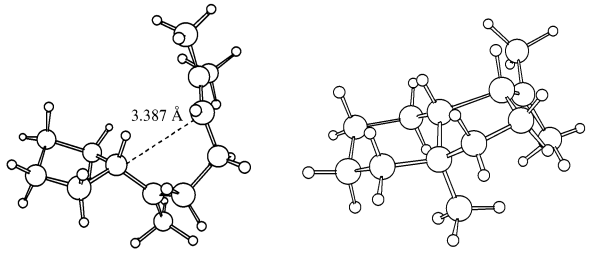


Figure 2. DFT structures of reactant **6** and product **8** of the concerted cyclization reaction in which rings C and D are formed in concert.

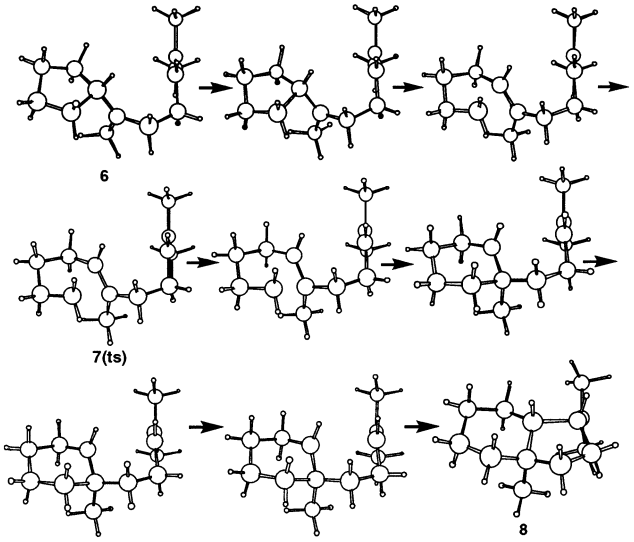


Figure 3. Geometries of selected points of the 3-21G IRC pathway of **7(ts)**.

to form ring C is well underway in the transition structure with the newly forming cyclohexane bond somewhat shorter than the breaking bond in the cyclopentane ring. The nascent six-membered ring (C) is clearly seen to be in a chair conformation. The five-membered ring (D) is closing in such a way to yield the expected trans stereochemistry of the bicyclic ring system. In the transition structure the newly forming carbon−carbon bond of ring D has a C−C distance of 3.079 Å with the sp^2 carbon of the original double bond having undergone very little rehybridization.[15]

Examination of the normal mode of the imaginary frequency of **7(ts)** shows that this transition structure likely links the two carbocations **6** and **8** (see Figure 2). This was confirmed by intrinsic reaction coordinate calculations[16] (see Figure 3). Close examination of the IRC indicates that as the C ring expands the double bond moves closer to the developing positive charge in ring C. As it approaches, its orientation is suggestive of π-complex formation with very little rehybridization of the sp^2 carbons.[17] Further along the path (beyond the transition structure) the original double bond carbon which is involved in the formation of the D ring begins to rehybridize toward sp^3.

Reactant **6** was calculated to lie 7.8 kcal/mol[18] in energy below that of the transition structure **7(ts)** and the product **8** to lie 3.5 kcal/mol below that of the reactant **6**. The relatively low activation energy for the conversion of **6** to **8** suggests that, once the cyclopentylcarbinyl carbocation **3** is formed in the lanosterol biosynthesis, it will very quickly undergo a concerted ring expansion and ring closure to give the protosterol carbocation **5**. Hence, we propose that the "cascade" arising from the protonation of squalene epoxide occurs *without* the necessity of going through the higher-energy intermediate carbocation **4** (with the concomitant violation of Markovnikov's rule). Furthermore, it is suggested that the primary role of the enzyme in the C-ring expansion and D-ring formation is to "hold" the substrate in the proper conformation for the completion of the cascade leading to the tetracyclic system.

Supporting Information Available: Cartesian coordinates, energies, and zero-point energies for structures **6**−**8** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Two excellent reviews: (a) Abe, L.; Rohmer, M.; Restwich, G. D. *Chem. Rev.* **1993**, *93*, 3. 2189. (b) Wendt, K. U.; Schulz, G. E.; Corey, E. J.; Liu, D. R. *Angew. Chem., Int. Ed.* **2000**, *39*, 2812.

(2) (a) van Tamelen, E. E.; Willet, J.; Schwartz, M. Nadeau, R. *J. Am. Chem. Soc.* **1966**, *88*, 5937. (b) van Tamelen, E. E.; Lees, R. G.; Grieder, A. *J. Am. Chem. Soc.* **1974**, *96*, 2255. (c) van Tamelen, E. E.; James, D. R. *J. Am. Chem. Soc.* **1977**, *99*, 950. (d) van Tamelen, E. E.; Hopla, R. E. *J. Am. Chem. Soc.* **1979**, *101*, 6112.

(3) van Tamelen, E. E. *J. Am. Chem. Soc.* **1982**, *104*, 6480.

(4) (a) Corey, E. J.; Virgil, S. C.; Cheng, H.; Baker, C. H.; Matsuda, S. P. T.; Singh, V.; Sarshar, S.*J. Am. Chem. Soc.* **1995**, *117*, 11819. (b) Corey, E. J.; Cheng, H. *Tetrahedron Lett.* **1996**, *37*, 2709.

(5) Nishizawa, M.; Takenaka, H.; Hayashi, Y. *J. Am. Chem. Soc.* **1985**, *107*, 522.

(6) Johnson, W. S.; Lindell, S. D.; Steele, J. *J. Am. Chem. Soc.* **1987**, *109*, 5852

(7) (a) Corely, E. J.; Cheng, H.; Baker, C. H.; Matsuda, S. P. T.; Li, D.; Song, X. *J. Am. Chem. Soc.* **1997**, *119*, 1277. (b) Corey, E. J. Staas, D. D. *J. Am. Chem. Soc.* **1998**, *120*, 3526.

(8) Jenson, C.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1997**, *119*, 10846.

(9) Gao, D.; Pan, Y.-K.; Byun, K.; Gao, J. *J. Am. Chem. Soc.* **1998**, *120*, 4045.

(10) 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.; and Pople, J. A. *Gaussian 98W*; Gaussian, Inc.: Pittsburgh, PA, 1998. Becke's three-parameter hybrid method (ref 11) with the Lee−Yang−Parr correlation function (ref 12) and the 6-31G* basis set (ref 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.

(14) Structure **7(ts)** is similar to the nonclassical carbocation proposed by van Tamelen (ref 3) as an *intermediate* for the formation of rings C and D.

(15) The analogous transition structure was found with MP2/6-31G*, and it had a somewhat shorter C−C distance of the forming D ring (2.875 Å). Otherwise the MP2 structure was very similar to the DFT structure.

(16) The IRC calculations were carried out on the SCF/3-21G optimized transition structure. The SCF/3-21G structures of **6**−**8** were found to be very similar to the DFT optimized structures.

(17) This is reminiscent of π-complexes found as *intermediates* by Dewar and Reynolds in their MINDO calculations in their study of biomimetic cyclizations.

(18) The reported DFT energies contain zero-point energy corrections.

JA026850R