ORGANIC LETTERS

2003
Vol. 5, No. 2
165−167

Formation of the C Ring in the Lanosterol Biosynthesis from Squalene

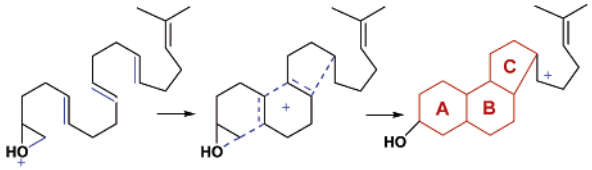
B. Andes Hess, Jr.*

Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37235

bahessjr@aol.com

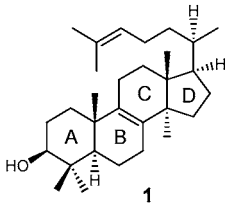
Received December 11, 2002

ABSTRACT

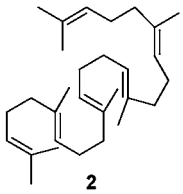


Ab initio calculations were performed on a cyclohexane derivative to elucidate the mechanism of the formation of the five-membered C ring in the biosynthesis of lanosterol from squalene. A conformational analysis of the side chain containing the double bond indicated that the conformer that should give rise to the cyclized C ring is not a minimum on the potential surface. Consequently, it is suggested that it is very likely that C-ring formation occurs in concert with formation of the A and B rings.

It has been well-established[1,2] that in the biosynthesis of lanosterol (**1**) at least one carbocation intermediate is formed in the cascade of reactions following the epoxidation and protonation of squalene (**2**) that gives rise to the tetracyclic ring system (A−D) of steroids.[3] Corey and co-workers have

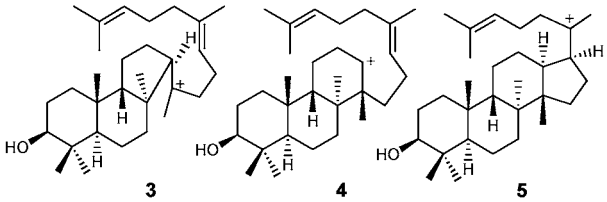


1



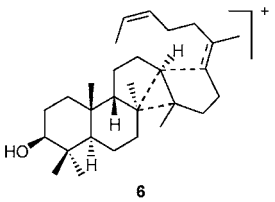
2

shown that ring C is first formed as a carbocation intermediate with a five-membered ring (**3**)[2] and concluded that **3** undergoes a ring expansion to **4** before the five-membered D-ring (**5**) is formed. Corey's findings were supported by the theoretical studies of Jenson and Jorgensen.[4] Very



3 **4** **5**

recently we have suggested that conversion of **3** to **5** might instead involve expansion of the C-ring of **3** in concert with the formation of the five-membered D-ring in **5**, via transition structure **6**.[5] This concerted mechanism avoids the energeti-



6

cally unfavorable C-ring expansion with the conversion of

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

(2) (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.

(3) 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.

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

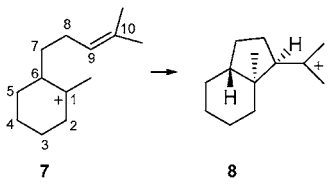
(5) Hess, B. A., Jr. *J. Am. Chem. Soc.* **2002**, *124*, 10286.

10.1021/ol027449c CCC: $25.00
Published on Web 01/03/2003

the tertiary carbocation **3** to the less stable secondary carbocation **4**.

Here will be addressed the mechanism of the formation of the five-membered C-ring intermediate **3**, for which there is relatively little experimental or theoretical evidence. Previously Corey showed that protonation of 2,3-squalene epoxide and the formation of ring A are a concerted process.[6] In two theoretical studies the same conclusion was reached, and in both it was suggested that B-ring formation is also likely to be in concert with A-ring formation.[4,7] The question that remains unanswered is whether the C ring is also formed in concert with the A and B rings to give in essentially one step the first confirmed intermediate (**3**) in the lanosterol biosynthesis.

To attempt to answer this question ab initio calculations were undertaken on the ring closure of carbocation **7** to give the bicyclic carbocation **8**.[8−11] For the five-membered C ring



7 **8**

to form with a *trans* ring juncture to the B ring in the squalene biosynthesis, the C ring must adopt a boat conformation. Hence all calculations were performed on **7** with a boat conformation. For the side chain to close to form **8**, it must adopt the appropriate conformation such that the double bond can "attack" the electron-deficient C1. There is only one viable staggered conformation about the C6−C7 bond, the one in which the two hydrogens of C7 are bisected by the C6−C5 bond. The other two staggered conformations about this bond suffer steric interactions with the A and B rings (that is, if the A ring is considered). In the case of rotation about the C8−C9 bond, only one "staggered" conformer is free of steric interactions when rotation occurs about the C7−C8 bond, and that is the one that allows the ring closure to occur. The problem of conformational analysis of the side chain is therefore reduced to considering conformers arising from rotation about the C7−C8 bond.

Examination of the C7−C8 bond in a model of **7**, which has the "best" conformers about the C6−C7 and C8−C9 bonds as discussed above, indicates the three staggered conformers and the three eclipsed transition structures linking them do not suffer any significant steric strain other than that from the eclipsing about the C7−C8 bond in the eclipsed conformers. SCF/3-21G calculations were used to search for these six conformers. Five of them were found without difficulty and are shown in Figure 1 (**9**−**13**), with **10** and

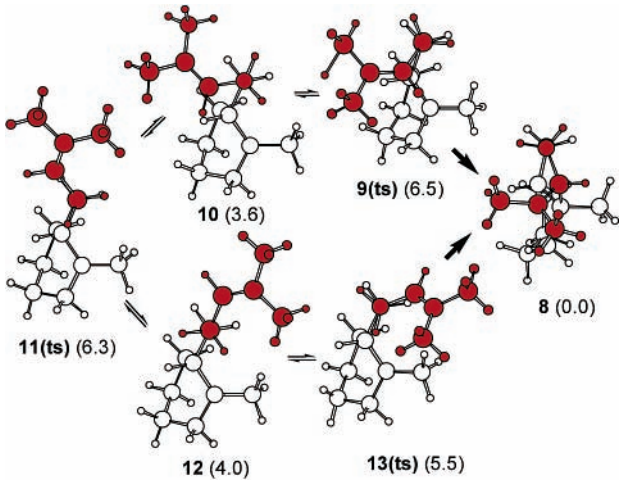


Figure 1. Conformers (**10** and **12**), transition structures (**9**, **11**, **13**), and the ring-closed product (**8**) of **7**. The "rotating" group is highlighted in red. Structures shown are those from HF/3-21G optimizations. Relative energies (B3LYP/6-31G* with ZPE corrections) are given in parentheses in kcal/mol.

12 being two of the staggered conformers. They are linked by the eclipsed transition structure **11** as was seen from the normal mode of its one imaginary frequency. Transition structures **9** and **13** are linked, as expected, in one direction to **10** and **12**, respectively. It was thought that in the other downhill direction they would lead to the third staggered conformer. This is the conformer that would be expected to give rise to the ring closure of **7** to **8**, since it would have carbons C1 and C9 in close proximity. IRC calculations indicated that initially rotation about the C7−C8 bond occurs, which appeared to be leading to the third elusive staggered conformer. However, it was found in both cases that, rather than reaching this conformer, the pathways led instead to the bicyclic product **8** (see Figure 1). Furthermore using as a starting geometry either **9** or **13** in which a 60° rotation was made about C_7-C_8 (in an attempt to find the third staggered conformer), geometry optimization led only to ring-closed **8**. This is strongly suggestive that this third staggered conformer does not exist as a stable minimum.

The failure to locate the third staggered conformer has strong implications about the mechanism of the formation of ring C in the biosynthesis of lanosterol. It strongly suggests that as the positive charge is developing at tertiary carbon C1 during the formation of ring B, ring closure of ring C begins. Hence, it is predicted that ring C formation is

(6) (a) Corey, 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.

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

(8) 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. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*; Gaussian, Inc.: Pittsburgh, PA, 1998.

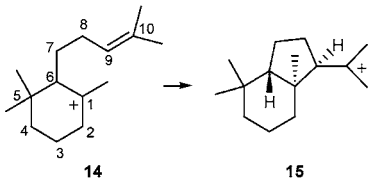
(9) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.

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

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

concomitant with formation of rings A and B, and therefore there exists no carbocation intermediates between 2,3-oxidosqualene and the tertiary carbocation **3**. This does presume that the enzyme holds squalene in the proper orientation. On the other hand, as seen from the relative energies of **8** and **9**−**13** (see below), even if squalene is only loosely held by the enzyme, it is still likely that no intermediate carbocation exists containing only the rings A and B.

The structures shown in Figure 1 were used as starting points for DFT geometry optimizations. In all cases but one (see below) the found DFT geometries were very similar to those obtained at the HF level with the 3-21G basis set. DFT energies relative to the product **8** are given in Figure 1. Conformers **10** and **12** are predicted to be only about 4 kcal/mol higher in energy than product **8**. Furthermore the barrier to conformational change between **10** and **12** as well as to their reactions to give **8** are quite small. A referee suggested that these energy differences will surely be influenced by dialkyl substitution at C5 in **7**, principally by hyperconjugative effects. The corresponding dimethyl DFT structures and energies of staggered conformer **10** (**14**) and product **8** (**15**) were obtained. The energy difference between these two



14 **15**

structures (3.62 kcal/mol) was found to be essentially unchanged from that obtained without the two extra methyl groups (3.55 kcal/mol).

An interesting result is the DFT geometry of **8,** which has a very long C−C bond (Figure 2).[12] This newly formed bond varies in length depending upon the method of calculation but in all cases is unusually long. As previously noted by Pan and Gao, hyperconjugation is the likely source of the elongation of such bonds in carbocations.[7] However the long C−C bond in **8** obtained with the DFT method suggests that other factors may also be involved. Examination of a model of **8** indicates that steric and angle strain may also play a

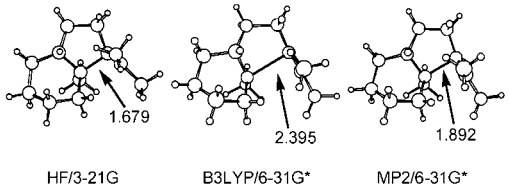


Figure 2. Optimized structures of **8** (distances in Å).

role in the elongation of this bond. This is supported by calculations on the tetramethyl cyclopentylcarbinyl carbocation **16** (see Figure 3) for which the three methods, unlike for **8**, give very similar results.[13]

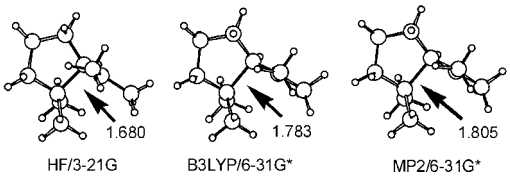


Figure 3. Optimized structures of **16** (distances in Å).

In conclusion, calculations performed for the model system **7** predict that there should be a very low activation barrier for the formation of the five-remembered C ring in the lanosterol biosynthesis. It is suggested that formation of rings A−C is very likely to be a concerted process, once the epoxidized squalene is protonated by the enzyme, which means that carbocation **3** is the first *intermediate* in the cascade of reactions leading to the formation of rings A−D in protosterol.

Supporting Information Available: Cartesian coordinates, energies, and zero-point energies for structures **8**−**16**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL027449C

(12) In fact at first this structure was thought to be a π complex; however, no bicyclic structure could be found with a "normal" C−C bond length.

(13) It is difficult to assess which of the correlated methods (DFT or MP2) gives the more realistic structure for **8**. If the DFT method is closer to reality, then this has definite implications about the mechanism of the ring expansion of ring C and formation of ring D. Future studies will address this point.