ON THE CONCERTED RING OPENING OF PROTONATED SQUALENE OXIDE AND A-RING FORMATION IN THE BIOSYNTHESIS OF LANOSTEROL

B. Andes Hess, Jr.

Department of Chemistry, Vanderbilt University, Nashville, TN 37235, U.S.A.; e-mail: hessba@ctrvax.vanderbilt.edu

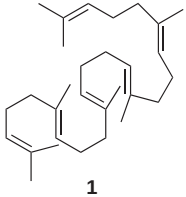
Received July 29, 2002
Accepted October 21, 2002

I dedicate this paper to Petr, Ivan and Miro whom I had the fortunate opportunity to meet in the early seventies during my year-long National Academy exchange visit to the Heyrovský Institute in Prague, and with whom I have maintained contact over the past thirty years.

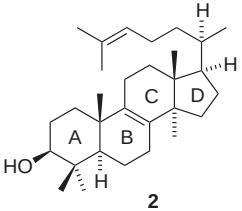
Density functional calculations were performed on a model system of squalene oxide to study the mechanism of the formation of ring A in the biosynthesis of lanosterol from squalene. When (2*Z*)-6,7-epoxy-3,7-dimethyloct-2-ene was protonated, it was calculated to undergo a very facile ring opening of the oxirane in concert with the formation of the six-membered ring of the 4-(hydroxymethyl)-1,2,3,3-tetramethy1cyclohexyl cation. A study of the reaction pathway (IRC) indicates a very early transition structure in which the carbon–carbon double bond participates anchimerically in the ring-opening of the protonated oxirane. It is suggested that the primary role of the enzyme in this first step of the biosynthesis of lanosterol is protonation of the oxirane ring along with holding the substrate in the proper conformation for the concerted ring-closure to occur. The similarity between this mechanism and that recently proposed for concerted C-ring expansion and D-ring formation in the biosynthesis of lanosterol is discussed.

Keywords: Steroids; Terpenoids; Biosynthesis; DFT calculations; Ab initio; Reaction mechanism; Epoxides; Cyclizations.

While the remarkable transformation of squalene (**1**) to lanosterol (**2**) has fascinated both organic and biochemists for more than fifty years[1], it has

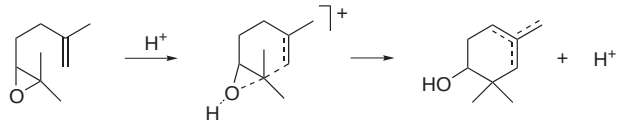


1



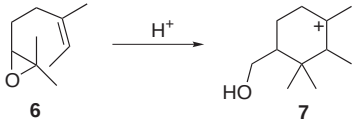
2

only been in the last few years that definitive progress has been made on the details of the mechanism of the cascade of reactions which leads to the formation of the tetracyclic system, the precursor to steroids. In 1998 Corey[2], based on kinetic studies of the oxirane system **3**, concluded (Scheme 1) that "in the acid-catalyzed cyclization of 5,6-unsaturated oxiranes to form a six-membered ring, the oxirane cleavage and cyclization



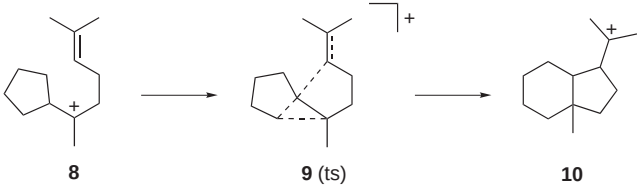
SCHEME 1

events are concerted". Hence **3** on protonation gives rise to the isomeric olefins **5** *via* transition structure **4**. Corey concluded that the ring-opening of the protonated 2,3-epoxidized squalene and cyclization of the A-ring in the enzymatic conversion of squalene to lanosterol is also a concerted reaction. In the same year Pan and Gao[3] reported *ab initio* calculations on the acid-catalyzed reaction on the related system **6** and found that the ring closure of **6** to **7** proceeded in a concerted fashion (Scheme 2).



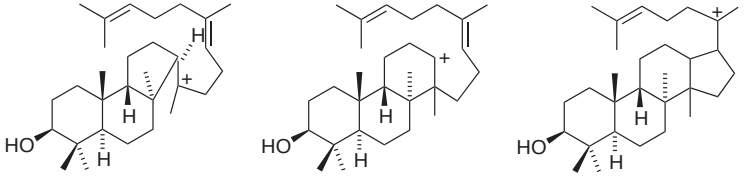
SCHEME 2

Very recently we reported[4] the results of a density functional study on the concerted ring expansion of the cyclopentyl carbinyl carbocation **8** with formation *via* transition structure **9** of the bicyclic tertiary carbocation **10** (Scheme 3). These results suggest a possible answer to the question of how the intermediate **11** in the biosynthesis of lanosterol is converted to the intermediate **13**. It had earlier been proposed[5] that this conversion pro-



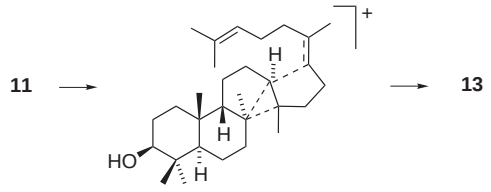
SCHEME 3

ceeds first through the ring-expanded **12** which subsequently undergoes an intramolecular cyclization to give **13**. However, this mechanism would require the unprecedented rearrangement of a tertiary carbocation **11** to a



11 **12** **13**

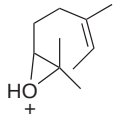
secondary carbocation **12**. Our results provide an alternative mechanism which avoids this unprecedented step (Scheme 4).



SCHEME 4

In fact the conversions of **6** to **7** and **8** to **10** share a common feature, the participation of the double bond in an anchimeric fashion. In the former case, the double bond of **6** assists in the ring opening of the protonated oxirane, while in the latter case the double bond of **8** assists in the ring expansion of the five- to six-membered ring. Because of this we decided to undertake calculations of the same type as those performed for the conversion of **6** to **7** for the conversion of **8** to **10**. Gao and Pan had performed Hartree–Fock (RHF) calculations with the 6-31G* basis set on the former conversion with single-point DFT calculations at the HF geometry, while we had carried out DFT (B3LYP/6-31G*//B3LYP/6-31G*) on the latter conversion. Furthermore while Gao and Pan's calculations were performed with formic acid as a catalyst, it is known that such oxiranes are quite stable in glacial acetic acid[6,7]. As they noted in their paper, this is consistent with their quite high calculated activation energy for the acid-catalyzed reaction of oxirane **6**. In the enzymatic reaction Corey has proposed the acidity of the catalyzing carboxylic acid is enhanced by the presence of a protonated histidine[6,8]. As a consequence Gao and Pan also performed RHF calculations on the cyclization of protonated **6**. However, they did not lo-

cate the actual transition structure for the conversion of the protonated oxirane to the cyclohexane derivative. The calculations reported below were therefore done on protonated oxirane **14** which would yield **7** on ring opening of the oxirane and cyclization.



14

COMPUTATIONAL METHODS

Calculations were performed using GAUSSIAN 98W[9]. The density functional method was employed using Becke's three-parameter hybrid method[10] with the Lee–Yang–Parr correlation function[11] and the 6-31G* basis set[12]. All stationary points were characterized by computation of second derivatives. Zero-point energies were calculated with unscaled B3LYP/6-31G* frequencies obtained analytically with G98W. Internal reaction coordinate calculations[13] were used to determine the reaction pathway.

RESULTS AND DISCUSSION

A transition structure was located (**15**) which appeared to be that which connects the protonated oxirane **14** with cyclized **7** (see Fig. 1). It is seen to be an "early" transition structure in that the C–C bond which is forming the cyclohexane ring is quite long[14] (3.240 Å) and the breaking C–O bond of the oxirane is 1.927 Å. Furthermore the carbon–carbon double bond has increased only 0.005 Å in length from that of the starting material. In con-

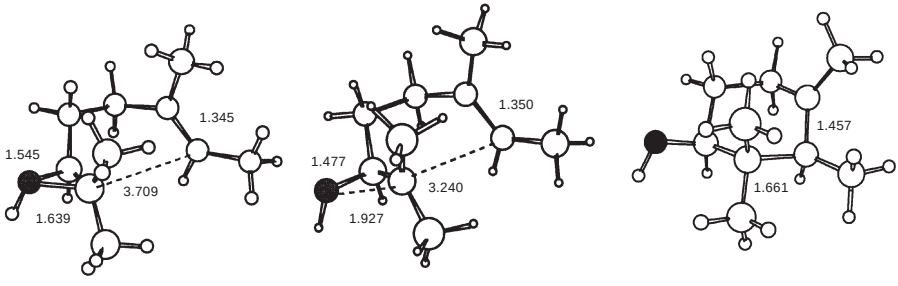


FIG. 1
Structures (B3LYP/6-31G*//B3LYP/6-31G*) of the protonated oxirane **14**, the cyclohexane product **7** and the transition structure **15** linking them. Bond distances are given in Å

trast to this Pan and Gao calculated a significantly shorter C–C forming bond of the cyclohexane ring (1.944 Å) in their formic acid catalyzed reaction. This is not surprising, since in their transition structure the hydrogen of formic acid is 1.403 Å from the oxygen, whereas in **15** the O–H bond is 0.977 Å in length. That is their transition structure is much further along the reaction pathway. In order to determine if this significant difference in the two transition structures was due to the method of calculation used to optimize the geometries of the transition structures, we also optimized structures **7**, **14**, and **15** at the RHF level (HF/6-31G*//HF/6-31G*) and these optimized structures are depicted in Fig. 2. It is immediately seen by comparison of the two figures that there are no major differences in the DFT and RHF structures.

In Table I are given the energies of **7** and **15** relative to the protonated oxirane **14**. It is seen from the Table that both RHF and DFT methods pre-

TABLE I
Relative energies of the three stationary points (corrected for zero-point energy with DFT frequencies)

Species	RHF/3-21G	RHF/6-31G*	B3LYP/6-31G*
14	0.0[a]	0.0[b]	0.0[c]
15	1.6	0.4	0.4
7	–22.3	–17.0	–13.0

[a] Total energy = –461.475216 a.u.; [b] total energy = –464.042260 a.u.; [c] total energy = –467.154317 a.u.

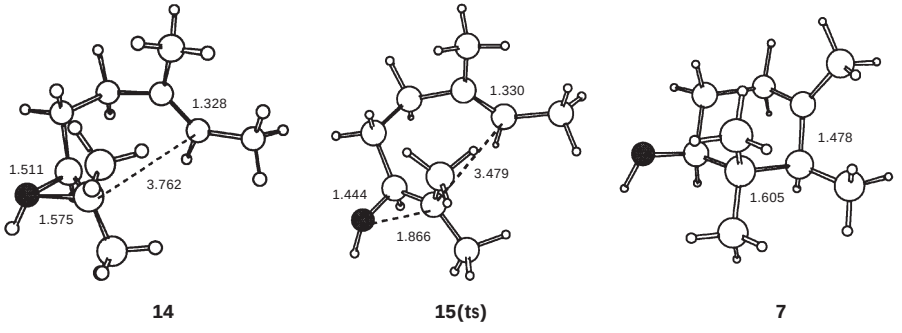


FIG. 2
Structures (HF/6-31G*//HF/6-31G*) of the protonated oxirane **14**, the cyclohexane product **7** and the transition structure **15** linking them. Bond distances are given in Å

dict a very low activation energy for the concerted ring-opening of the epoxide and the formation of the cyclohexyl carbocation product. Both methods also predict the product to be significantly more stable than the reactant (an exothermic reaction).

In order to confirm that transition structure **15** indeed connects the reactant **14** and product **7**, IRC calculations were undertaken at the RHF/3-21G level. The results of these calculations are summarized in Fig. 3, and relative energies of the three stationary points are given in Table I. It is seen that the 3-21G optimized structures are in good qualitative agreement with the RHF/6-31G* and DFT structures shown in Figs 1 and 2, which suggests that the IRC done at the lower level should give a reasonably good qualitative picture of the reaction pathway. The results of the IRC confirm the "early" nature of the transition structure in that the distance between the two car-

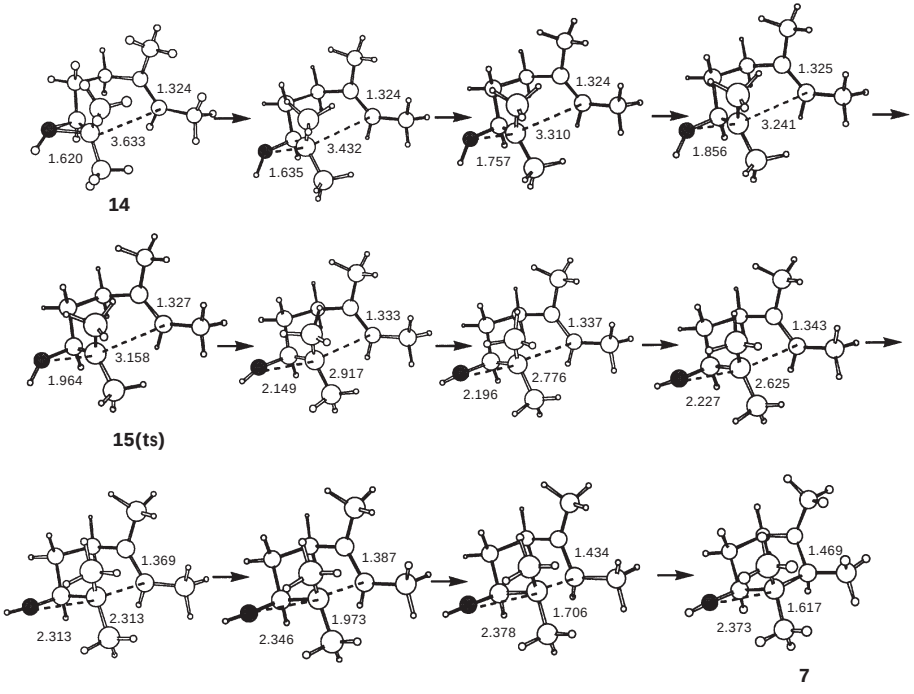
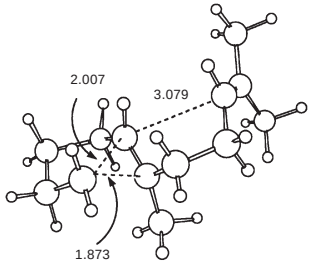


FIG. 3
Selected points along the IRC pathway (HF/6-3-21gGHF) for the reaction of **14** *via* transition structure **15** to yield **7**. Distances shown are in Å

bons which form the cyclohexane ring during the course of the reaction decreases by only 0.475 Å between the reactant **7** and the transition structure **15**, while between **15** and product **14** it undergoes a decrease of 1.541 Å. The main change between reactant and transition structure is the ring opening of the protonated oxirane ring with the C–O bond length increasing by 0.344 Å. Beyond the transition structure the oxirane ring continues to open while the doubly bound carbons approach more closely the developing positive charge on C-2. In fact, in the early stages after passing the transition structure, these two sp^2 carbons remain very close to being equidistant from C-2 as well as undergo very little rehybridization, which is suggestive of a π-complex[15]. However as the reaction proceeds further, C-7 begins to approach C-2 faster than C-6 does and C-7 begins more distinctly its rehybridization to sp^3. What is remarkable is that the C–C double bond begins its interaction with the developing positive charge at a quite large distance. This was also found to be the case for the concomitant C-ring expansion and D-ring formation mentioned in the introduction[4]. The transition structure **15** in fact has many similarities to the transition structure **16** found for the C- and D-ring formation (Fig. 4). In transition structure **16**, as in **15**, the assisting double bond is quite distant from the carbon with the developing positive charge as well as the sp^2 carbons of the double bond being essentially equidistant from this carbon. In addition, the carbons of the double bond in **16** are also essentially unhybridized. Hence we conclude that for the these two processes (epoxide ring-opening and ring-expansion), the mechanisms are closely related. That is, the double bond in both cases participates in an anchimeric manner, not only presumably lowering the energy of the process, but also directing the stereochemistry.



16

FIG. 4
The transition structure (B3LYP/6-31G*) for ring expansion of the C-ring and formation of the D-ring[4]. Distances shown are in Å

Conclusions

DFT calculations on a system which models the A-ring formation in the biosynthesis of lanosterol from squalene confirm the earlier results[3] of Pan and Gao. Given the very low calculated activation energy for the concerted ring-opening of the protonated oxirane and six-membered "A-ring" formation, we suggest that the major role of the enzyme in this first step of the cascade of reactions which leads to the formation of the tetracyclic ring-system of lanosterol is the protonation of the oxirane of 2,3-epoxidized squalene. The enzyme is presumably also responsible for holding the substrate in the proper conformation for the ring closure to occur. Finally the similarity between the mechanism of A-ring formation and that of C-ring expansion and D-ring formation in the same cascade of reactions is noted.

REFERENCES AND NOTES

1. See reviews: a) Abe L., Rohmer M., Restwich G. D.: *Chem. Rev. (Washington, D. C.)* **1993**, *93*, 2189; b) Wendt K. U., Schulz G. E., Corey E. J., Liu D. R.: *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 2812.
2. Corey E. J., Staas D. D.: *J. Am. Chem. Soc.* **1998**, *120*, 3526.
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**, *124*, 10286.
5. 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.
6. Corey E. J., Cheng H., Baker C. H., Matsuda S. P. T., Li D., Song X.: *J. Am. Chem. Soc.* **1997**, *119*, 1277.
7. Goldsmith D. J.: *J. Am. Chem. Soc.* **1962**, *84*, 3913.
8. Corey E. J., Cheng H., Baker C. H., Matsuda S. P. T., Li D., Song X.: *J. Am. Chem. Soc.* **1997**, *119*, 1289.
9. 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 98W*. Gaussian, Inc., Pittsburgh (PA) 1998.
10. Becke A. D.: *J. Chem. Phys.* **1993**, *98*, 5648.
11. Lee C., Yang W., Parr R. G.: *Phys. Rev. B: Condens. Matter* **1988**, *37*, 785.
12. Hariharan P. C., Pople J. A.: *Theor. Chim. Acta* **1973**, *28*, 213.
13. a) Gonzalez C., Schlegel H. B.: *J. Chem. Phys.* **1989**, *90*, 2154; b) Gonzalez C., Schlegel H. B.: *J. Phys. Chem.* **1990**, *94*, 5523.

14. Pan and Gao (ref.[3]) estimated this bond distance to be 3.2 Å by optimizing structures along the "reaction pathway" with fixed C–C distances of the forming cyclohexane bond (the first figure in their supplementary information).
15. Dewar M. J. S., Reynolds C. H.: *J. Am. Chem Soc*. **1984**, *106*, 1744.