Theoretical Evidence for a Concerted Mechanism of the Oxirane Cleavage and A-Ring Formation in Oxidosqualene Cyclization

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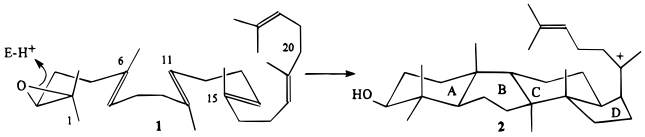
The conversion of 2,3-oxidosqualene (**1**) to lanosterol, catalyzed by lanosterol synthase, is one of the most remarkable processes in biochemistry. The reaction is characterized by a precise



enzymatic control with the formation of four rings and seven new stereocenters.[1] Over the past four decades, the quest for a fundamental understanding of the enzymatic mechanism in steroid biosynthesis has inspired numerous experimental investigations.[2−4] Early work and recent studies by Corey et al. have established that the cyclization involves discrete carbenium ion intermediates in the formation of **2**, which undergoes a series of 1,2-methyl and hydride shifts to yield lanosterol.[2,3] In this paper, we present computational evidence supporting a concerted mechanism for the oxirane cleavage and A-ring formation.

To address the question of oxirane activation, and to determine whether epoxide opening occurs prior to or concurrently with cyclization by C_2-C_7 bond formation, transition structures and products for the oxirane cleavage and the cyclohexyl ring formation of (3*S*)-2,3-oxido-2,6-dimethyloct-6-ene (**3**) have been determined with and without the presence of a Brønsted acid catalyst, using restricted Hartree−Fock (RHF) calculations and the 6-31G* basis set.[5] Electron correlation effects are included in single-point energy calculations with the hybrid HF and density functional theory, B3LYP/6-31+G*//HF/6-31G*.[6]

Transition structures and stable geometries for the formic acid-catalyzed and uncatalyzed reactions of **3** have been determined

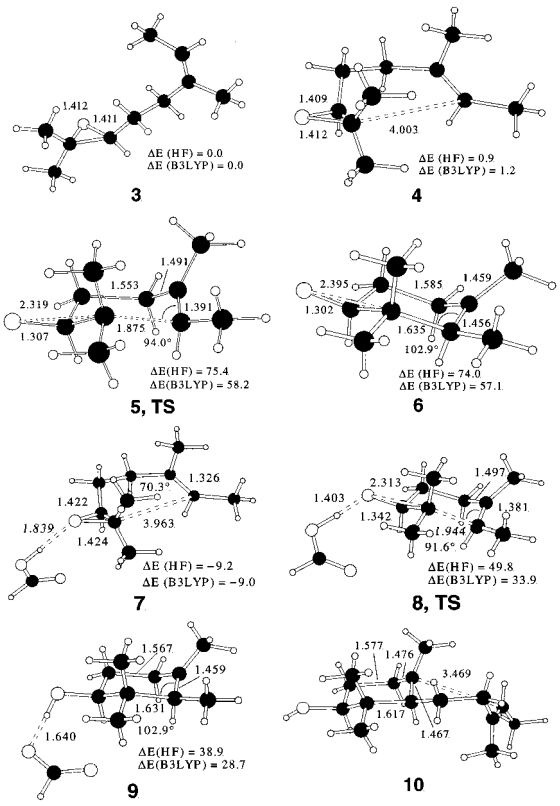


Figure 1. RHF/6-31G* optimized structures and selected geometric values for the catalyzed and uncatalyzed oxirane cleavage. Distances are given in angstroms, and energies are given in kilocaleries per mole at the RHF/6-31G* and B3LYP/6-31+G* level.

at the RHF/6-31G* level (Figure 1). Both transition structures **5** and **8** have been verified by vibrational frequency calculations. The forming C−C bond in **5** has a length of 1.875 Å, which is only 0.24 Å longer than that in the carbenium ion intermediate **6**, and is much shorter than a typical value for C−C bond formation reactions. For example, the length of the C−C bond being formed is 2.27 Å in the transition state for the Claisen rearrangement of allyl vinyl ether.[7] The oxirane ring is essentially split in **5**, with a C_2-O distance of 2.319 Å. For the catalyzed reaction, the C_2-C_7 bond length in **8** is 1.944 Å, which is 0.07 Å greater than the uncatalyzed case. At the transition state, the hydrogen bond distance between formic acid and the epoxy oxygen is shortened to 1.403 Å from 1.839 Å in **7** due to increased H-bonding interactions. Both carbenium ions **6** and **9** exhibit strong hyperconjugation with the alignment of the cationic 2p orbital and the C−C bond, resulting in elongated C_2-C_7 distances (1.631 and 1.635 Å).[8]

In view of the large differences between the transition structures and reactants, intrinsic reaction paths (IRC) were determined at the RHF/6-31G* level to establish the connectivities.[9] The reaction profiles and several selected structures are shown in Figure 2. The IRC calculations showed that the transition structures collapse to the initial folded pre-chair conformation for both reactions, despite the large $O-C_2$ distance at the transition structures. Transition structure **5** also leads to the final cation **6**, whereas the IRC profile of transition structure **8** involves a concerted proton transfer from HCO_2H to the epoxy oxygen,

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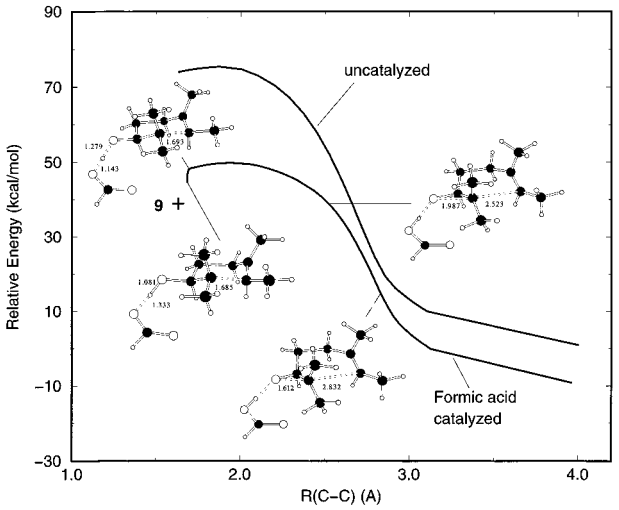

Figure 2. Potential energy change along the gas-phase reaction paths for the catalyzed and uncatalyzed oxirane cleavage from RHF/6-31G* calculations. A few selected structures are also shown.

which further facilitates the A-ring formation. Although the proton-transfer step is smooth in the IRC calculations (Figure 2), the reaction profile cannot be completed on the product side from **8** because the formate ion moves abruptly toward the cationic center at C_6. The last configuration from the IRC calculations shows that the proton is already fully transferred and the structure is essentially the A-ring carbenium ion. Optimization of this structure constrained by fixing the hydrogen bond to be linear resulted in structure **9**, which is 5 kcal/mol lower in energy than **8**. The IRC path calculations demonstrate that both catalyzed and uncatalyzed reactions are concerted in the oxirane cleavage and cyclohexyl ring formation. This is in accord with the conclusion of Corey et al. on the basis of Michaelis−Menten kinetic data.[3e] In that study, the CH_3 group at the C_6 position in **1** was replaced by Cl and H. The values of V_{max}/K_m were found to be proportional to the carbocation stabilizing ability of CH_3, Cl, and H and the nucleophilicity of the substituted C−C double bonds, suggesting that the nucleophilic attack at the oxirane is concerted.[3e]

The lowest-energy conformer of **3** that was found in the conformational search is only 0.9 to 1.2 kcal/mol lower in energy than the folded pre-chair conformation **4**. Nucleophilic attack of the oxirane by the C_6-C_7 double bond gives transition structure **5**, which leads to the formation of a zwitterionic intermediate **6**. The activation energy for this process is computed to be 74.5 and 57.0 kcal/mol from **4** at the RHF/6-31G* and B3LYP/6-31+G* levels, respectively. Notably, product **6** is only ca. 1 kcal/mol below the transition structure, a feature typical of charge separation processes in the gas phase.[10] Because of the development of charges at the transition state, the reaction is predicted to be significantly accelerated in polar solvents.[10] In combination with the RHF/6-31G* vibrational frequencies, the computed $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$, and $\Delta G^{\ddagger}$ for the reaction at 298 K are 55.4 kcal/mol, −11.5 cal/(mol·K), and 58.8 kcal/mol, respectively.

The high energy barrier is overcome by protic activation of **1** to initiate cyclization in lanosterol biosynthesis. Corey et al. identified a highly conserved aspartic acid residue to be essential for catalysis from site-directed mutagenesis experiments.[3e,3f] To model the aspartic acid residue in the enzyme, formic acid was used in the present computational model for oxirane activation. The interaction energy for the bimolecular complex (**7**) between formic acid and **4** is found to be −9.2 and −9.0 kcal/mol at the HF/6-31G* and B3LYP/6-31+G* levels. Significantly, the activation barrier is lowered by ca. 15 kcal/mol in comparison with the uncatalyzed system. At the B3LYP/6-31+G* level along with the RHF/6-31G* vibrational frequencies, the predicted $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$, and $\Delta G^{\ddagger}$ relative to **7** are 40.3 kcal/mol, −11.1 cal/(mol·K), and 43.6 kcal/mol, respectively. Inclusion of the solvent effect in acetonitrile by using the polarizable continuum model (PCM) at the B3LYP/6-31G* level further reduces $\Delta G^{\ddagger}$ by 10.8 kcal/mol to a value of 32.8 kcal/mol.[11] Experimentally, **1** and similar oxiranes are stable for many hours in glacial acetic acid, and trichloroacetic acid is required for the catalyzed oxirane cleavage.[3e,12] The still rather high activation free energy for the formic acid-catalyzed reaction in acetonitrile (32.8 kcal/mol relative to **7**) is consistent with the experiments. Corey et al. proposed that the enhanced acidity required for catalysis in the enzyme can be achieved by the presence of a protonated histidine forming an Asp:His salt bridge.[3e,f] This has been confirmed by the crystal structure of squalene cyclase.[4] A limiting case of strong Brønsted acid catalysis is demonstrated by the protonated oxirane, which undergoes oxirane cleavage by nucleophilic attack from the π bond with a barrier of only ca. 0.6 kcal/mol at the B3LYP/6-31+G*//HF/6-31G* level. Furthermore, the reaction changes from a highly endothermic process for **3** to a reaction exothermic by −15.5 kcal/mol. Thus, the results suggest that acid catalysis becomes most effective when the pK_a of the Brønsted acid is equal to or lower than that of the TS **5**.

The propagation of the A-ring carbenium ion intermediate was examined by considering the reaction of the oxidosqualene fragment **10** to B-ring formation. The key issue is the existence or absence of a barrier to the reaction, which is addressed by determination of the reaction profile by using RHF/3-21G optimizations and B3LYP/6-31G* energy calculations. At the RHF/3-21G//3-21G level, the energy change for the conversion of **10** to the B-ring product **11** is exothermic by −6.7 kcal/mol with a small barrier of 0.7 kcal/mol at a distance of 2.9 Å. With inclusion of electron correlation at the B3LYP/6-31G*//HF/3-21G level, a shallow minimum (−2.3 kcal/mol) at a C_6-C_{11} distance of 2.6 Å is found, which collapses to the product **11** with a barrier of about 1 kcal/mol. This feature has been observed in other cation−olefin addition reactions by Jenson and Jorgensen.[13] The relatively low exothermicity in B-ring formation (−2.4 kcal/mol) is due to increased ring strain and steric effects of the twist boat conformation in the product. It is noted that similar tertiary cation reactions give energy changes of ca. −10 kcal/mol.[13] In the enzyme active site, carbenium ion propagation may be further assisted by cation−π interactions with properly aligned aromatic residues.[14] In fact, numerous aromatic residues are found at the active site in the crystal structure of squalene cyclase.[4] Similarly, they have been identified in lanosterol synthase from studies of mechanism-based inhibitors, site-directed mutagenesis, and amino acid sequence analysis.[3f]

The present computational studies established that the electrophilic activation of oxirane cleavage and A-ring formation are concerted for both uncatalyzed and formic acid-catalyzed reactions, consistent with experiments. Transition state stabilization by catalytic Brønsted acid depends critically on the strength of its acidity. The high energy barrier for the uncatalyzed oxirane cleavage is reduced to an essentially barrierless process for the protonated oxirane. In addition, B-ring formation proceeds in concert with the closure of the A-ring.

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Supporting Information Available: RHF/6-31G* optimized structures in the Z-matrix (8 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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