FORMATION OF RING A IN THE BIOSYNTHESIS OF HOPANOIDS FROM SQUALENE. A DENSITY FUNCTIONAL STUDY

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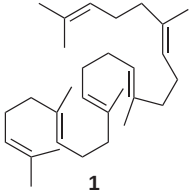
I dedicate this paper to Rudolf and Milena on the occasion of his "diamond" birthday. I first had the good fortune to meet Rudolf thirty years ago when I spent a year in his group at the J. Heyrovský Institute in Prague as a National Academy of Science Exchange Scientist. Over the years since then I have greatly benefited from his friendship and his "gentle" scientific guidance. I raise a toast to Rudolf, an outstanding scientist and my friend for so many years.

Density functional calculations were carried out on the ring closure of the 2,6-dimethyloct-6-en-2-yl cation to a chair conformer of the 1,2,3,3-tetramethylcyclohexyl cation as a model for the first step in the biosynthetic cyclization of squalene to the triterpene hopanoids. The concerted reaction was found to have an activation energy of 4.6 kcal/mol and to be exothermic by 11.5 kcal/mol.

Keywords: Triterpenes; Steroids; Biosynthesis; Squalene; Cyclizations; Reaction mechanism; DTF calculations; *Ab initio* calculations.

The remarkable transformation of squalene (**1**) to many different triterpenes has fascinated chemists for over fifty years[1]. It is known that bacterial cyclases initiate the cyclization of squalene by protonation of its 2,3-double bond followed by cyclization to the pentacyclic hopanoids such as hopene (**2**). In the case of steroids, the 2,3-double bond of squalene is first epoxidized by an enzyme, followed by protonation of the epoxide and sub-



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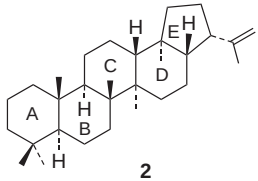

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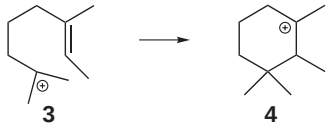
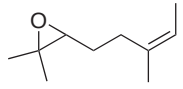
sequent cyclization. While there has been a number of other theoretical studies directed toward the understanding of the cyclization of squalene to the steroids[2], none has been reported for the cyclization of squalene to the hopanoids. Herein we report calculations on the model system **3** and its cyclization to the cyclohexyl cation **4** in order to study the formation of



3 **4**

ring A in the hopanoid biosynthesis. We have previously reported[2c] calculations on the formation of ring A of the steroids on the model system **5** that lend support to the concerted nature of the cyclization and protonation of



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the epoxide. In both cases the formation of the A ring is thought to give rise to the chair conformer of a cyclohexane ring. Knowledge of these A ring formations will be useful in the further study of these systems, in particular the formation of the B rings, which are known to occur differently in the biosynthesis of the steroids and hopanoids. In the steroid cyclization of squalene the B ring is formed in a boat conformation, while in the hopanoids, a chair conformation is formed:

steroid cyclization

hopanoid cyclization

COMPUTATIONAL METHODS

Calculations were performed using Gaussian 98W [3]. The density functional method was employed using Becke's three-parameter hybrid method[4] with the Lee–Yang–Parr correlation function[5] and the 6-31G* basis set[6]. 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[7] were used to determine the reaction pathway.

RESULTS AND DISCUSSION

Ring A is presumably formed in a chair conformation, and with this in mind a preliminary search for a transition structure was carried out with SCF/3-21G calculations. A transition structure (**6**) was located and SCF/3-21G IRC calculations showed that it linked a chair conformation of the cyclohexyl cation **4** with a conformer of **3**. Each of these structures was in turn reoptimized with DFT calculations and the structures obtained are shown in Fig. 1. The conversion of **3** to transition structure **6** is primarily a rotation about the C2–C3 bond in **3**, which has the effect of moving the positively charged carbon atom in **3** closer to the double bond. Beginning with the DFT transition structure **6**, a DFT IRC calculation (Fig. 2) indicated that there is a smooth transition from **6** to the cyclohexyl cation in a chair conformation **4**. It is seen from transition structure **6**, with its rather long distance between the two carbons that form the C–C bond, that the interaction of the positively charged carbon with the double bond begins at a rather long distance. Going from **6** to **4**, this distance decreases in a straightforward fashion to give the cyclohexane ring. The DFT pathway was found to be very similar to that obtained at the SCF level.

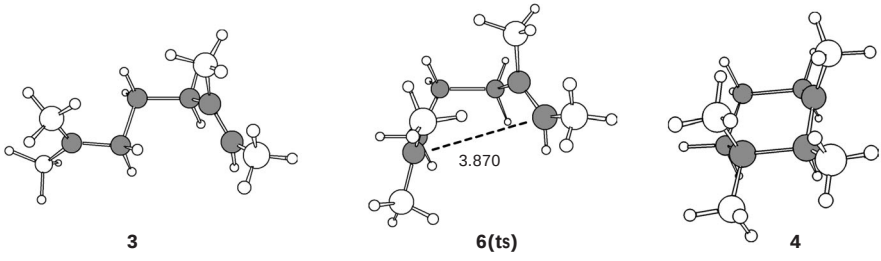

FIG. 1
Structures (B3LYP/6-31G*) of the cations **3** and **4**, and the transition structure **6(ts)** which links them. The distance between the two carbons of the forming bond is given in Å

However there was found to be a difference in the pathway between **3** and transition structure **6** computed with DFT from that computed at the SCF level. It was found that with DFT there existed an extremely shallow minimum (less than 0.01 kcal/mol), **7**, on a very flat portion of the reaction pathway (Table I). Structure **7** is linked with **3** *via* transition structure **8**, which lies only 0.1 kcal/mol higher in energy than **7**. Furthermore when zero point energies were included, these two stationary points (**7** and **8**) both were actually slightly higher in energy than the transition structure **6**. In Fig. 3 are shown two perspectives of each of the structures **6**–**8**. It is seen that on going from **8** to **6** the main movement within the structures is rotation about the C3–C4 bond. Structure **8** can be arrived at by starting with

TABLE I
Relative energies of the stationary points **3**–**7** in kcal/mol (energies in parentheses are corrected for zero point energy with DFT frequencies)

Species	RHF/3-21G	B3LYP/6-31G*
3	0.0[a]	0.0[b] (0.0)[c]
4	–22.4	–14.3 (–11.5)
6(ts)	5.1	5.1 (4.6)
7	–	5.0 (4.7)
8(ts)	–	5.0 (4.7)

[a] Total energy = –387.314857. [b] Total energy = –392.222979 a.u. [c] Total energy with zero point correction = –391.957242 a.u.

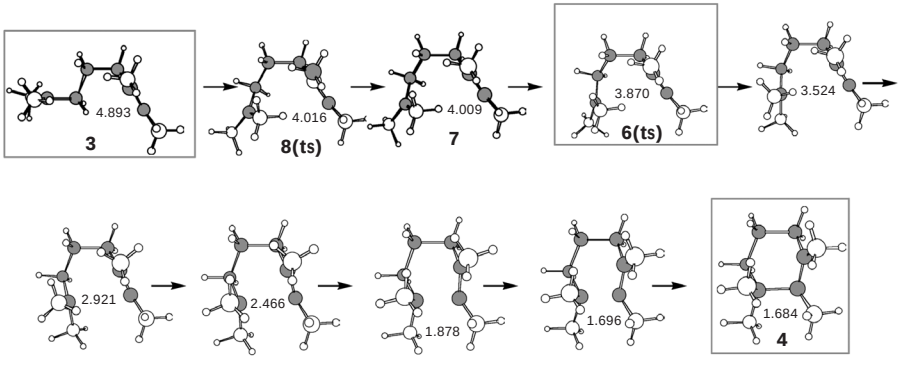

FIG. 2
The reaction pathway for the cyclization of **3** to the chair cyclohexyl cation **4**. The distances between the two carbons of the forming bonds are given in Å

conformer **3** and rotating about this bond. During this rotation the structure must pass through an eclipsed conformer with respect to the C3–C4 bond. Apparently during this rotation the interaction begins between the positively charged carbon and the double bond. While the energy, due to steric hindrance, would presumably increase during this rotation to the eclipsed conformer of the eclipsing hydrogens, this is offset by the favorable interaction of the double bond with the positively charge carbon atom. The result of this is an extremely flat potential energy surface around the eclipsed conformer leading to transition structure **6**.

In Fig. 4 transition structure **6** is compared with the transition structure (**9**) found previously[1c] for the cyclization of the protonated epoxide **5**. It is seen that transition structure in the former case (**6**) occurs "earlier" along the reaction pathway (forming bond of 3.870 Å) than in the latter case (forming bond of 3.479 Å). This is not surprising, since in the case presented here (**6**), the reactant has presumably almost a full positive charge on carbon 2, whereas for the epoxide case the corresponding carbon has

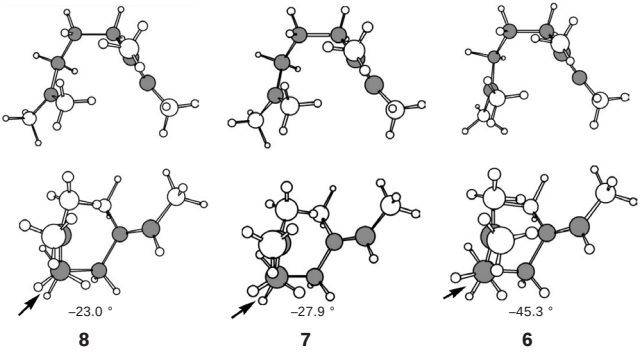

FIG. 3
The structures of **6**–**8**. Dihedral angles (H–C–C–H) are indicated

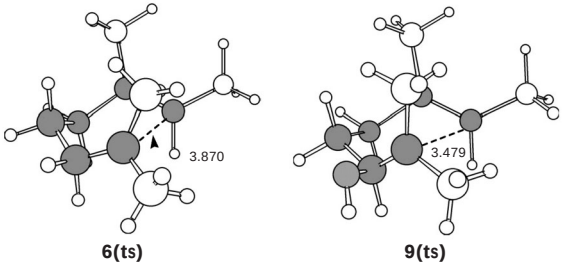

FIG. 4
Structures (B3LYP/6-31G*) of the transition structures **6** and **9**. The distances between the two carbons of the forming bonds are given in Å

only a partial positive charge in the transition structure (the protonated, partially ring-opened epoxide). In both cases it is easily seen from Fig. 4 that the transition structures are leading to a chair conformation of the cyclohexyl cations.

CONCLUSIONS

It has been shown that a transition structure **6** exists for a concerted reaction for the cyclization of a model system of the formation of the A ring in the hopanoid biosynthesis from squalene. Comparison of **6** with the transition structure found previously for a model system for the initial cyclization of squalene oxide to give steroids indicates that the two concerted reactions are closely related.

Note Added In Proof

Rajamani and Gao have very recently reported (*J. Am. Chem. Soc.* **2003**, *125*, 12768) molecular dynamics simulations on the carbocation cyclization of squalene to hopene. They reported that the formation of the A ring leads to a distinct monocyclic carbocation intermediate, which corresponds to our structure **4**.

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