Formation of the B Ring in Steroids and Hopanoids from Squalene

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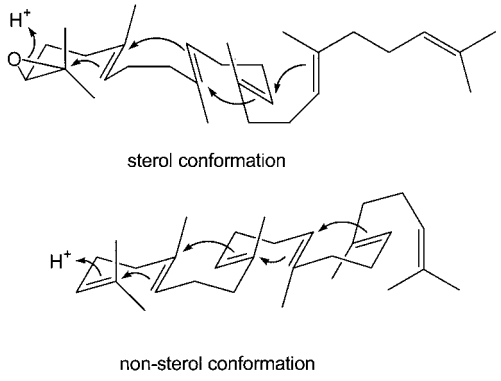
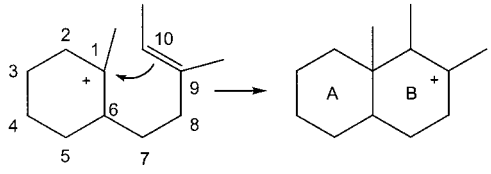
A theoretical conformational study based on density functional calculations provides evidence that the sterol and non-sterol cyclizations of squalene to triterpenes are controlled by conformational effects as has been previously suggested. It was found that different conformers of a model system of squalene give rise to the chair–boat conformation found in the steroids and the chair–chair conformation of the pentacyclic 3-deoxytriterpenes for their A and B rings. It is suggested that the enzymes play a key role in holding the substrate in the proper orientation for these cyclizations to occur. (© Wiley-VCH Verlag GmbH & Co. KGaA, 69451 Weinheim, Germany, 2004)

Introduction

It has been well established that squalene is the precursor to many naturally occurring tripterpenes.[1] Eukaryotic oxido-squalene cyclases convert squalene through its epoxide to the tetracyclic protosterol cation, while the bacterial cyclases convert squalene directly to pentacyclic 3-deoxytriterpenes (e.g., hopanoids). These two cyclizations also differ in other aspects, the most notable being the conformation of the ring B of the polycyclic products. In the case of the steroid biosynthesis ring B is formed with a boat conformation, whereas in the hopanoids this ring has a chair conformation. This results in a chair−boat−chair conformation of rings A−C in the former case, and a chair−chair−chair conformation in the latter (see Scheme 1). It has been conjectured that the enzymes hold the squalene precursors in different conformations to allow the two stereochemically different cyclizations to occur. In both cases the cyclizations involve carbocations in which either a forming or a formed carbocation interacts with a neighboring double bond to bring about ring-closure. Previous ab initio studies indicate that these ring closures are likely to proceed without the intervention of reactive intermediates.[2,3] Similar studies (density functional theory) have also been reported on the closure of rings A,[4] C,[5] and D.[6] Herein a detailed theoretical conformational study[7−11] of the model system **1** in its ring closure to **2** is reported, the results of which can account for these two stereochemically different pathways in the biosynthesis of steroids and hopanoids.



Scheme 1



1 **2**

Density functional theory (DFT) was used throughout to locate the stationary points on the potential surface. It is felt that for this type of conformational study, DFT will provide a good qualitative description of the potential surfaces studied. In support of this is Wiberg's finding that DFT results for the rotation of the ethyl group in equatorial and axial ethylcyclohexanes were very similar to those obtained with MP2 calculations.[12]

Results and Discussion

First to be considered is the sterol ring-closure of **1** in which ring A is in a chair conformation and ring B is

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formed in a boat conformation. Examination of a model of **1** indicates that for the ring closure to occur only one of the possible staggered conformations of the C6−C7 bond can lead to ring closure. In order for the boat conformer to be formed in the cyclization, a particular conformer of the C8−C9 bond is also required. Hence, the conformational study is reduced to locating the conformers about the C7−C8 bond of **1**. This is a typical butane-like C−C bond, and as a consequence one would expect to find three staggered conformers. In fact, a careful density functional theory (DFT) search for these three conformers yielded only two conformers, **3** and **4** (see Figure 1). An eclipsed conformer (**TS34**) was located, and it is the transition structure linking **3** and **4**. A second eclipsed transition structure (about the C7−C8 bond) was also found (**TS35**), from which its structure one would expect to be the transition structure that links **3** with the elusive third staggered conformer of **1**.

A DFT intrinsic reaction coordinate (IRC) calculation initially appeared to be leading to this third staggered conformer. However, the potential surface is very flat in the region where the staggered conformer would be expected, and the IRC proceeded to a chair−boat conformer of **2** (structure **5** in Figure 1). A third transition structure (**TS46**) was located, but surprisingly, it was not eclipsed about the C7−C8 bond, but rather is a staggered conformation in which there has been only a 2° rotation around the C7−C8 bond from that of the staggered conformer **4**. However, the distance between C1 and C10 has decreased by more than 0.2 Å, which suggests that this transition structure might lead directly to the cyclized product. This was confirmed by an IRC calculation in that the pathway led directly to a second chair−boat conformer of **2** (structure **6** in Figure 1). Conformer **6** is calculated to be 2.4 kcal/mol more stable than **5**. A transition structure (**TS56**) linking these two chair−boat conformers of **2** was found, and it was only 0.3 kcal/mol higher in energy than **5**.

Conformational considerations similar to those for the formation of the chair−boat discussed above hold for the conformers of **1** that would yield the chair−chair conformer of **2**. In order for the B ring to close to a chair conformation, the C_4 group attached at C8 must be rotated by 180° from those conformers discussed above (**3** and **4**) that lead to the chair−boat conformation of **2**. Again two staggered conformers were found (**7** and **8**) and an eclipsed

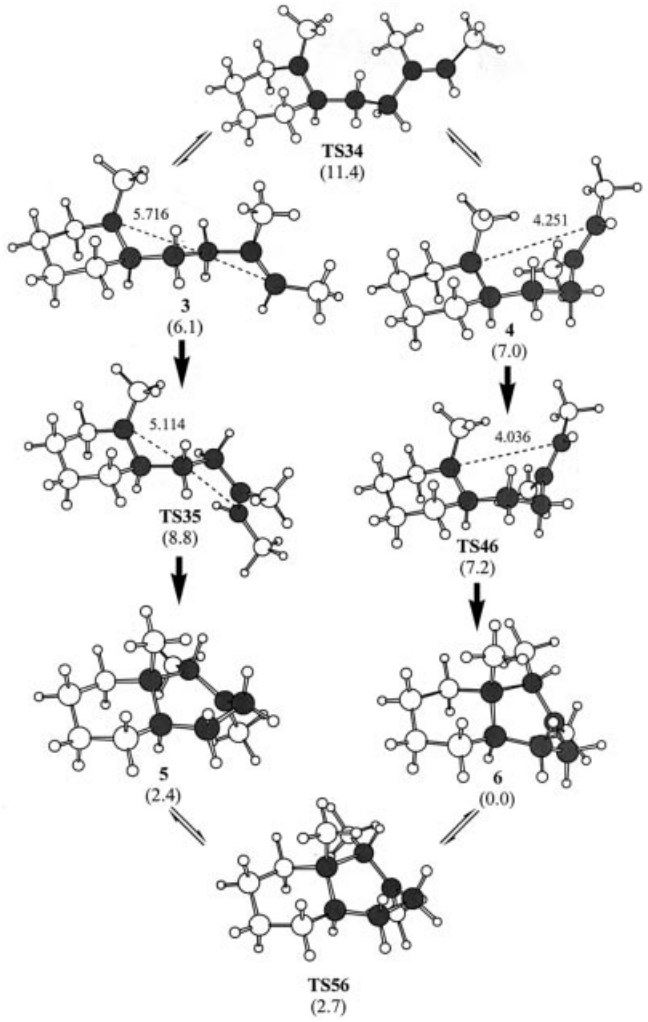


Figure 1. The formation of the sterol B ring from **1**; relative energies (in parentheses) are in kcal/mol; the dotted lines show the forming bonds, and these distances are given in A

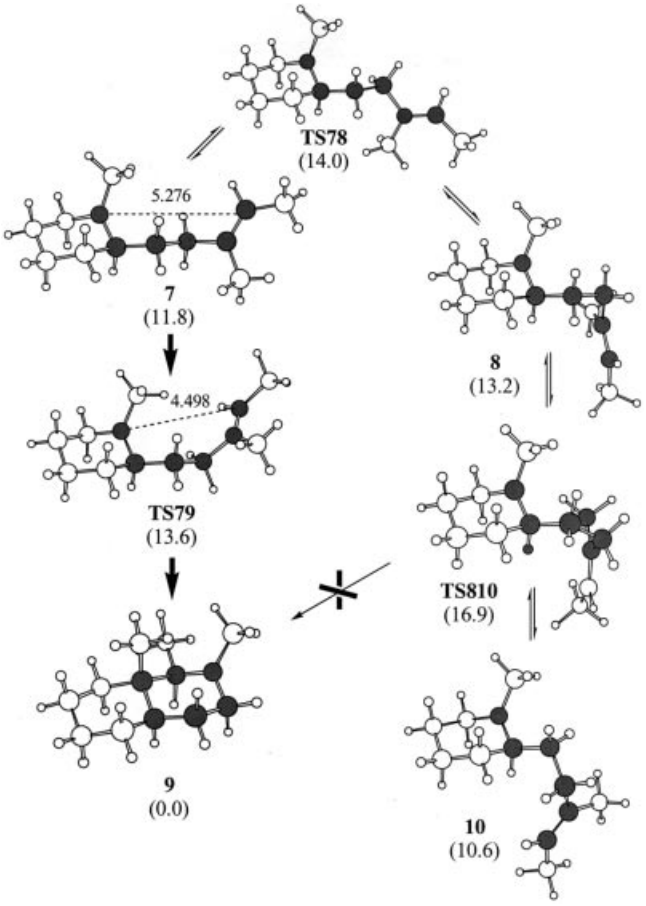


Figure 2. The formation of the nonsterol B ring from **1**; relative energies (in parentheses) are in kcal/mol; the dotted lines show the forming bonds, and these distances are given in A

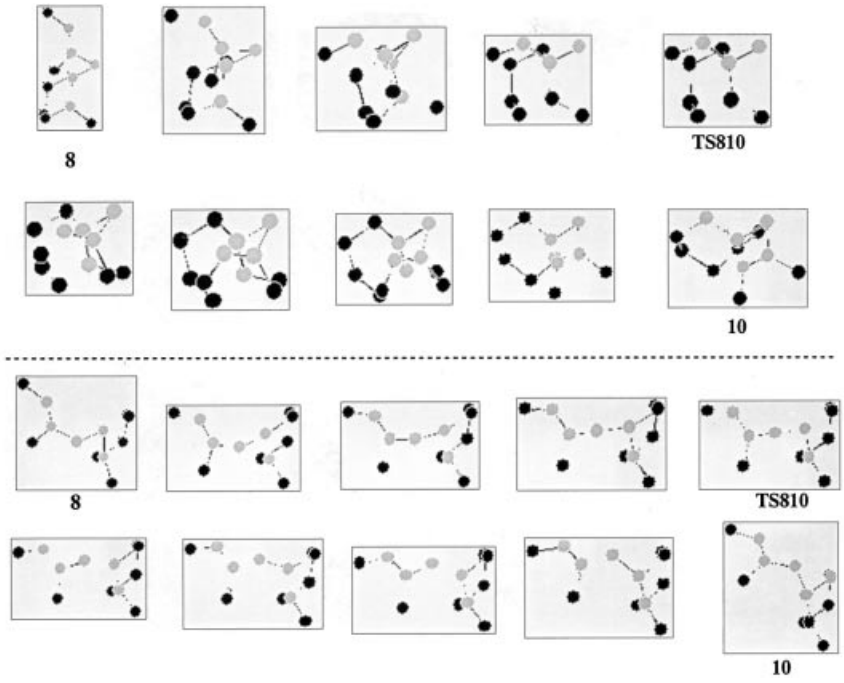


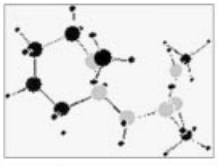
Figure 3. Views of points along the IRC pathway (SCF/3-21G) for the conversion of **8** into **10** through the transition structure **TS810**; the top views show the rotation about the C7−C8 bond and the bottom views show the rotation about the C6−C7 bond

transition structure (**TS78**) that links them (see Figure 2). A second eclipsed transition structure (**TS79**), which as in the case of the chair−boat cyclization, did not lead to a third staggered conformer, but instead to the chair−chair structure **9**.

A third eclipsed transition structure (**TS810**) was located, but it did not collapse to conformer **9** as indicated by an IRC calculation. Instead continued rotation around the C7−C8 bond from **TS810** is accompanied by simultaneous rotation around the C6−C7 bond. The net effect is a simultaneous conformational change about the C6−C7 and C7−C8 bonds. In retrospect, this is not a surprising result since the C1 and C9 methyl groups begin to impinge on one another as further rotation occurs about the C7−C8 bond. The second rotation (C6−C7) occurs to relieve this steric strain (see Figure 3).

Examination of the energies of the conformers shown in Figure 2 again indicates that going from the staggered conformer **7** to the chair−chair bicyclic **9** is a low energy process, having an activation energy of only 1.8 kcal/mol. The chair−chair conformer **9** was calculated to be 5.6 kcal/mol lower in energy than the higher energy chair−boat conformer **6**. This is in remarkably good agreement with the experimental value of 5.1 ± 0.9 kcal/mol for the energy difference between chair−chair and chair−boat *trans*-decalin.[13]

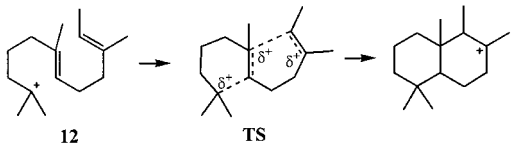
These conformational results can provide some insight into the mechanism of the formation of the A and B rings in the biosynthesis of steroids and hopanoids. It has previously been suggested that the ring closure of these two rings might very well be a concerted rather than a step-wise process, though it was noted that "*It is not known, however, whether there is any temporal overlap between the formation of the A and B rings.*"[1b] It is suggested here that the failure to find the third staggered conformer in the conformational results presented above for either the chair−boat or chair−chair formation of the B ring may be taken potentially (see below) as computational support for the concerted nature of the A and B ring closures. A possible explanation for the failure to find these two conformers is that the proximity of the C9−C10 double bond to the positive charge on C1 in **1** would be such that this conformer would simply collapse to conformer **2**. If one takes structure **4**, and rotates it clockwise about the C7−C8 bond by 120°, one obtains staggered conformer **11**. It can be seen that the positively charged carbon atom in **11** is indeed in close proximity to the double bond.



Conclusion

This detailed conformational analysis of the ring closures of the B rings in the steroid and hopanoid pathways indicates that in both cases the staggered conformer that would be expected to give rise in one case to a boat conformer of the B ring and in the other case to a chair conformer of the

B ring does not probably exist, because of the close proximity of the positively charged carbon atom to a double bond. This result suggests that a concerted pathway for the formation of rings A and B in the biosynthesis of steroids and hopanoids might exist. It is quite probable that the "missing" third staggered conformers in Figures 1 and 2 would exist in the absence of a nearby positive charge. If the enzyme holds the squalene chain in such a conformer, during the ring-closure of ring A the double bond in the chain, which gives rise to the B ring, would be in close proximity to the developing positive charge on ring A; and it is likely B ring formation would begin prior to completion of the A ring formation (hence concerted formation of A and B rings).[14] However, these results by no means require that A and B ring formation is a concerted process in the enzymatically controlled cyclizations of squalene to the steroids and hopanoids. It is suggestive that if these are step-wise processes (with the intermediacy of a carbocation arising from the formation of ring A), then the intermediate carbocation is likely formed in a conformer that must undergo a rotation about a C−C bond (energy barrier) for formation of the B ring. A very recent QM/MM study reported by Rajamani and Gao on the formation of the hopanoid ring system from squalene found the formation of rings A and B to be a stepwise process, which involved an activation energy of only 4 kcal/mol. However, the AM1 method used might not properly describe the interaction of a carbocation with a double bond. A further test of the concertedness of the A−B ring formation would be to study the "double" ring closure of a system such as **12**. If a transition structure for the "double" ring closure of the A and B rings in **12**, in which the side chain had the appropriate staggered conformation about the C7−C8 bond, could be located; this would confirm the existence of a concerted pathway, though this need not necessarily be the pathway actually followed in the enzymatic cyclization. To make the final determination which pathway is operative would require modeling studies in which both pathways (concerted and stepwise) were examined.



12 **TS**

Acknowledgments

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[7] All calculations were performed with Gaussian98W: M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres, C. Gonzalez, M. Head-Gordon, E. S. Replogle, J. A. Pople, Gaussian, Inc., Pittsburgh PA, **1998**. Becke's three-parameter hybrid method (ref.[8]) with the Lee−Yang−Parr correlation function (ref.[9]) and the 6-31G* basis set (ref.[10]) were employed. All relative energies given in the figures include zero-point energy corrections. Unless otherwise specified in the text, IRC calculations were performed at the SCF/3-21G level.

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[11] Cartesian coordinates, imaginary frequencies for transition structures and energies may be found in the Supporting Information for all structures reported here (for Supporting Information see also the footnote on the first page of this article).

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[13] J. L. Margrave, J. M. Frisch, R. G. Bautista, R. L. Clarke, W. S. Johnson, *J. Am. Chem. Soc.* **1963**, *85*, 546−548. While they did not report this particular energy difference, it can easily be obtained from their data. They were more interested in obtaining the energy difference between chair and boat cyclohexane. This agreement provides additional support for the choice of the DFT method for the calculations reported here.

[14] Note added in proof (March 23, 2004): Schulz has recently published the X-ray structure of 2-azasqualene cocrystallized with squalene-hopene cyclase (D. J. Reinert, G. Balliano, G. E. Schulz, *Chem. Biol.* **2004**, *11*, 121−126). Examination of this crystal structure suggests that indeed the enzyme "holds" the squalene in the proper conformation for the A- and B-ring formation to be a concerted process.

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