THE CONCERTED NATURE OF THE ENZYMATIC CYCLIZATION OF RINGS A-D OF SQUALENE TO HOPENE

B. Andes HESS, Jr.[a],* and Lidia SMENTEK[a,b]

[a] *Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37235, U.S.A.;*
e-mail: b.andes.hess@vanderbilt.edu

[b] *Institute of Physics, N. Copernicus University, 87-100 Toruń, Poland;*
e-mail: lidia.smentek@vanderbilt.edu

Dedicated to Professor Rudolf Zahradník on the occasion of his 80th birthday.

A conformational analysis of squalene encapsulated in squalene-hopene cyclase has been performed based on Schulz's X-ray structure and our DFT calculations. Based on this analysis it is concluded that the formation of rings A-D in the cyclization of squalene are likely to be a concerted but highly asynchronous reaction.
**Keywords**: Squalene; Steroid biosynthesis; Hopene; Carbocation cyclizations.

Over the past several years the mechanism of the enzymatic cyclization of squalene oxide and squalene to the steroid and hopene structures has received renewed scrutiny[1]. Of greatest importance is the report by Schulz[2] of the X-ray structure of 2-azasqualene encapsulated in squalene-hopene cyclase. There are three interesting aspects of this mechanism, the overall energetics of the cyclizations, the conformational aspects giving rise to the known stereochemistry of the cyclized products and the question of whether the cyclization proceeds in a concerted or stepwise fashion[3]. The first one of these has very recently been extensively addressed by Matsuda in a computational study of the overall energetics of the cyclization of squalene to hopene[4]. He performed a very careful analysis of the results of a number of computational methods and concluded that the energy release is approximately 50 kcal/mol for squalene cyclizations. This result is very close to the 48 kcal/mol previously suggested by Schulz[2]. In the present report we focus on the conformational aspects of these cyclizations as well as on the concerted versus step-wise process of the cyclizations.

RESULTS AND DISCUSSION

Schulz's extraordinary X-ray of 2-azasqualene encapsulated in squalene-hopene cyclase provides a starting point for the study of the conformational aspects. The X-ray gives a "snapshot" of a critical point in the cyclization process by "freezing" the 2-azasqualene within the enzyme, presumably in a conformation very similar to that existing for squalene itself just before the cyclization begins.

One can ask the question whether this conformation is a natural one or rather forced by the internal structure of the enzyme cavity. In order to answer this question, the X-ray structure (x, y and z coordinates of all atoms other than hydrogen) was used as a starting geometry for the complete DFT optimization of protonated squalene (**1**).
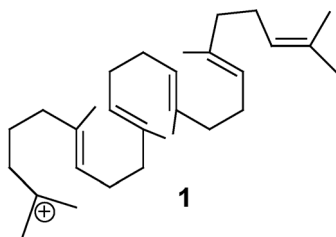

Hydrogens were added to the carbon backbone taking into account whether the carbons were sp³ or sp² hybridized. The resulting DFT (B3LYP/6-31G*)[5–8] optimized structure is depicted by **2** [9].
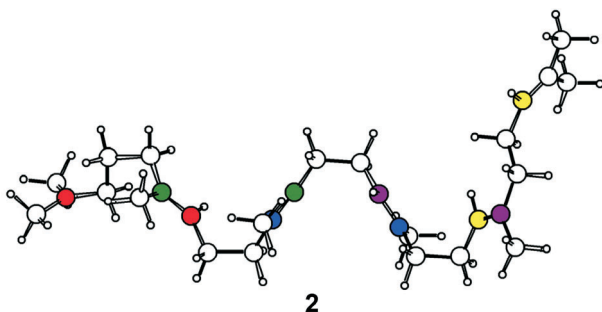
We note that Matsuda has determined that while the B3LYP/6-31G* method is not reliable as far as energies are concerned, geometries obtained in this way are reliable[4]. The atoms of the same color in **2** indicate which pairs of atoms close to form rings during the cyclization of squalene to hopene. A comparison of **2** (without hydrogens) with the structure found

by Schulz (**3**) is shown below. Dihedral angles about the individual bonds are also given.

It can be immediately seen that there is a great similarity between these two structures. *This indicates that the encapsulated squalene is likely to be in a conformation that is not "forced" by the structure of the enzyme, but simply held by it.* Closer examination of **2** and **3** shows that not only do their conformations appear to be quite similar, but furthermore they have the proper orientation for sequential closure of rings A-D in the formation of hopene[2].


2

3
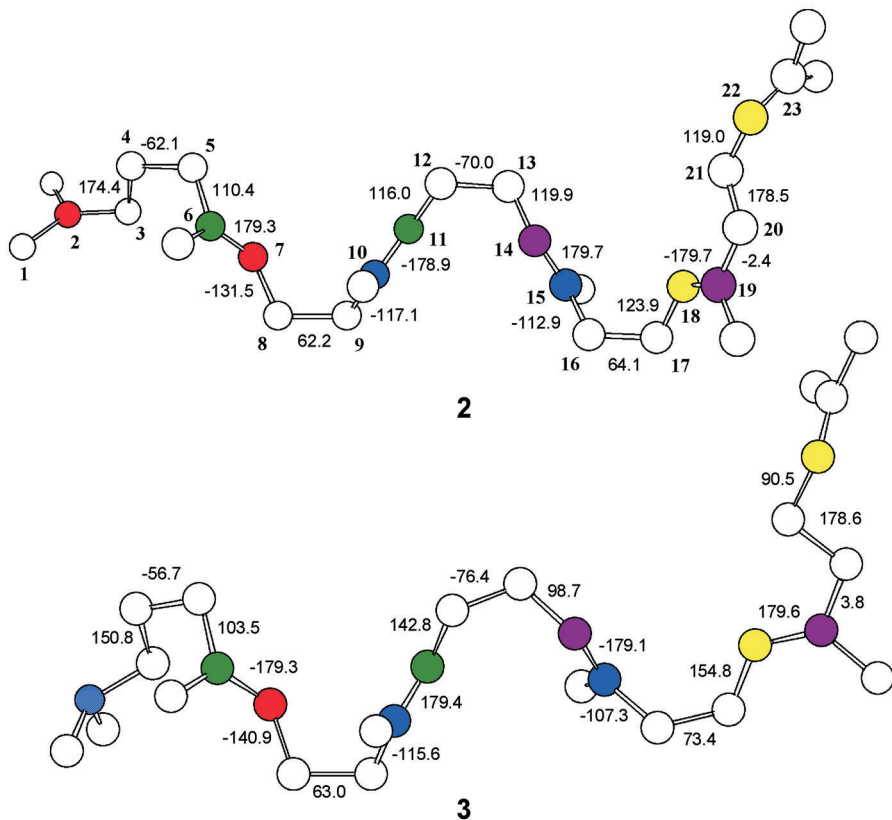
Critical to these ring closures are the conformations about the bonded pairs of sp^3 hybridized carbons in the backbone of squalene. Structures **4** (DFT) and **5** (X-ray) depict two views of the first ten carbons atoms of each chain.

Even though a nitrogen atom has replaced C2 in **5** (shown as a blue atom), it can be seen that the two structures are remarkably similar. The

structures on the left have the C4–C5 bond perpendicular to the plane of the page. There are three possible conformations about this bond, only one of which can give rise to ring closure. This is because of the proximity of the two carbons involved in bond formation to yield ring A. Both structures **4** and **5** are present in this unique conformation, the one which is capable of allowing the ring closure to occur.

4

5
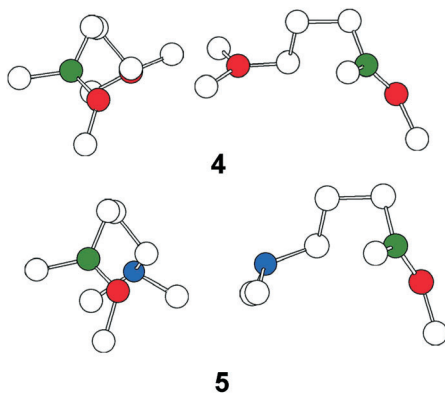
A similar situation is found for the conformation about the C8–C9 bond in the chain of squalene, the conformation of which is critical for the formation of ring B of hopene. Depicted in structures **6** (DFT) and **7** (X-ray) is the fragment of the squalene chain involved in ring B formation (green carbons give rise to the new single bond). Again this is the only one of three possible conformations about the carbon–carbon single bond that could lead to B ring closure. The similarity between the calculated and observed structures is again remarkable.

6

7
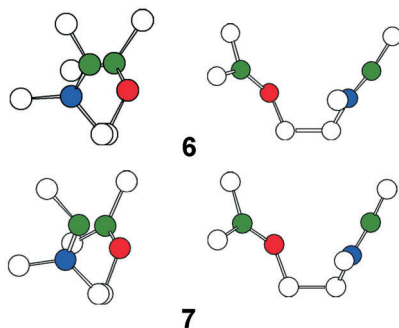
We have previously studied the ring closure of rings A and B using the model system **8** to yield bicyclic **9**. A transition structure (**10**) was located, which linked **8** and **9** in a concerted but asynchronous reaction[10].


8   **9**
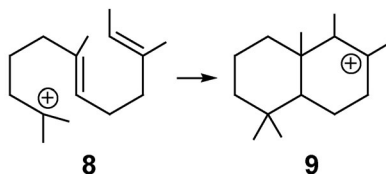
The reaction was found to be highly asynchronous[10], with the formation of the A ring close to completion before the B ring begins forming. Below, the corresponding fragments of the DFT structure (**11**) and X-ray structure (**12**) are compared with that of transition structure **10**. There is a marked similarity of the geometry of those carbons of these three structures that will give rise to the B ring. Hence one can conclude that the enzyme is holding squalene in a conformation that will give rise to the concerted formation of rings A and B. That is, as the charge is transferred to carbon 6 in the formation of ring A, the C10–C11 double bond required for formation of ring B is held in a position such that it can begin to interact with this forming positive charge, which will give rise to a concerted but asynchronous ring closure of rings A and B.

11   **12**

10
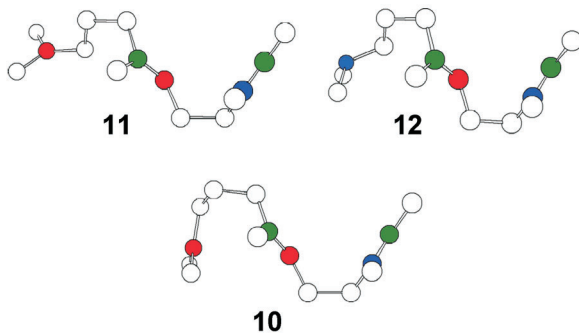
Structures **13** (DFT) and **14** (X-ray) show a perspective about the C–C single bond, which is critical to the closure of ring C. It is thought that ring C is first formed as a five-membered ring (between the blue C10 and purple C14 carbon atoms on the left of **13** and **14**), which would subsequently undergo a ring enlargement during the course of the formation of ring D (see below).

13

14
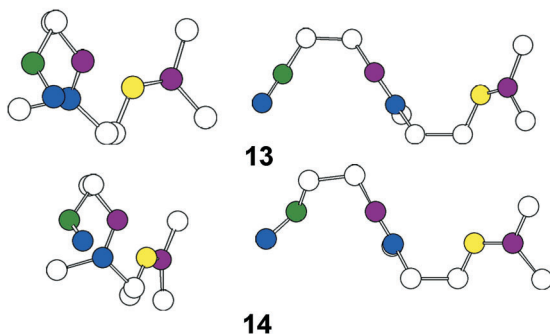
We have previously carried out a detailed conformational analysis[11] of the model system **15**, which can undergo ring closure to give the 6-5 ring system (**16**). In particular three conformers were examined that might exist about the C–C single bond that connects the C=C double bond to the six-membered ring. Two of these conformers were found to exist, while the third one indeed did not, since all attempts to locate this third conformer ended with the structure collapsing to bicyclic **16**.

15   16
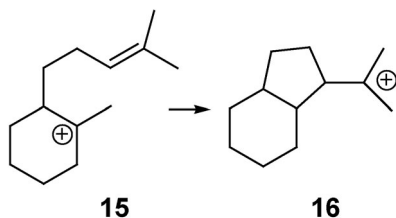
Structure **17** is an approximation of what this elusive conformer of **15** would look like if it were to exist. Note its similarity to the corresponding fragment of the X-ray structure of 2-azasqualene depicted by **18**, in particular the five carbons shown at the top of the structure, which are those carbons that will form the five-membered C ring. Hence it is extremely likely that ring C (five-membered) is formed in concert with ring B, since

17   18
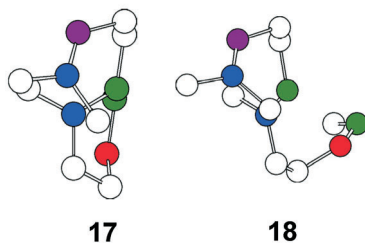
squalene is held by the enzyme in the very conformer about the C–C single bond that will collapse to form ring C as the positive charge is built up on the forming adjacent ring B. Even if the C and D ring formations are also concerted this discussion is still germane, since it is likely that in the concerted cyclization the formation of one ring is almost complete (see above) before the second ring begins to form.

The next ring formation (D) requires a ring expansion of ring C to a six-membered ring. This is a process that is not favored energetically, because it is anti-Markovnikov in nature (conversion of a tertiary carbocation to a secondary one). This high-energy step can be avoided if the ring expansion occurs in concert with formation of ring D as we have previously suggested[12,13]. We located a transition structure (**19**) for the concomitant ring expansion and ring closure of model system **20** to give the bicyclic product **21**.

20   **19(ts)**   **21**
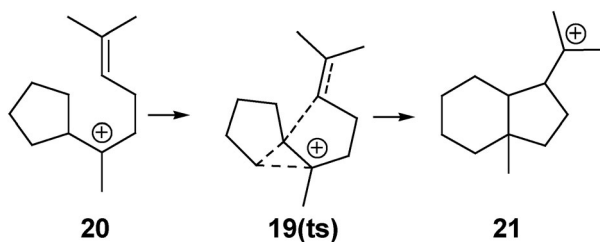
Hence the six-membered C ring in the squalene cyclization might be formed in concert with the formation of the five-membered D ring. The question arises whether the squalene cyclization "pauses" at the completion of the formation of the five-membered C ring or whether the formation of the five-membered D ring is part of an overall concerted process of cyclization of rings A-D. Schulz has suggested[2] based on his X-ray results that, because the C14 cation is well positioned to interact with the C18–C19 double bond, it is likely that rings C and D are formed in a concerted fashion. His suggestion is supported by comparison of structure **20** with the appropriate fragment of the azasqualene from its X-ray structure (**22**). It can be seen that the conformation about the C16–C17 single bond in **22** is very close to the corresponding bond in **20**. Hence this fragment of squalene is at least in part properly positioned by the enzyme for the concerted formation of rings C and D.

Only a rotation about the C17–C18 single bond is required to bring the C18–C19 double bond into the proper orientation for interaction with the developing carbocationic at C14. Though this is supportive of Schulz's suggestion, it is still possible that there might be a "pause" in the cascade of

22

20
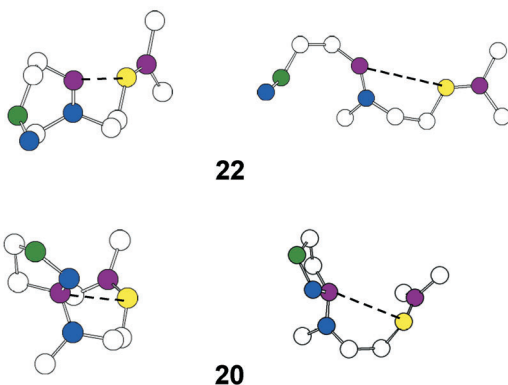
ring closures at the five-membered C ring followed by some reorganization of the remaining carbons of squalene before the final cyclizations of the D and E rings. In a recent report it was suggested that this indeed might be the case[14]. However, what is very likely, as noted by Schulz[2], is that while the ring expansion of the D ring is presumably concomitant with formation of the E ring, it does require significant conformational reorganization before this process may occur. Hence it is higly probable that the cascade of reactions "pauses" with formation of the five-membered D ring.

CONCLUSIONS

In summary, it may be concluded from this detailed conformational analysis that the enzyme "holds" squalene in a conformation that allows the concerted, but presumably asynchronous, formation of rings A-C. It is also likely that even the formation of the five-memberd D ring (in concert with the ring expansion of the initially formed five-membered C ring) might be part of an overall concerted process for the formation of rings A-D in the cyclization of squalene.

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