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ORIGINAL PAPER

Do the substituent effects affect conformational freedom of squalene in hopene biosynthesis?

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Abstract The analysis of biochemical processes is one of the main challenges for modern computational chemistry. Probably the biggest issue facing scientists in this case is the number of factors that have to be taken into account, as even those factors that do not seem to be meaningful may eventually be crucial. Such a belief led to the investigation on the substituent effects during squalene cyclization process. We focused on the formation of lanosterol ring A through squalene epoxide and an analogue process observed in bacteria, leading to the hopene formation without an intermediate oxide. Interestingly, our results indicate that, opposite of chemical intuition, a more substituted chain is more likely to adopt a conformation suitable for the cyclization process. Presumably the rational for this behavior is the presence of intermolecular CH···π interactions between the hydrogen atoms from methyl groups and the squalene π bonds in the open-chain structure. The effect seems to have a firm impact on the hopene formation process. Calculations were performed using two different methods: MP2 and M06-2X, combined with the cc-pVDZ basis set.

Keywords Conformational freedom · Hopene · Lanosterol · Squalene · Substituent effects

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Introduction

It is known that the shape of a flexible, multi-substituted molecule is a resultant of steric interactions between its substituents themselves as well as the main chain, and effects of sterically demanding end - groups [1]. This dependency gives chemists a great spectrum of possibilities. Unfortunately, controlling the conformation of open chain molecules by the type and location of its substituents occurs to be a highly demanding task, in most cases hardly possible. So far there are only a few examples of successful designing of open chain compounds with preferred conformation by using substituent effects [2, 3]. Nevertheless, nature succeeded in developing molecules with a preference to adopt the conformation which is optimal for the function they may serve. It is especially true when it comes to the specific enzyme – ligand recognition. One of these reactions, the enzymatic polycyclization of squalene to lanosterol or hopene (Fig. 1), may be considered. The product specificity and high stereoselectivity are believed to be achieved by several factors, such as forcing the substrate to occupy a prefolded conformation, progression of the reaction through rigidly held, partly cyclized carbocationic intermediates, and stabilization of the intermediate carbocations [4]. Although these factors can explain selectivity of the squalene cyclization process, it is also worth considering a peculiar and rather neglected factor - the substituent effect. The ability of designing open chain compounds with

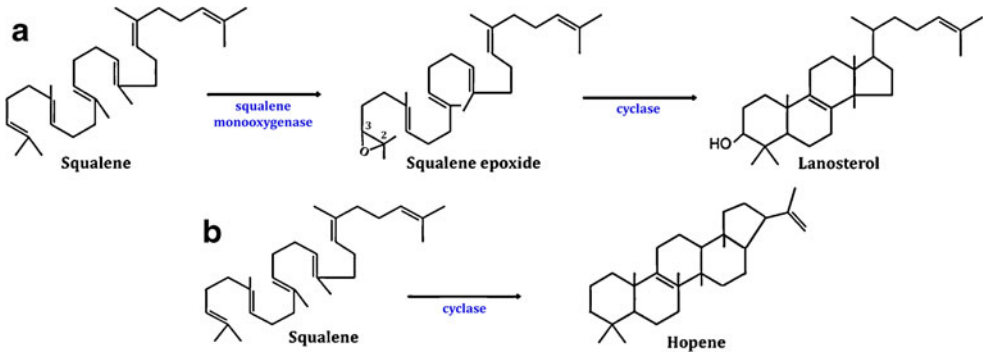

Fig. 1 The squalene cyclization process: (**a**) in eukaryotic organisms, (**b**) in bacteria

preferred conformation by proper displacement of substituent groups may be very useful, especially in a bioactive agents designing. Therefore it is worth studying mechanisms employed by nature.

The main aim of this study was to gain information about substituent effects affecting molecule conformational freedom by analyzing the polycyclization process leading from squalene to lanosterol either hopene formation.

Computational details

Calculations were performed using Gaussian03 package [5]. Structures were studied with two different methods: the second-order Møller-Plesset perturbation theory (MP2) [6], and M06-2X density functional [7], combined with the cc-pVDZ basis set [8]. As the object of interest was only the part of squalene molecule that is converted into the lanosterol/hopene A ring, in order to facilitate calculations the model molecules (Fig. 2, R is in fact a hydrogen atom) were used. First, the relaxed potential energy surface scan for rotation around C3-C4 bond for Sq, Sq_1, Sq_2, Sq_3, Osq, Osq_1, Osq_2 and Osq_3 structures was carried out with 10° step size, to determine the preferable C2-C3-C4-C5 dihedral values. In order to approach the stable conformation nearest to the closed ring for all Sq_X and Osq_X molecules, optimization of structures in which the initial values of C-C-C angles and C-C-C-C dihedrals were set up to those observed in the closed ring molecule, in the chair conformation (111° for C-C-C angle and 55° or −55° for C-C-C-C dihedral) was performed.

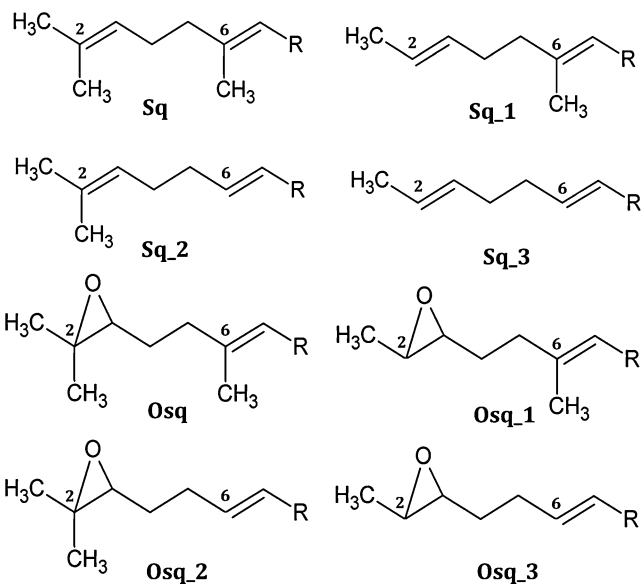

Fig. 2 The investigated molecules. The digits refer to the atoms numbering

Theoretically this procedure could have been altered by performing the full relaxed potential energy surface scan for rotation around C3-C4 and C4-C5 bonds, followed by an analysis of a three dimensional PES. However for such a large molecule it would be a too time-demanding task. Briefly, 8 (models) x 36 (rotation around C3-C4 with 10° step) x 36 (rotation around C4-C5 with 10° step) give 10 368 structures for optimization. Such a cost does not seem to be justifiable, as it is highly unlikely to provide new insights into the substituent effects.

Additionally, for all structures we decided to perform the potential energy surface scan around C4-C5 bond, with the C2-C3-C4-C5 dihedral frozen and corresponding to that observed in the cyclic form (55°).

Results and discussion

The main aim of this study was to gain information about the substituent effects affecting polycyclization process leading from squalene to either lanosterol or hopene formation. There is a wide variety of studies treating in details the enzymatic squalene cyclization [4, 9], and the authors did not wish to repeat them. We focused on the formation of the lanosterol ring A through squalene epoxide and its comparison with analogue process observed in bacteria, leading to the hopene formation without an intermediate oxide (Fig. 1) [10]. When analyzing the substituent effects affecting formation of the lanosterol/hopene ring A, one may consider the influence of methyl

groups at the C2 and C6 position as they are not directly involved in the cyclization process. Although the bacterial squalene cyclases do not employ exactly the same substrate conformation as the eukaryotic enzyme, the conformation of squalene in the part responsible for A-ring formation is the same in both cases [4].

Subsequently, the following models were investigated (Fig. 2):

- **Sq** which corresponds to the squalene molecule,
- **Sq**_1 which corresponds to the squalene without methyl group at the C2 position,
- **Sq**_2 which corresponds to the squalene without methyl group at the C6 position,
- **Sq**_3 which corresponds to the squalene without methyl groups at the C2 and C6 position,
- Similarly built models: **Osq**, **Osq**_1, **Osq**_2 and **Osq**_3 corresponding to 2,3-(S)-oxidosqualene and its derivatives.

The calculations were performed at two different levels of theory, i.e., *ab initio* MP2 method and hybrid meta DFT method - M06-2X were used. In both cases from the derived data the same conclusions could be drawn. Some differences in results related to the values of rotational energy barriers and depth of local energy minima. These differences on average did not exceed 10% of relative energy values and there were practically no differences between geometry of stable conformers. For the sake of clarity, here we present data obtained only by one method (MP2), all charts and tables for calculations by M06-2X method are available as supplementary materials (online resource 1).

First for the listed structures we began with the relaxed potential energy surface scan for rotation around the C3-C4 bond. That step gave us a general overview on the influence of substituent groups on the molecule conformational freedom and preferred values of the C2-C3-C4-C5 dihedral (Fig. 3, Fig. 4), which for the closed ring structure, in the chair conformation equals around 55° [11].

In the case of the **Sq_X** models no crucial differences between conformers energies, as well as rotational energy barriers, have been noticed. The main difference seems to be in the highest energy barrier, which is around 2 kJ mol^{-1} higher for the **Sq** and **Sq_2** models than for the rest. For the molecules with a methyl group at the C2 position local energy minimum in the range between 0° and 180° is observed for around 100°, while for the other molecules around 110° (Table 1).

Data obtained for **Osq_X** models confirm the firm influence of C2 methyl substituent group on the molecule conformer population as the plots of relative energy upon the rotation around C3-C4 bond are entirely different in two of four cases. The **Osq_1** as well as **Osq_3** molecule, i.e.,

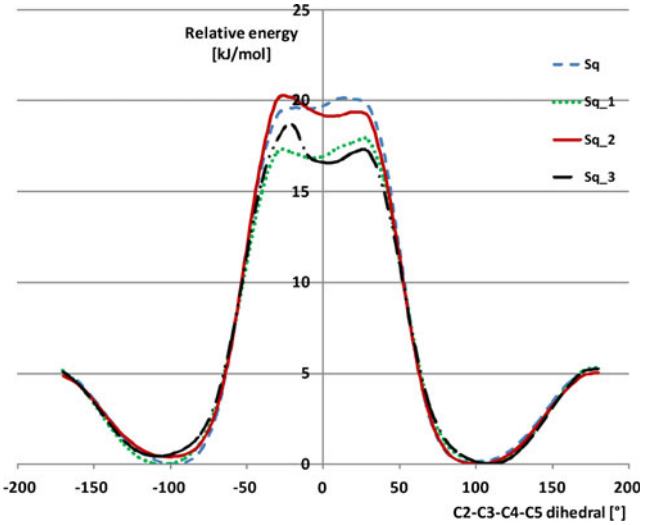

Fig. 3 Relative energy of the Sq_X conformers upon the rotation around C3-C4 bond

the structures without a substituent at the C2 position, have three local minima for the C2-C3-C4-C5 dihedral values around: -140°, -30° and 90°. However, in the presence of a methyl group at the C2 position (**Osq**, **Osq_2**) only two stable conformations can be observed, for the −140 and 90 ° C2- C3-C4-C5 dihedral values (Table 1). Interestingly, while the highest rotational energy barrier for the **Osq** and **Osq_2** structures increased by 9 kJ mol^{-1}, compared to the **Osq_1** and **Osq_3** molecules, the lowest rotational energy barrier decreased by 3 kJ mol^{-1}.

The first step of our procedure did not bring any clear answers on the substituent effects affecting cyclization of squalene and a rotation around only one bond was investigated. To avoid a highly computationally demanding determination of three-dimensional PES for rotation around

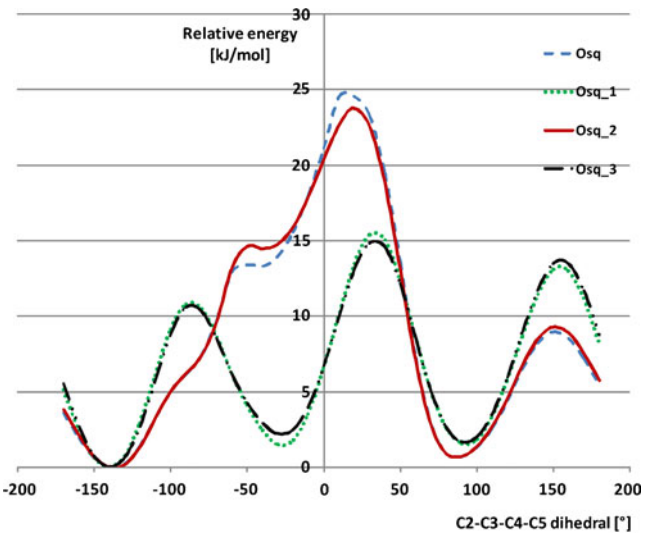

Fig. 4 Relative energy of the Osq_X conformers upon the rotation around C3-C4 bond

Table 1 Local energy minima along the C3-C4 bond rotation path

Structure	C2-C3-C4-C5 dihedral value [°]	ΔE[1)] [kJ/mol]
Sq	-100	0.0
	100	0.1
Sq_1	-110	0.0
	110	0.0
Sq_2	100	0.0
	-100	0.4
Sq_3	110	0.0
	-110	0.4
Osq	-140	0.0
	90	0.7
Osq_1	-140	0.0
	-30	1.5
	90	1.6
Osq_2	-140	0.0
	90	0.7
Osq_3	-140	0.0
	90	1.7
	-30	2.2

[1)] ΔE – relative energy

the C2-C3 and C3-C4 bonds, an alternative procedure was conducted. To determine and compare stable conformations nearest to the closed ring structure, in the presence or absence of particular substituent, we optimized **Sq_X** and **Osq_X** molecules in which the initial values of C-C-C angles and C-C-C-C dihedrals were set up to those observed in the closed ring molecule (111° for C-C-C angle and 55° or −55° for C-C-C-C dihedral) [11].

The obtained results were surprising and definitely did not correspond to our intuition, since it occurred that the distance between atoms which form a bond during the cyclization process (C2 and C7) is the shortest for the highest substituted chain. Relevant data are presented in Table 2. These findings seemed to be completely opposite

Table 2 The crucial parameters of the stable conformations nearest to the closed ring structure

Structure	C2 – C7 distance [pm]	C2-C3-C4-C5 dihedral [°]	C3-C4-C5-C6 dihedral [°]
Sq	347	94.9	-69.7
Sq_1	433	123.4	-56.0
Sq_2	362	95.5	-68.7
Sq_3	421	117.0	-63.8
Osq	356	95.4	-66.7
Osq_1	366	93.9	-61.2
Osq_2	374	95.5	-64.8
Osq_3	376	93.4	-62.6

with the knowledge on aliphatic cyclic structures (e.g., cyclohexane) stability and influence of torsional and steric strains. In the case of the investigated structures higher substituted chains adopt conformations more suitable for formation of six-membered ring, while it is widely known that due to the presence of torsional and steric strains substituted aliphatic six-membered rings are less stable than non-substituted ones [12]. Further investigations however showed that this knowledge does not deny our findings.

The largest differences were observed in the group of squalene derivatives (**Sq_X**), as for the **Sq** structure (i.e., the model that corresponds to the squalene molecule) the distance between atoms which form a bond during the cyclization was more than 80 pm shorter than for the **Sq_1** structure (i.e., the model that corresponds to the squalene molecule without methyl group at C2 position). Interestingly, removing a methyl group at the C6 position in the chain did not cause such a severe effect and the C2- C7 distance increased only by 15 pm. Derived data suggest strong similarity between **Sq** - **Sq_2** and **Sq_1** - **Sq_3** molecules. It seems to confirm the leading influence of the C2 methyl group on the squalene preorganization during cyclization process. When it comes to the oxidosqualene derivatives (**Osq_X**) the substituent effect has been noticed too. Nevertheless, it is much weaker and the differences in distance between C2 and C7 atoms are up to 20 pm. Probably it is the result of the dominant imprint of an oxygen atom.

Because our findings seemed not to stand with the knowledge on the stability of a substituted six-membered rings, there was a need to perform some additional calculation. The direct comparison of molecules total energies in a conformation strictly corresponding to that observed in the closed ring structure was impossible, as particular structures have a different number of atoms. Therefore an alternative approach was utilized. The potential energy surface scan around the C4-C5 bond with frozen C2-C3-C4-C5 dihedral, equal to that observed in the cyclic form in the chair conformation (55°), was performed. Next the relative energies of conformers with both dihedrals (C2-C3-C4-C5 and C3-C4-C5-C6) equivalent to those observed in the cyclic structure were compared (Fig. 5, Fig. 6). In line with the expectations, molecules with a methyl substituent at the C2 position, in both **Sq** and **Osq** cases, occurred to be much less stable than the others (Table 3). The differences equal around 12 kJ mol^{-1}. This can be easily explained by the presence of repulsion forces corresponding to the 1,3 – diaxial interactions in closed ring structures and goes with the known facts on aliphatic cyclic structures stability. However one should notice that the presence of a methyl group at the C2 position generally does not result in the increase of the rotation energy barrier along the preferred path. Comparing to a non-substituted

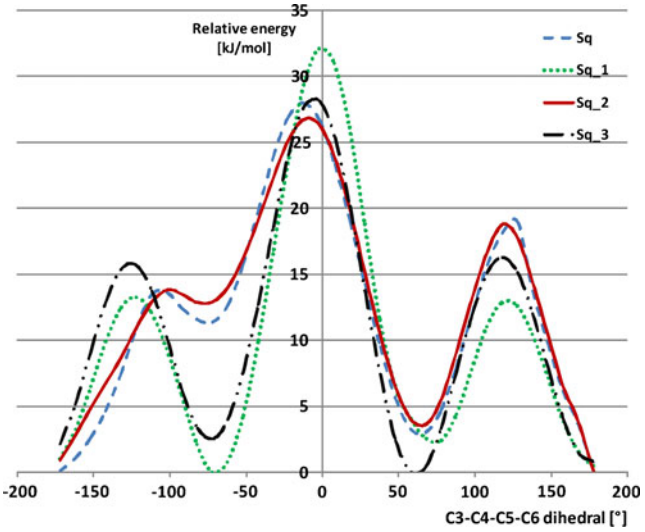

Fig. 5 The relative energies of Sq_X conformers upon the rotation around the C4-C5 bond with the C2-C3-C4-C5 dihedral frozen (55°)

chain (**Sq_3**) for the **Sq_2** molecule, i.e., the one with a methyl substituent at the C2 position, we can even observe some lowering of the rotation energy barrier. The same effect was observed for the **Osq_2** molecule.

The data suggest that although methyl substituents in the squalene chain decrease the closed ring structure stability, they do not hinder the preorganization process. On the other hand, findings on the stable conformations nearest to the closed ring structure showed that the distance between atoms which form a bond during the cyclization process is the shortest for the highest substituted chain (Table 1). It suggests then that the methyl substituent located at the C2 position in a squalene chain allows the molecule to adopt a conformation more suitable for the cyclization, although the

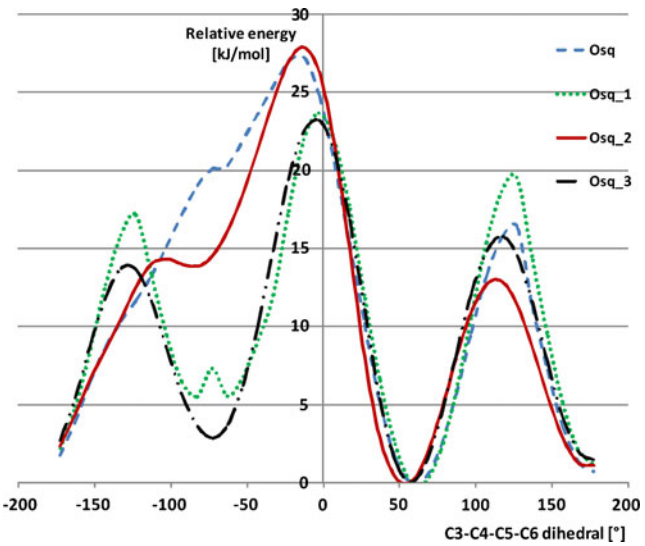

Fig. 6 The relative energy of Osq_X conformers upon the rotation around the C4-C5 bond with the C2-C3-C4-C5 dihedral frozen (55°)

Table 3 Local energy minima along the C4-C5 bond rotation path

Structure	C3-C4-C5 dihedral value [°]	ΔE[1)] [kJ/mol]
Sq	179	0.0
	69	3.0
	-71	11.3
Sq_1	-71	0.0
	179	0.5
	79	2.4
Sq_2	179	0.0
	69	3.6
Sq_3	59	0.0
	179	0.8
	-71	2.5
Osq	58	0.0
	178	0.7
Osq_1	68	0.0
	-82	5.5
	-62	5.5
	178	1.1
Osq_2	58	0/0
	178	1.1
Osq_3	58	0.0
	178	1.4
	-72	2.8

1) ΔE – relative energy

cyclization product is less stable. The rational for this behavior seems to be the presence of CH···π interactions [13, 14] between the hydrogen atoms from the C2 methyl group and the squalene π bond in the open-chain molecule (Fig. 7).

Conclusions

The three step calculation procedure on the substituent effects, affecting conformational freedom of squalene, led us to the following conclusions. First of all, opposite to a chemical intuition, a more substituted chain is likely to adopt a conformation more suitable for the cyclization process. It can be clearly seen from the analysis of stable conformations nearest to the closed ring structure in which

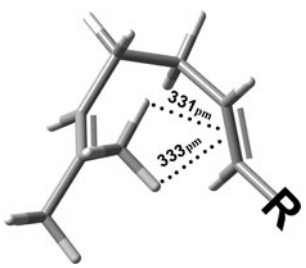

Fig. 7 Stabilizing CH···π interactions. The molecule (Sq_2) is in the stable conformation nearest to the closed ring structure (Table 1). The distance was measured between hydrogen atoms and the point located in the center of the C6-C7 π bond

the distance between atoms forming a bond during the cyclization process is in the shortest for the highest substituted chain. A methyl group at the C2 position seems to have a crucial influence on the effect. For the molecule without the C2 methyl group, in the stable conformation nearest to the closed ring structure, the distance between atoms which form a bond during the cyclization process is about 80 pm greater than the corresponding distance in molecule containing the methyl group at C2 position. However, removal of the second methyl substituent, located at the C6 position, results in the distance increasing only up to 20 pm. Obtained data show that in the presence of the C2 methyl substituent, energy barriers along the preferred rotation path are generally the same as for the non-substituted chain, or even lower. Interestingly, observed effects of stabilizing pre-cyclization conformers by methyl substituents seem to not stand with the knowledge on aliphatic cyclic structures (e.g., cyclohexane) stability. It is widely known that due to the presence of torsional and steric strains substituted aliphatic six-membered rings are less stable than non-substituted one. On the contrary, in the case of the investigated structures higher substituted chains adopt a conformation more suitable for the formation of a six-membered ring. Although at first glance these facts seem to contradict each other, the discrepancy is only apparent. When all dihedrals strictly correspond to those observed in the cyclic structure, conformers with a methyl substituent at the C2 position become unstable. Consequently there is no mismatch between the obtained data and known information on cyclic structures stability. Most probably the methyl substituent at the C2 position in a squalene chain allows the molecule to adopt a conformation more suitable for the cyclization, although the cyclization product is less stable. The rational for this behavior seems to be the presence of intermolecular CH···π interactions between the hydrogen atoms from C2 methyl group and the C6 - C7 π bond in the non-cyclic structure. The effect seems to have firm impact on the hopene formation process but not in the case of oxidosqualene cyclization where the oxygen atom seems to have dominant influence.

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