The Radical Transformation in Artemisinin: A DFT Study

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The 6,7,8-trioxybicyclo[3,2,2]nonane model molecule has been used to study the reaction mechanism of the radical transformations in artemisinin. The transition state species and the details of the potential energy surfaces of the intramolecular 1,5-hydrogen shift and the homolytic cleavage of the C−C bond in artemisinin have been predicted at the B3LYP/6-31G(d,p) level. A low value of free energy of activation (6.4 kcal/mol) has been found for the intramolecular 1,5-hydrogen shift process. The similarity between the model molecule and artemisinin give the first theoretical support to the suggestion that the energy barrier would not be as high as in an open chain species. The structural details of the O-centered radical and the corresponding transition state revealed in this study indicate that the collinear prerequisite is not necessary and the nonlinear transition state is possible for the 1,5-H shift in artemisinin. Also, the critical distance between the transferred hydrogen atom and the receptor oxygen atom could be longer than 2.1 Å. The lifetimes of 3.4×10^{34} s and 8.3×10^{32} s at 30 K for the O-centered radicals suggest that it is possible to observe the O-centered radicals experimentally at low-temperature.

Introduction

Artemisinin has been used as an effective anti-malarial drug for a long time. The structures of artemisinin and its derivatives are rather unique among the natural products.[1,2] Their unusual 1,2,4-trioxane ring system has been proven to be critical for the antimalarial activity.[3,4]

The mode of the action of artemisinin has been suggested to involve two distinct steps.[3−6] Cleavage of the endoperoxide bridge in the 1,2,4-trioxane is catalyzed by intraparasitic iron and heme to generate unstable free radical intermediates in the first step and the resulting free radical alkylates specific malaria proteins in the second step. Several free radical intermediates have been proposed based on the experimental studies of the mechanism of the anti-malarial action of artemisinin.[3−11] Experiments suggest that the first event in the radical forming process is the reduction of the endoperoxide bond by ferrous ion to produce O-centered radicals. These reactive radicals immediately rearrange to C-centered radicals. Depending on the different forms of the O-centered radical, the transformation from an O-centered radical to the C-centered radical can be either an intramolecular 1,5-hydrogen atom shift process or a homolytic cleavage of the C−C bond (Scheme 1).[3,5,7] Evidence for the intramolecular 1,5-hydrogen atom shift process was obtained by using a sterochemical probe and by the radical trapping experiment.[7−9] Further evidence for the importance of the homolytic C−C bond cleavage of an O-centered radical has been reported recently.[10,11] While the proposed mechanism which assumes that the C-centered radicals can be formed through a 1,5-hydrogen atom shift has been wildly accepted, a number of questions concerning the details of the intramolecular 1,5-hydrogen transfer process still remain.[13] The predicted atomic distance between the transferred H atom and the O radical site is longer than the critical distance of 2.1 Å.[12,13] Also, the energy barrier was expected to be too high.[13] Furthermore, due to the high reactivity of the radicals, the O-centered radicals suggested in the mechanism have not been detected directly by experiments. Computational studies on these intermediate radicals enable us to reveal the details of their structures, stability, and reactions. Such information on the radical transferring process will be helpful in understanding the bio-action phenomena of artemisinin.

In the previous studies, the structures and stability of the O-centered and the C-centered free radical intermediates have been investigated based on the model molecule 6,7,8-trioxybicyclo[3,2,2]nonane using the density functional theory (DFT) at the B3LYP/6-31G(d) level (Scheme 2).[12] At the semiempirical and HF/3-21G levels, Thomson, Corey, and Zerner calculated the properties of a very similar model molecule which was labeled as BC in their paper.[14] The justification of such a simplification is based on the results of the previous studies of the structure and activity correlation of various tricyclic trioxanes which reveal that certain rings in artemisinin and its derivatives are redundant and that their activity can be represented by bicyclic trioxanes.[15,16] The reliability of such a simple model also has been justified by the good agreement between the 6,7,8-trioxybicyclo[3,2,2]nonane model molecule and artemisinin for their geometric parameters and the vibrational behaviors.[12,14,17]

In this paper, we report the results of a theoretical investigation of the reaction process of the intramolecular 1,5-hydrogen shift and the homolytic cleavage of the C−C bond for the model molecule of 6,7,8-trioxybicyclo[3,2,2]nonane. Attention has been focused on the details of the potential energy surfaces of both intramolecular 1,5-H shift and the homolytic C−C cleavage processes. We try to address the following questions. (1) Is there

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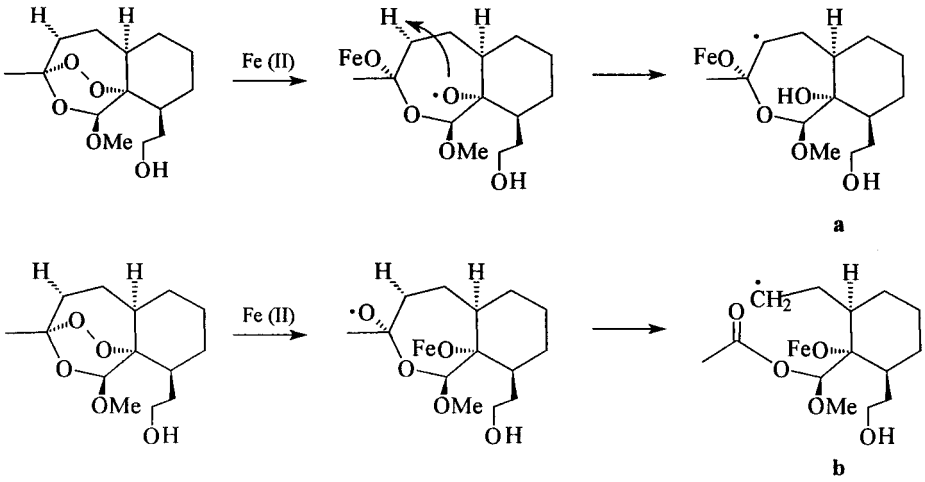
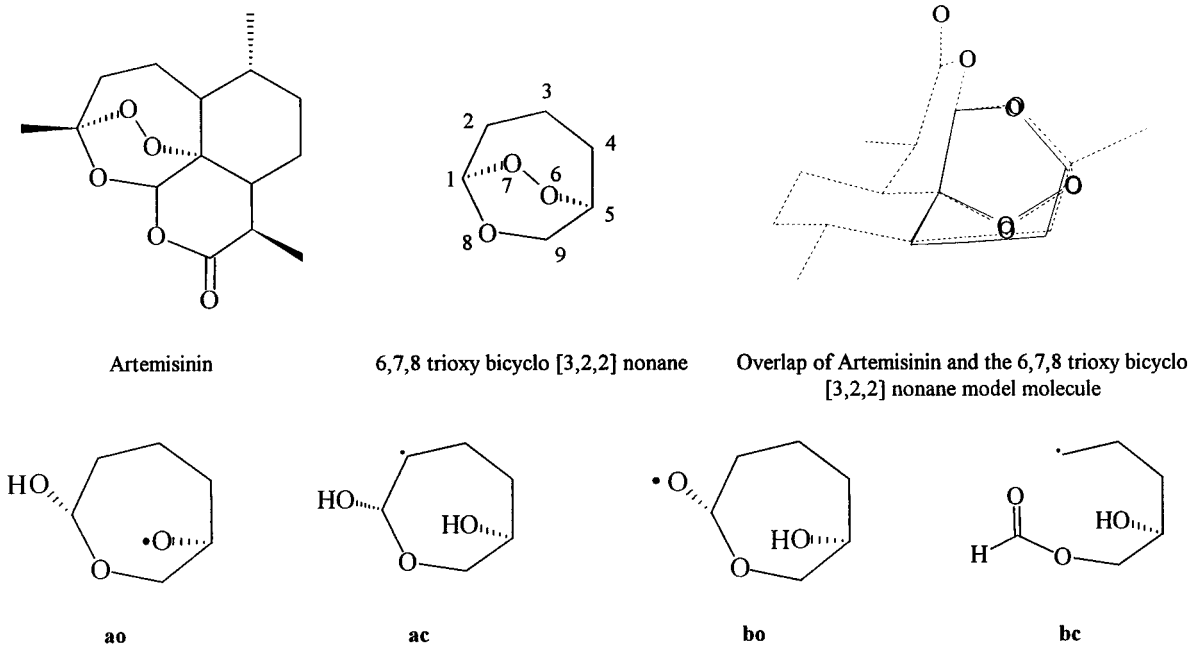
SCHEME 1. The C-Centered Free Radicals Formed through either a 1,5-Hydrogen Atom Shift (a) or a Homolytic C−C Bond Cleavage (b) of an O-Centered Radical Produced in the Reduction of the Edoperoxide Bond by Ferrous Ion[a]



[a] From the figure, it is clear that the only important part of the C-centered radical forming procedure is bicyclic trioxane.

SCHEME 2. The Structures of Artemisinin, 6,7,8-Trioxybicyclo[3,2,2]nonane, and the Corresponding O-Centered and C-Centered Radicals



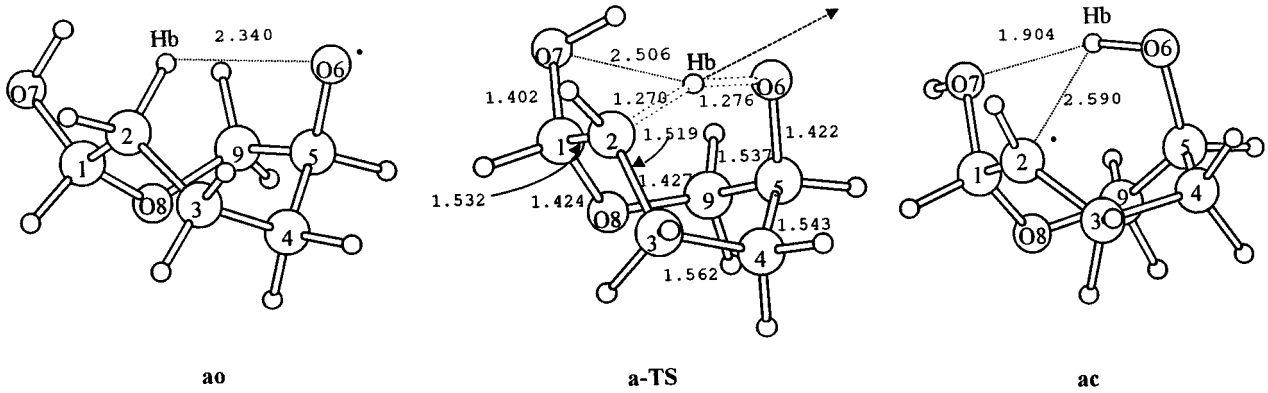
a high energy barrier to block the intramolecular 1,5-H shift? (2) Is it possible to detect experimentally the O-centered radicals?

Method of Calculation

The molecular structures of the studied free radicals have been fully optimized by analytical gradient techniques using the DFT method with Becke's three-parameter (B3)[18] exchange functional along with the Lee−Yang−Parr nonlocal correlation functional (B3LYP).[19,20] On the basis of the previous theoretical studies of artemisinin,[12,17,21,22] the B3LYP/6-31G(d) approximation seems to be the lowest, computationally efficient level of theory able to predict reliable information for such models. To have a better description of the properties related to the hydrogen atoms, the p-like polarization functions were added for the H atoms in the present study. The standard 6-31G(d,p) basis set was then applied in the calculations. Harmonic vibrational analysis predictions of the infrared frequencies and intensities have been performed at the B3LYP/6-31G(*d*,*p*) level for the structures optimized at the same theoretical level. The Gaussian 94 program package[23] was used in the reported calculations.

Results and Discussion

1. Intramolecular 1,5-Hydrogen Transfer. The optimized structures of the O-centered and the C-centered radicals (**ao**, **ac**) as well as the corresponding transition state (**a-TS**) are depicted in Figure 1. The geometric parameters of **ao** and **ac** are consistent with the previous B3LYP/6-31G(d) prediction.[12] An atomic spin analysis based on the Mulliken method has been carried out in order to check the radical atom site. The predicted atomic spin densities of 0.86 on the O6 atom of the radical **ao** and 1.00 on the C2 atom of **ac** at the B3LYP level confirm the free radical structure of these species. In the transition state, the electron of Hb is strongly coupled with both O6 and C2 atoms, resulting in the spin densities of 0.48 on the C2 atom and of 0.51 on the O6 atom. The vibrational frequency analysis



ao **a-TS** **ac**

Figure 1. Geometry of the O-centered radical (**ao**), C-centered radical (**ac**), and the transition state (**a-TS**) corresponding to the intramolecular 1,5-H shift process. The arrows in the transition state represent the vibration mode corresponding to the imaginary frequency.

TABLE 1: Selected Geometric Parameters of the Radicals (ao, ac) and the Transition State (a-TS) Corresponding to the Intramolecular 1,5-H Shift Process[b]

	ao	a-TS	ac	artemisinin[a]
R_{C1-C2}	1.531	1.532	1.496	1.547
R_{C2-C3}	1.536	1.519	1.488	1.540
R_{C3-C4}	1.550	1.562	1.561	1.548
R_{C4-C5}	1.547	1.543	1.541	1.555
R_{C5-C9}	1.566	1.537	1.536	1.539
R_{C5-O6}	1.370	1.422	1.424	
R_{C1-O7}	1.407	1.402	1.409	
R_{C1-O8}	1.425	1.424	1.433	1.440
R_{C9-O8}	1.417	1.427	1.441	1.400
R_{C2-Hb}	1.100	1.270	2.590	
R_{O6-Hb}	2.340	1.276	0.972	
R_{O7-Hb}	2.613	2.506	1.904	
$\angle_{C2HbO6}$	119.1	145.3		
$\angle_{C4C5C9}$	112.5	113.2	114.9	111.2

[a] Optimized at the B3LYP/6-31G(d) level, ref 21. [b] Atomic distance in angstroms and atomic angle in degrees. For atomic labels, see Figure 1.

of the transition state shows the only imaginary frequency of 1426*i* cm^{-1}. The corresponding vibrational mode clearly demonstrates the process of transfer of Hb between the O6 and C2 atoms (Figure 1).

All three structures adopt the boatlike conformation. The selected geometrical parameters of the radicals (**ao, ac**) and the transition state (**a-TS**) are listed in Table 1 along with the corresponding part of artemisinin for comparison. The atomic distance between the transferred hydrogen atom (Hb) and the radical site O6 amounts to 2.340 Å, 0.24 Å longer than the critical value of 2.1 Å as suggested by Jefford et al.[15] The C2−Hb-O6 atomic angle is calculated to be 119° in **ao**. In the transition state **a-TS,** the atomic distance between the Hb and O6 atoms is found to be 1.276 Å and the distance between the Hb and C2 atoms is 1.270 Å. It is worth noting that the O6−C2 atomic distance in the transition state is 2.419 Å, about 0.6 Å shorter than that in **ao**. The boat form of the O-centered radical twists slightly in the 1,5-H shift process. There are no substantial changes in the geometry of the C4−C5−C9 part of the model. This part is bonded to the closed-chain system in artemisinin. The twisting is expected to cost no extra energy in artemisinin. This can be justified according to the overlap of **a-TS** on the artemisinin (Figure 2). The geometry of the part that is related to the C4−C5−C9 fragment in the model molecule is basically the same as in artemisinin. It is important to note that the value of the C2−Hb-O6 bond angle in the transition state **a-TS** is about 145°, which indicates that the collinear transition state is not necessary for the intramolecular 1,5-H shift process as suggested by Wu et al.[7] A large O7−Hb atomic distance of 2.506 in **a-TS** implies little influence of O7 on the hydrogen transfer process. However, the O7 atom is hydrogen bonded to Hb in the C-centered radical **ac**.

TABLE 2: Total and Relative Energies of the Radicals and the Transition States

Total Energy (Hartree)[a]			
	E	E_0	G
ao	−460.8570379	−460.687142	−460.721222
bo	−460.8594445	−460.688653	−460.722341
Relative Energy (kcal/mol)			
	ΔE	ΔE_0	ΔG
ao	0.00	0.00	0.00
ac	−5.16	−4.83	−4.48
a-TS	7.22	5.31	6.36
bo	0.00	0.00	0.00
bc-1	−10.07	−14.25	−16.00
bc-2	−12.19	−14.77	−16.70
b-TS1	7.76	6.10	6.14
b-TS2	−3.54	−6.52	−8.58
Activation Energy (kcal/mol)			
	ΔE	ΔE_0	$\Delta G^{\ddagger}$
ao ⇒ a-TS	7.22	5.31	6.36
ac ⇒ a-TS	12.38	10.14	10.84
bo ⇒ b-TS1	7.76	6.10	6.14
bc-1 ⇒ b-TS1	17.83	20.35	22.14
bc-1 ⇒ b-TS2	6.53	7.73	7.42
bc-2 ⇒ b-TS2	8.65	8.25	8.11

[a] E_0, zero-point corrected energy; G, free energy.

The total and relative energies of the radicals and the transition state intermediate are given in Table 2, and the potential energy surface of the intramolecular 1,5-H shift is shown in Figure 3. The C-centered radical is 5.2 kcal/mol more stable than the O-centered radical **ao**. The free energy difference between **ao** and **ac** amounts to 4.5 kcal/mol. The energy barrier from **ao** to **ac** is predicted to be 7.2 kcal/mol. The corresponding free energy of activation amounts to 6.4 kcal/mol. On the basis of the experimental results, Wu et al. suggest that the activation energy in artemisinin would not be higher than in open chain compounds.[7] The overlap between the model molecule and artemisinin in Scheme 2 enables us to estimate that the activation energy in artemisinin is also approximately 7 kcal/mol. The activation energy of the reversed reaction (12.4 kcal/mol) does not favor the process of transferring Hb back to C2. Because of the high reactivity of the radicals, instead of the reversed H atom transfer, other subsequent reactions should predominate. The free energy of activation obtained from the calculation enable us to estimate the reaction rate. The rate constant for

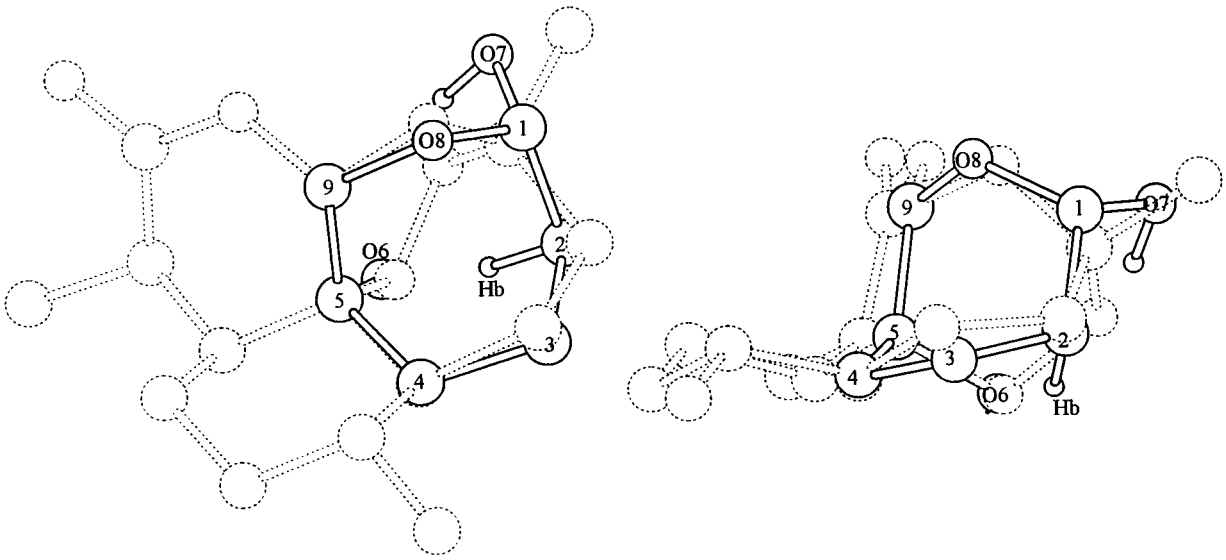


Figure 2. Different views of the overlap of artemisinin and the transition state (**a-TS**) in the intramolecular 1,5-H shift process. The geometry of the C9−C5−C4 fragment in the model molecule is basically the same as in artemisinin.

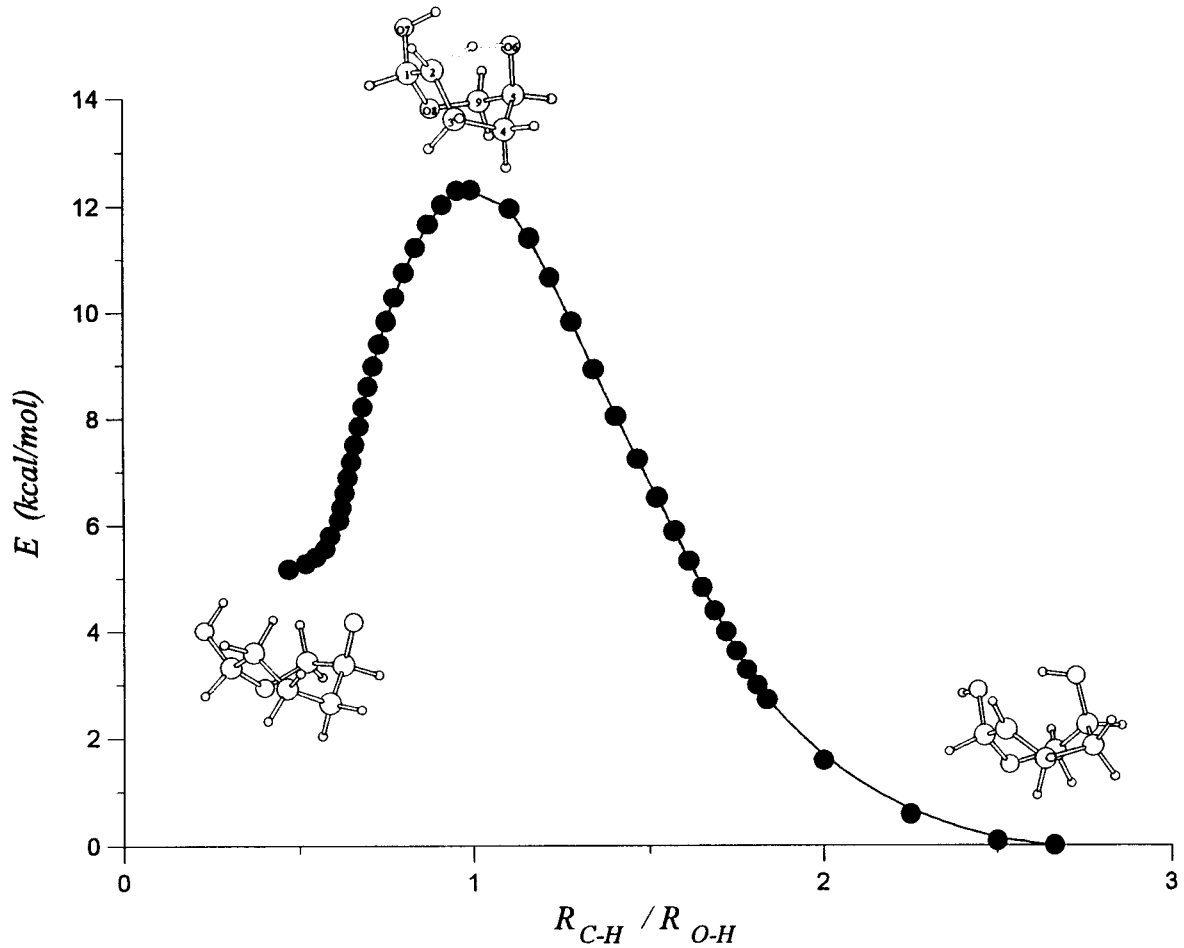


Figure 3. The reaction potential energy surface of the intramolecular 1,5-H shift. Left is the O-centered radical **ao,** and right is the C-centered radical **ac**.

the classical 1,5-H shift is calculated to be 1.3×10^8 s^{-1} and 2.9×10^{-35} s^{-1} at 298 and 30 K, respectively. This corresponds to the lifetimes of 8×10^{-9} s and 3.4×10^{34} s at 298 and 30 K, respectively. Hence, based on the classical rate theory, one would expect that the corresponding O-centered radical in artemisinin could be detected experimentally at low-temperature.

2. Homolytic C−C Cleavage. The transition state of the homolytic C−C cleavage has been located for the **bo** radical (Figure 4). Moving along the reaction coordinate on the direction of the transition vector of the transition state, the IRC (intrinsic reaction coordinate) calculations at the same theoretical level lead to another local minimum corresponding to a C-centered radical (**bc-1**, see Figure 4). The predicted structural parameters are given in Figure 4. The atomic spin density of 0.85 on the

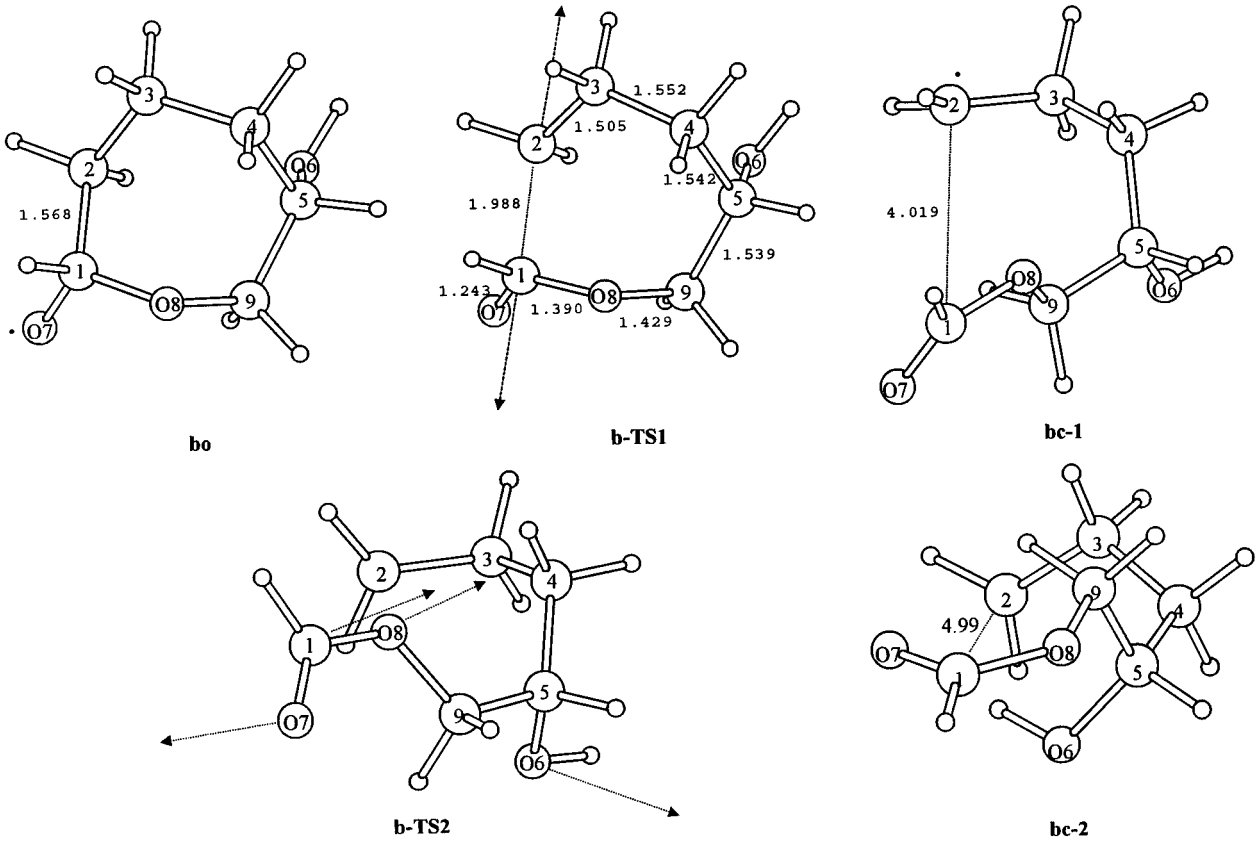


Figure 4. The geometry of the O-centered and C-centered radicals and the related transition states in the homolytic cleavage of the C−C bond process. The arrows in the transition states represent the vibration mode corresponding to the imaginary frequency.

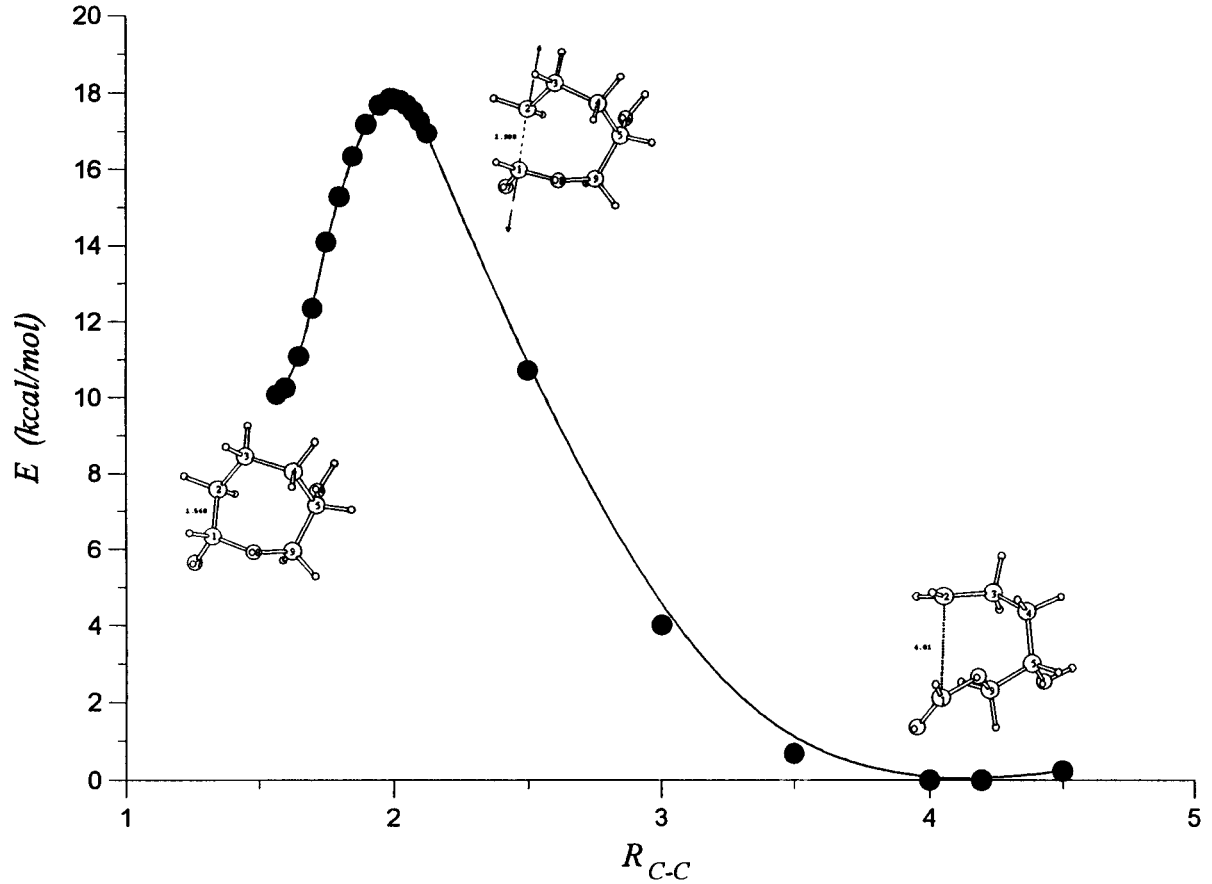


Figure 5. The reaction potential energy surface of the homolytic cleavage of the C−C bond process. Left is the O-centered radical **bo,** and right is the C-centered radical **bc-1.** R_{C-C} is in Å.

O7 atom of the radical **bo** and 1.06 on C2 atom of **bc-1** indicate that the free radicals are localized. In the transition state (**b-TS**), the electron on C1 is coupled with the electrons of both O7 and C2 atoms, resulting in spin densities of 0.61 on C2 and of 0.40 on O7. A vibrational frequency analysis of the transition state shows the only imaginary frequency of 470*i* cm^{-1}. The corresponding vibrational mode characterizes the process of cleavage of the C1−C2 bond as shown in the Figure 4.

The atomic distance between the C1 and C2 atoms in **b-TS** amounts to 1.988 Å, which is about 0.43 Å longer than the C1−C2 bond in the O-centered radical **bo**. The corresponding C-centered radical structure located in this study differs from the one obtained in the previous investigation (**bc-2**). The homolytic C−C cleavage leads to an open chain structure that is quite flexible. Accordingly, more local minima are expected. One of them depicted in Figure 4 corresponds to the C-centered radical predicted in the previous study (**bc-2**) which is 2.12 kcal/mol more stable than **bc-1**. The transition state between **bc-1** and **bc-2** has been found (**b-TS2**) to have the vibrational mode corresponding to the rotation of O7−C1−O8 around the C5−C9 axis, which characterized by the only imaginary frequency of 110*i* cm^{-1}. The energy barrier between these structures is about 6.5 kcal/mol.

The potential energy surface of the C−C cleavage procedure is given in Figure 5. The related activation energy of the reaction is predicted to be 7.8 kcal/mol. The resultant C-centered radical **bc-1** has a lower energy than the **bo** (by 12.2 kcal/mol). The molecular geometry of the **bc-1** radical is significantly different from that predicted for the C−C cleavage transition state, the C1−C2 atomic distance in **bc-1** is about 4.0 Å. On the basis of these results, the possibility of the reversed reaction can be ruled out.

The approximate classical homolytic C−C cleavage rate is calculated to be 1.9×10^{8} s^{-1} and 1.2×10^{-33} s^{-1} at 298 and 30 K, respectively. The corresponding lifetimes are 5×10^{-9} s and 8.3×10^{32} s at 298 and 30 K, respectively. Consequently, one would expect that the related O-centered radical might be detected experimentally at low temperature.

Conclusions

The transition states and the potential energy surfaces of the intramolecular 1,5-hydrogen shift and the homolytic cleavage of the C−C bond in artemisinin have been obtained for the 6,7,8-trioxybicyclo[3,2,2]nonane model molecule. The present study shows the following.

(1) A low activation free energy of 6.4 kcal/mol in the intramolecular 1,5-hydrogen shift process gives the first theoretical support to the suggestion of a low energy barrier. The good overlap of the model molecule and artemisinin confirms that this activation energy barrier in artemisinin will have similar value. We estimate that the free energy of activation should be about 7 kcal/mol in artemisinin.

(2) The lifetimes of 3.4×10^{34} s and 8.3×10^{32} s at 30 K for O-centered radicals **ao** and **bo** suggest that the O-centered radicals could be detected experimentally in low temperature.

This calculation study also reveals some important details on the intramolecular 1,5-H shift process. The structural details of the O-centered radical and the corresponding transition state revealed in this study indicate that the collinear prerequisite is not necessary and a nonlinear transition state is possible for the 1,5-H shift in artemisinin. Also, the critical distance between the transferred hydrogen atom and the receptor oxygen atom could be longer than 2.1 Å.

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