Antimalarial endoperoxides: synthesis and implications of the mode of action

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Dedicated to Professor Gerasimos J. Karabatsos on the occasion of his 70th birthday
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Abstract

6,7-Dioxabicyclo[3.2.2]non-8-ene **2** and 1-isopropyl-4-methyl-2,3-dioxabicyclo[2.2.2]oct-5-ene (ascaridol) **3** were prepared as simplified, endoperoxide versions of clinically used antimalarial drugs. Fourier transform infrared (FTIR) technique in conjunction with $^{18}O_2$-enriched compound **2** has been applied in probing the bonds of the endoperoxide moiety and the bonds of the rings owing to the presence of the O–O, the C–O, the O–O–C as well as the C=O modes in the spectrum. The endoperoxide moiety is especially useful in this regard because the homolytic cleavage of the O–O bond can be characterized and hence can be used to assess the vibrational properties of the O- and C-centered radicals and subsequently that of the C–C bond cleavage. The cleavage of the O–O bond, and the ability to correlate vibrational properties of the reaction products with structural properties of the isolated products suggest that infrared spectroscopy is an appropriate tool to study the mode of action of antimalarial endoperoxides.

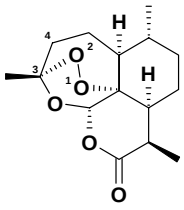
Keywords: FTIR spectroscopy, $^{18}O_2$ endoperoxide, O–O bond cleavage mechanism

Introduction

Malaria is one of the most widespread parasitic diseases caused by invasion protozoan parasites of the class of Plasmodium into the human body.[1] It is estimated that there are 240 million people who are chronically affected, and there are 120 million new cases reported every year.[2,3] There is a general consensus that antimalarial peroxides, both of natural and synthetic origin, kill the Plasmodium parasite through a mode of action that is entirely different from that of the traditional quinoline-based drugs such as quinine and chloroquine.[4] While the mechanism of action of the antimalarial endoperoxides is not completely understood, there is growing evidence that the initial key step is the reductive cleavage of the O–O bond of the endoperoxide moiety,

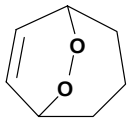
leading to oxygen- and then carbon-centered radicals that subsequently cause the biologically relevant damage of the malarial parasite. Artemisinin **1**[5] and its semisynthetic dihydro derivatives posses an endoperoxide function as the essential structural component of their activity. Clinical trials of artemisinin derivatives are now being sponsored by the World Health Organization and clinical studies using suppositories containing water-soluble sodium artesunate have produced striking malarial cure rates.[6] The extraction and the cost of artemisinin derivatives is a limiting factor to the wide use of these efficient drugs in tropical and subtropical regions, where the malaria is endemic and chloroquine-resistant strains are spreading. Therefore, there is a need for new, low-cost synthetic drugs with pharmacological properties similar to those of artemisinin. Rational design and laboratory synthesis of structurally simpler endoperoxides have led to various peroxide compounds, different from artemisinin, some of which have excellent antimalarial activity.

Several important properties of artemisinin have been determined from studies of simpler trioxane analogs. It has been established by Posner and co-workers that deoxygenation of 1,2,4 trioxanes into corresponding 1,3-dioxolanes occurs via an unzipping-zipping process.[7] The Fe(II)-induced cleavage of the peroxide bond in trioxane tosylate leads through the C–C bond cleavage to a ring-contracted tetrahydrofuran acetal and then produces a stable electophilic tetrahydrofuran aldehyde. On the other hand, the 1,5-H shift process produces stable 4-hydroxy deoxytrioxane as a mixture of two diastereomers. These findings led these authors to conclude that the reduction of the endoperoxide by Fe(II) follows a different mechanistic course leading to different products than the endoperoxide cleavage by non Fe(II) reducing agents.[8] Recently, the use of the $^{18}O_2$ enriched trioxane alcohol allowed us to detect the heme Fe(IV)=O intermediate in the artemisinin/hemin dimer reaction.[9]



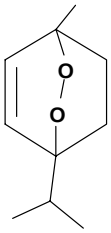
1

artemisinin



2

6,7-dioxabicyclo[3.2.2.]non-8-ene



3

ascaridole

The Fourier transform infrared (FTIR) technique has been applied to the study of some 1,2,4-trioxanes and has been found to be powerful in probing the bonds of the endoperoxide moiety and the bonds of the rings owing to the presence of the O–O, the C–O, the O–O–C as well as the C=O modes in the spectrum. The endoperoxide moiety is especially useful in this respect because the homolytic cleavage of the O–O bond can be characterized and hence can be used to assess the vibrational properties of the O- and C-centered radicals, and subsequently that of the 3-C–4-C bond cleavage. In our previous work,[10] we applied the FTIR approach to further characterize the reactions of artemisinin and its synthetic analog trioxane alcohol with Fe(II) and

Fe(III). Although the Fe(III)/artemisinin reaction is slower, we showed that cleavage of the O–O bond occurs and the three products formed have vibrational characteristics similar to those found in the products of the Fe(II)/artemisinin reaction. The product ratios in the Fe(II) and Fe(III) reactions was depended on the oxidation state of the iron and thus on whether the activation follows the 1,5 H-shift or the C_3-C_4 cleavage pathway. In the case of the Fe(II)-induced cleavage of trioxane alcohol, three major products were detected and their characteristic C=O vibrations are located at 1717 and 1740 cm^{-1} and that of the hydroxyl (OH) at 3433 cm^{-1}. We also applied laser photolysis FTIR techniques to monitor the FTIR spectra of artemisinin photoproducts, and found that the O–O bond is photolabile. The artemisinin photoproducts have vibrational characteristics which are similar to those observed in their corresponding Fe(II)- and Fe(III)-induced reductive cleavage products. From the different product ratios observed in the Fe-induced cleavage of the endoperoxide and that in the photolytic cleavage we infer that there is a mechanistic difference between the two processes. These results also provide important links between metabolites and chemical reaction products that have been observed in the antimalarial mode of action of trioxanes. Following our search for novel compounds with high antimalarial activity, in the work presented here compounds **2** and **3** were prepared and characterized.

Materials and Methods

Artemisinin **1** (98 %, Aldrich Chemical Company) was used without further purification. 6,7-Dioxabicyclo[3.2.2.]non-8-ene **2** and its ^{18}O-^{18}O labeled analog were synthesized by introducing $^{16}O_2$ (99 %, Messer) and $^{18}O_2$ (97 %, Isotec), respectively, to 1,3-cycloheptadiene (97 %) in the photosensitized oxygenation step. In the $^{18}O_2$ photooxygenation experiment, $^{18}O_2$ gas (25 mL) was anaerobically transferred to a tightly sealed evacuated flask containing a solution of 1,3-cycloheptadiene (100 mg) in dichloromethane (25 mL) and in the presence of methylene blue sensitizer (5 mg). The whole mixture was placed in an ice bath (0 °C) irradiated with a 500 W lamp for 25 min. The total yield of **2** was 30 % and the final isotope enrichment was ~ 70%. In the case of ^{16}O-^{16}O photooxidation a continuous flow of $^{16}O_2$ was affordable, and the yield approached 100 %. Ascaridole **3** was synthesized in a similar way by introducing $^{16}O_2$ to α-terpinene (85 %), during the photooxygenation step.

The FTIR spectra were recorded at 293 K from thin film samples placed on a AgCl window, at 2 cm^{-1} spectral resolution with a Bruker Equinox IFS 55 FTIR spectrometer equipped with a liquid nitrogen cooled mercury cadmium telluride (MCT) detector. Typically, 100 scans were recorded and averaged for each spectrum.

Results and Discussion

The FTIR spectra presented here and the frequencies of the modes are fully consistent with earlier studies of artemisinin, and with the vibrational data reported for some 1,2,4 trioxanes by Jefford and co-workers.[11] The FTIR modes in the 700–1200 cm^{-1} range have been shown to be sensitive indicators to the O–O and C–O modes of the O–O–C unit, and at 1735 cm^{-1} to δ-lactone carbonyl ν(C=O) mode. Figures 1a and 1b show the FTIR spectra of artemisinin **1** and ascaridole **3**, respectively. The principal bands seen in Figure 1a are assigned in analogy to those vibrations found in other 1,2,4-trioxanes.[11] The O–O stretching and C–O stretching modes and bending modes of the C–O–O–C moiety are located at 723, 831, 883 and 1114 cm^{-1}, respectively; the C=O mode of δ-lactone at 1735 cm^{-1}. Based on $^{18}O_2$ enriched ascaridole we have assigned the 730 cm^{-1} mode to the O–O stretching. Figures 2a and 2b show the FTIR spectra of $^{16}O_2$- and $^{18}O_2$-enriched compound **2**, respectively. These data illustrate that the modes located at 718/701, 798/786 and 896/884 cm^{-1} arise from the O–O stretching of compound **2**.

Computational studies on the radical intermediates will enable us to reveal the details of their structures, stability, and reactions. Such information on the radical transfer-ring process will be helpful in understanding the bio-action phenomena of compounds **2** and **3**. Figure 3 depicts the calculated FTIR spectrum of compound **2** at the HF/6-311++G** level. We expect these theoretical calculations to form the basis for the details of the potential energy surfaces of both the intramolecular 1,5-H atom shift and the homolytic 3-C–4-C cleavage process. This way we will be able to address the following questions: (1) Is there a high energy barrier to block the intramolecular 1,5-H shift? (2) Is it possible to detect experimentally the O-centered radicals? Experiments to address these questions are in progress in our laboratory.

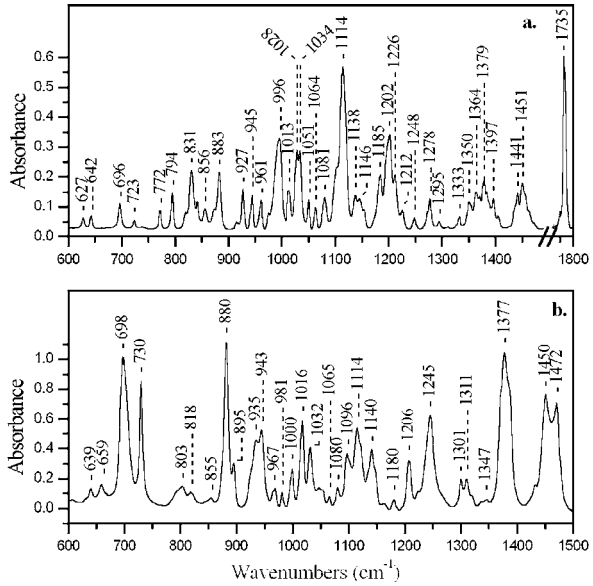


Figure 1. FTIR spectra of artemisinin (a) and ascaridole (b). The spectral resolution was 2 cm^{-1} and 100 scans were recorded and averaged.

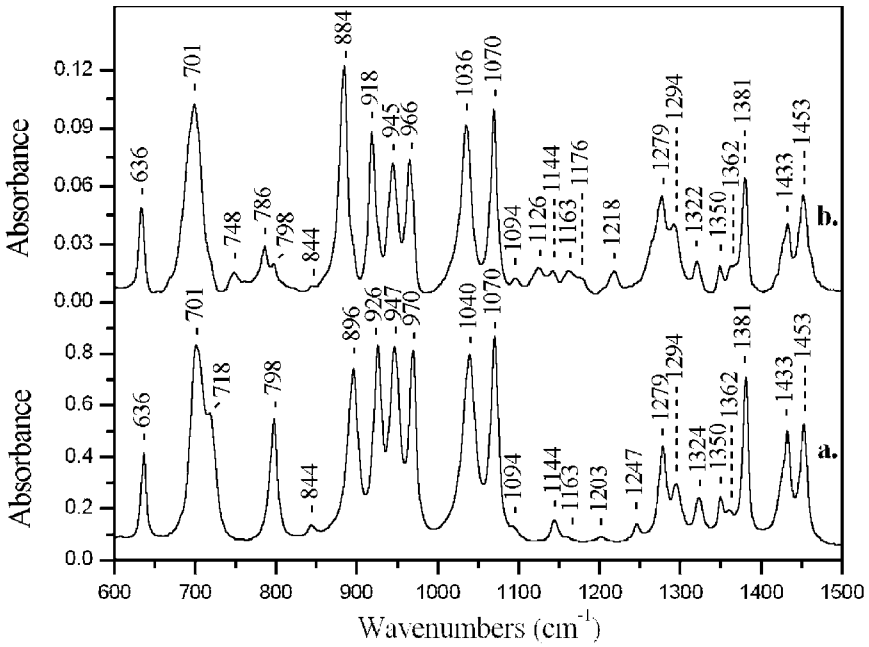


Figure 2. FTIR spectra of 6,7-dioxabicyclo[3.2.2.]non-8-ene **2** (a) and its ^{18}O-^{18}O labeled analog (b). The spectral resolution is 2 cm^{-1}, 100 scans were recorded and averaged.

In an effort to understand the progression of the intermediates in the O–O cleavage of artemisinin, we present a reaction scheme for artemisinin (Scheme 1). The structures of the intermediates and those of the final products were taken from our data and the literature. Although O- and C-centered radicals are depicted in Scheme 1, there is no evidence for the build-up of such intermediates in our previous measurements. The characterization of the O-centered radicals produced in the cleavage of the O–O bond as well as the detection of C-centered radicals, formed either through a 1,5-H shift or by homolytic 3-C–4-C bond cleavage remains problematic, because they exhibit very short lifetimes (10^{-6}–10^{-9} s). However, it has been shown in several 1,2,4-trioxanes like artemisinin, that only the pathways involving the 4-C radical intermediate (resulting from 1,5-H shift) is important for high antimalarial activity.[12,13] Recently, Gu *et al.*[14] in a DFT study showed that the rate constant for the classical 1,5-H shift is 1.3×10^{8} s^{-1} and 2.9×10^{-35} s^{-1} at 298 K and 30 K respectively. These authors concluded that the corresponding O-centered radical is detectable experimentally at low temperature. Moreover, they calculated the 3-C–4-C bond cleavage rate to be 1.9×10^{8} s^{-1} and 1.2×10^{-33} s^{-1} at 298 K and 30 K, respectively, and they reported that the C-centered radical is 5.2 kcal/mol more stable than the O-centered radical. Since there is experimental evidence both for the formation of a secondary 4-C radical and of an unstable epoxide by Wu *et al.*[15] and for the formation of a primary4-C radical,[16] their structures were used in Scheme 1. The epoxide was characterized by its IR spectrum giving rise to ν(OH) and ν(C=O) at 3500 and 1728 cm^{-1}, respectively.

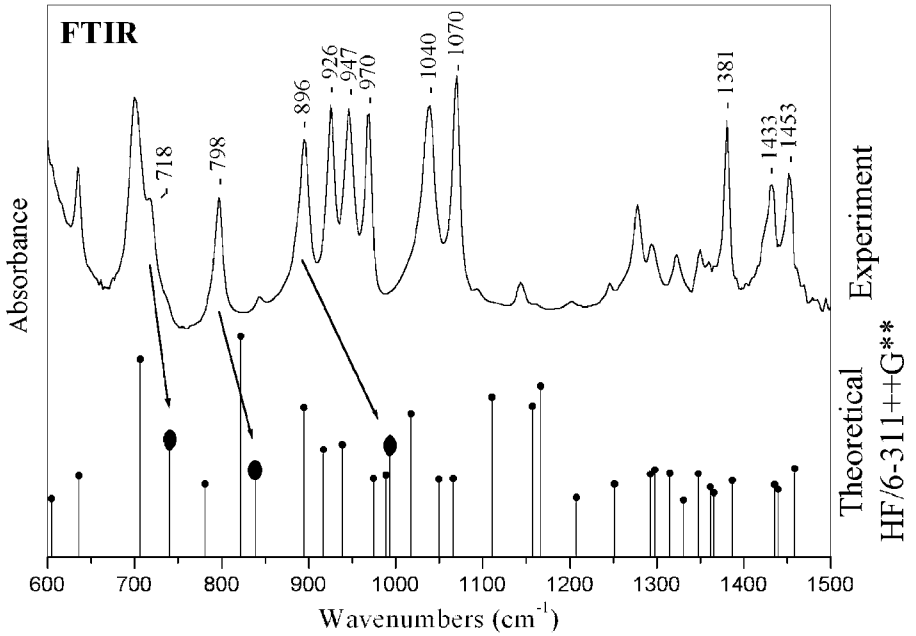
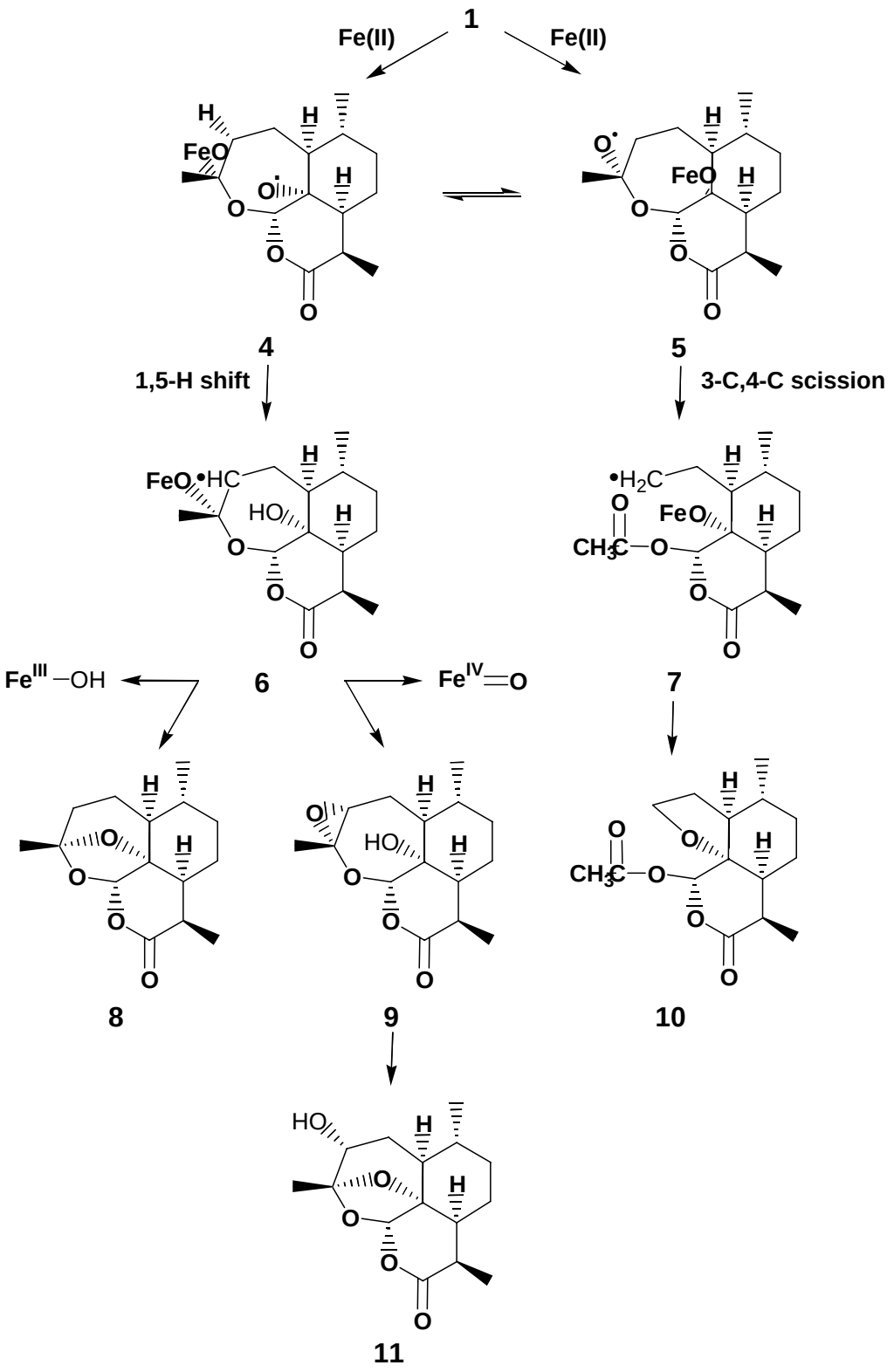


Figure 3. Comparison between the experimental and the theoretical FTIR spectrum of 6,7-dioxabicyclo[3.2.2.]non-8-ene **2**. The three marked modes in the calculated spectrum correspond to the O–O stretching vibration of the endoperoxide bridge, according to the molecular orbital analysis.

In the reaction scheme depicted in Figure 4, two separate pathways are indicated for the decay of the primary O-centered radicals. In the first pathway, if iron associates with O_1, then compound **5** is formed which is converted, via the C_3-C_4 scission, to the primary C_4 radical compound **7**. The latter compound is converted to compound **10**. On the other hand, if iron associates with O_2, then through the 1,5-H shift compound **4** is converted to the secondary C_4 radical compound **6**. The later compound can be either converted to the unstable epoxide **9**, with the concomitant formation of Fe(IV)=O or to the stable compound **8** and the formation of Fe(III)-OH. Compound **9** can be further converted to the final product **11**. Under all experimental conditions we used, there is no evidence for the build-up of either a secondary radical or of the unstable epoxide, due to the absence of a peak at 1728 cm^{-1}, thus we assume that their lifetimes are very short. In the case of Fe(II) reaction, the ratio of the I(1755 cm^{-1}, compound **8**)/I(1717 cm^{-1}, compound **10**) is 1.2 while in the photolysis products is 2.8. Consequently, under the conditions in which the O-O bond is cleaved photolytically, and in the absence of bound Fe to neither of the oxygens of the endoperoxide bridge, the dominant pathway is that in which compound **8** is formed.



Scheme 1. Proposed mechanism for the Fe(II)-induced activation of artemisinin **1**.

The reactions of compounds **2** and **3**, with both heme and non heme Fe(II) and Fe(III), and the comparison with the well-studied 1,2,4 trioxanes will contribute to a better molecular basis for rational design of new synthetic endoperoxide-containing antimalarial drugs. In addition, the photolytic cleavage of the O-O moiety will be monitored through the FTIR spectroscopy and properties about the nature of the bond and the way it affects the biological activity of the drugs will be revealed. These studies will ultimately lead to flow time-resolved step-scan FTIR experiments where the detection and the evolution of the radical intermediates will definitely resolve the reaction mechanism.[17] Finally, theoretical calculations focused especially on the energy barriers raised between the two allowed pathways will address some important details on the intramolecular 1,5-H shift process, as well as the structural details of the O- and C-centered radicals.

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