Evidence for Fe(IV)=O in the Molecular Mechanism of Action of the Trioxane Antimalarial Artemisinin

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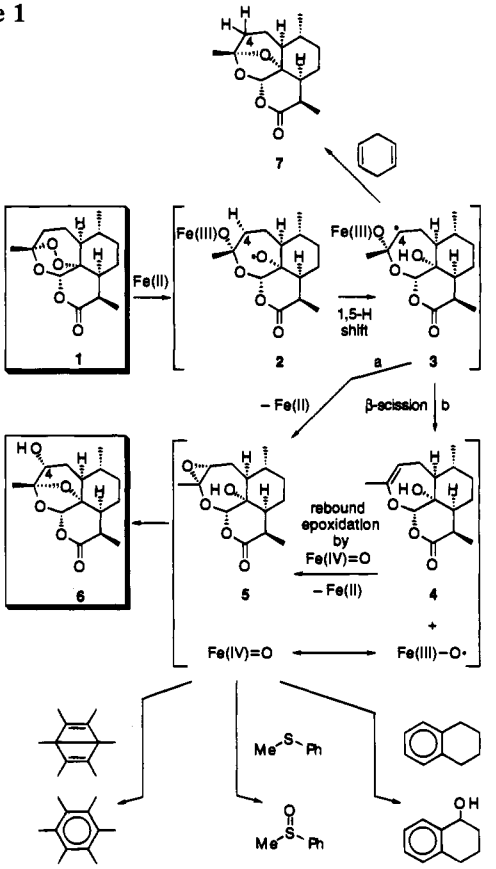
Received March 10, 1995

Among the 300–500 million people worldwide who are currently infected with malaria, about 2 million deaths, many of them children, occur each year.[1] As malaria parasites develop ever-increasing multidrug resistance to traditional alkaloidal antimalarial drugs,[2] artemisinin (quinghaosu, **1**), a non-alkaloidal endoperoxide natural product discovered in China, and related 1,2,4-trioxanes are increasingly being used for effective chemotherapy of malaria.[3,4] These organic peroxides, causing oxidative stress[5] to malaria parasites, apparently are reduced by the iron-rich parasites to form cytotoxic radical intermediates.[6] Using an oxygen-18-labeled trioxane[7] and some mechanism-based synthetic analogs,[8] we have shown that a carbon-centered radical, formed from an oxy radical *via* an intramolecular 1,5-hydrogen atom shift, is important for antimalarial activity.[9] We now report several kinds of evidence supporting the intermediacy of a high-valent, non-heme, iron–oxo species resembling that characteristic of monoxygenase metalloenzymes and known to cause oxidative damage to biological macromolecules.[10,11] It is proposed that such a high-valent iron–oxo species is formed *via* homolytic oxygen–carbon bond scission[12] from a β-ferryloxyethyl radical and that a highly electrophilic epoxide (*e.g.*, **5**, Scheme 1), a potent alkylating agent,[13] also is formed. A molecular mechanism representing these transformations and the types of evidence we have accumulated for a high-valent iron–oxo intermediate are summarized in Scheme 1. This molecular mechanism represents the *first report of generation of Fe(IV)=O during ferrous ion activation of a 1,2,4-trioxane* rather than, as usual, by heme protein (*i.e.*, cytochrome P_{450}) or metalloporphyrin model compounds activating dioxygen or hydrogen peroxide.[10,11]

Scheme 1



Evidence for a high-valent iron–oxo intermediate is provided by reactions characteristic of such a species, as follows: (1) hexamethyl Dewar benzene was rearranged into hexamethylbenzene (in 40% yield),[14,15] while the amount of C_4-hydroxylated artemisinin product **6** was diminished from 15–20% to 5–7% (Table 1); (2) methyl phenyl sulfide was oxygenated to the corresponding sulfoxide; and (3) tetralin (1,2,3,4-tetrahydronaphthalene) was oxidized into hydroxytetralin.[16] Three

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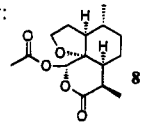
Table 1. Artemisinin Plus Iron(II) at 25 °C

0.7 equiv of iron(II)	additive	solvent	ratio of products[a] (6:7:8)	total yield[b] (%)
$FeBr_2$	—	THF	1:6:3	98%
$FeBr_2$	1,4-cyclohexadiene	THF		
	10% (v/v)		1:9:3	99%
	40% (v/v)		1:17:4	92%
$FeBr_2$	hexamethyl Dewar benzene	THF	1:11:3	88%
$FeCl_2{\cdot}4H_2O$	—	CH_3CN	1:0:6	78%
hemin, $PhCH_2SH$	—	THF	1:0.2:11	70%

[a] Ratio determined by ^1H NMR of the crude product mixture. The structure of **8** is shown below:

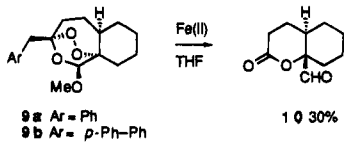


8

[b] Total yield of separately isolated and characterized products.

control experiments showed that product distributions were not affected by running these redox reactions in deoxygenated solvents.[16,17] Other control experiments showed also that these characteristic reactions required both artemisinin and ferrous ions. Additional evidence for the C_4-radical **3** in Scheme 1, besides that which we have provided from structural analogs,[8] is provided by a radical trapping experiment. Trapping of C_4-radical **3** was successful using the good hydrogen atom donor 1,4-cyclohexadiene[18] to increase the amount of reduced artemisinin **7** (Table 1). Formation of the C_4-hydroxylated product **6** having only $C_{4\alpha}$-hydroxyl stereochemistry[19] could result from one or both of two pathways, a or b, in Scheme 1: (a) direct intramolecular formation of α-oriented epoxide **5** with release of Fe(II), followed by internal S_N1-like opening of this epoxide by the free hydroxyl group in intermediate **5**, and/or (b) β-scisson of Fe(IV)=O from intermediate **3** and then intermolecular rebound epoxidation[10] of intermediate vinyl ether **4**. Although we do not now know whether only one or both of these pathways are responsible for formation of $C_{4\alpha}$-hydroxylated product **6**, it is clear from the evidence presented above that a high-valent iron−oxo species is an intermediate. Furthermore, high-valent iron−oxo species are known to epoxidize electron-rich carbon−carbon double bonds (*e.g.*, vinyl ether **4**) very easily.[10,11] When iron-based reduction of artemisinin was performed using ferrous chloride in the presence of water in acetonitrile,[20] no C_4-radical reduced product **7** was detected (Table 1). Iron-based reduction of artemisinin (**1**) was achieved also by using hemin plus benzyl mercaptan in THF[7,8] to approximate more closely the natural environment in the malaria parasite; although the ring-contracted product **8** shown in Table 1 predominated, C_4-radical-derived hydroxylated product **6** and reduced product **7** were still produced. Compounds **6** and **8** are produced also when artenisinin is thermolyzed.[19d] $C_{4\alpha}$-Hydroxylated product **6** is one of the *in vitro* metabolites of artemisinin formed in the presence of rat liver microsomes.[19]

When β-scission of Fe(IV)=O is preempted by β-scission of a good radical leaving group (*e.g.*, benzyl or *p*-phenylbenzyl, as in trioxanes **9**),[21] then the major product of iron reduction of the trioxane is norbenzyl lactone aldehyde **10**, almost none (1−3%) of a C_4-hydroxylated product like **6** is formed, and the parent trioxane (*i.e.*, **9a**) is devoid of antimalarial activity.[22] C_3-Benzylic trioxane **9b** reacted with ferrous bromide at 25 °C in THF to form, in about 35% total isolated yield, benzylic cleavage products (*i.e.*, *p*-phenylbenzyl alcohol and *p*-phenylbenzaldehyde); in this reaction (eq 1), added hexamethyl Dewar benzene was not rearranged into more than 5−7% of hexamethylbenzene, supporting a reaction pathway that does not involve more than a trace of Fe(IV)=O.



9a Ar = Ph
9b Ar = *p*-Ph–Ph

10 30%

(1)

These observations are especially significant because they represent (1) the first evidence for the intermediacy of high-valent iron−oxo species in the molecular mechanism of action of antimalarial 1,2,4-trioxanes like artemisinin; (2) the first evidence that high-valent iron−oxo species can be generated from a dialkyl peroxide rather than, as usual, from hydrogen peroxide, an organic hydroperoxide, or molecular oxygen; (3) the possibility that such Fe(IV)=O species are formed *via* homolytic oxygen−carbon bond scission from a β-ferryloxyethyl radical (*e.g.*, **3** in Scheme 1); and (4) the first explicit proposal of an antimalarial molecular mechanism involving highly electrophilic and cytotoxic alkylating epoxides like **5**. Similar alkoxy epoxides formed during metabolism of aflatoxin are potent alkylating agents.[23] This new understanding of antimalarial activity at the molecular level may help in the design of better chemotherapeutic trioxanes in the worldwide fight against malaria.

Acknowledgment. We thank the NIH (AI-34885) for financial support, Professors Craig Townsend, Anne-Frances Miller, and Kenneth Karlin (Department of Chemistry, The Johns Hopkins University) for discussions concerning high-valent iron−oxo species, and Dr. A. J. Lin (Walter Reed Army Institute of Research, Washington, DC) for a ^1H NMR spectrum of authentic $C_{4\alpha}$-hydroxylated product **6**.

Supplementary Material Available: Preparation of and spectroscopic data on **9b** and **10** (11 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

JA950795N

(15) A typical procedure is as follows: a flame-dried 10 mL round-bottomed flask was charged with iron(II) bromide (15 mg, 0.07 mmol) and THF (0.50 mL) under an argon atmosphere. The mixture was cooled to 0 °C. The flask was wrapped with aluminum foil to protect the reaction mixture from ambient light. Hexamethyl Dewar benzene (0.10 mL, 0.50 mmol) was then added to the reaction. A solution of artemisinin (28 mg, 0.10 mmol) in THF (0.50 mL) was precooled to 0 °C and added *via* cannula into the reaction mixture at 0 °C. The reaction was slowly warmed to room temperature over 45 min, stirred at room temperature for 15 min, cooled to 0 °C, quenched with water (3 mL), and diluted with ether (3 mL). Two layers were separated, and the aqueous layer was extracted with ether (3 × 5 mL). The combined organic layers were washed with brine (10 mL), dried over magnesium sulfate, filtered, and concentrated under reduced pressure to afford the crude products which were further purified by florisil column chromatography to give products **6**, **7**, **8**, and hexamethylbenzene[14] (40% yield based on hexamethyl Dewar benzene). These known products were fully characterized spectroscopically (For characterization of **6** and **8**, see ref 19d. For characterization of reduced product **7**, see: Xu, X.-X.; Zhu, J.; Huang, D.-Z.; Zhou, W.-S. *Tetrahedron* **1986**, *42*, 819.).

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