Evidence for the Importance of High-Valent Fe=O and of a Diketone in the Molecular Mechanism of Action of Antimalarial Trioxane Analogs of Artemisinin

Gary H. Posner,*[,†] Sheldon B. Park,[†] Lluïsa González,[†] Dasong Wang,[†] Jared N. Cumming,[†,‡] Donna Klinedinst,[§] Theresa A. Shapiro,[§] and Mario D. Bachi[||]

Department of Chemistry, School of Arts and Sciences, The Johns Hopkins University Baltimore, Maryland 21218
Department of Medicine, School of Medicine The Johns Hopkins University Baltimore, Maryland 21205
Department of Organic Chemistry The Weizmann Institute of Science, Rehovot, Israel

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Ancient Chinese folk medicine has led relatively recently to chemical identification of the 1,2,4-trioxane sesquiterpene artemisinin (qinghaosu, **1**), representing a new class of fast-acting, clinically useful, antimalarial drugs.[1,2] This natural endoperoxide and some related synthetic organic endoperoxides, causing oxidative stress to malaria parasites,[3] are reduced by the iron-rich parasites to form cytotoxic radical intermediates.[4] We have designed and synthesized some structurally simplified 1,2,4-trioxanes to determine whether they are reduced by ferrous iron to form the same kinds of radical intermediates as formed upon iron(II) reduction of artemisinin.[5,6] The simplified trioxanes we report here were formulated such that an intermediate carbon-centered radical would be intercepted before β-scission of Fe(III)−O$^{\bullet}$ [↔Fe(IV)=O] either by a competing β-scission of a better radical leaving group X$^{\bullet}$ from radical intermediate **2a** (Scheme 1A) or by a competing subsequent 1,5-hydrogen atom shift in radical intermediate **3a** (Scheme 1B); such mechanistically competing reactions were expected to *interrupt* formation of a high-valent iron−oxo species and thus to diminish or to undermine completely the antimalarial activity of these artemisinin analogs. Also, another analog was designed to have improved and therefore medicinally more desirable antimalarial potency by incorporating a structural feature that would *facilitate* β-scission of a high-valent iron−oxo species. We report here *in vitro* antimalarial activities that support the intermediacy of such high-valent iron−oxo species *via* the β-scission pathway in the antimalarial artemisinin analogs shown in Table 1. We report evidence also of a simple diketone as an unexpectedly antimalarial product formed upon iron(II) reduction of a trioxane.

Scheme 1

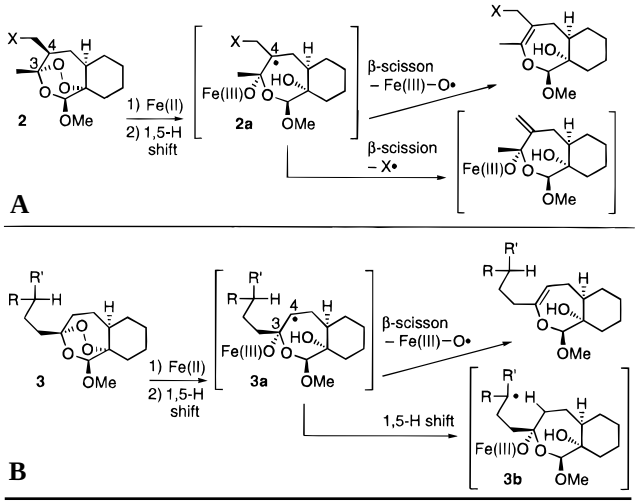
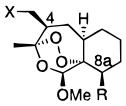


Table 1. Chemical Structure−Antimalarial Activity Relationships in Chloroquine-Sensitive *Plasmodium falciparum* (NF54) Parasites *in Vitro*[a]



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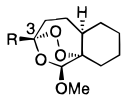

10-15

trioxane	X	R	IC_{50} ng/mL	IC_{50} nM
4	Ph	CH_2CH_2OH	3.0	8.3
5	Me_3Si	CH_2CH_2OH	86	240
6	Me_3Sn	CH_2CH_2OH	>1000	>2500
7	Ph	H	98	340
8	Me_3Sn	H	>1000	>2500
10		Me	220	960
11		Et	170	710
12		$(CH_3)_2CHCH_2CH_2$	46	160
13		$PhCH_2CH_2CH_2$	35	110
14		CH_2=CH	11	46
15		Ph	11	38
artemisinin (**1**)			3.0	11
arteether			1.9	6.1
chloroquine			2.6	5.0

[a] Antimalarial activity was determined as reported previously.[5] The standard deviation for each set of quadruplicates was ≤45% of the mean. R^2 values for the curves were ≥0.993.

Trioxanes **5** and **6** differ by only one atom: silicon *vs* tin. Despite this small structural difference within a complex organic endoperoxide, $C_{4\beta}$-silyl analog **5** has significant antimalarial activity, whereas tin analog **6** is virtually inactive (Table 1). Likewise, 8a-unsubstituted $C_{4\beta}$-stannyl analog **8** has virtually no antimalarial activity, whereas similar $C_{4\beta}$-substituted analog **7** is active. These results are consistent with selective β-scission of $Me_3Sn^{\bullet}$ [rather than Fe(III)−O$^{\bullet}$] from the intermediate C_4-carbon radical **2a** (X = Me_3Sn, Scheme 1A). Also, the absence (<0.1%) of hexamethyl Dewar benzene (HMDB) rearrangement during $FeBr_2$ reduction of $C_{4\beta}$-stannyl trioxane **8** is consistent with the absence of a high-valent iron−oxo intermediate.[6] As a control to show that the presence of a tin atom does not *necessarily* destroy a trioxane's antimalarial activity, tin-containing dihydroartemisinin analog **9** was synthesized[7] and was found to have measurable *in vitro* antimalarial activity (IC_{50} = 750 ng/mL, 1300 nM).

[†] Department of Chemistry, The Johns Hopkins University.
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[§] Department of Medicine, The Johns Hopkins University.
[||] The Weizmann Institute of Science.

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(6) Posner, G. H.; Cumming, J. N.; Ploypradith, P.; Oh, C. H. *J. Am. Chem. Soc.* **1995**, *117*, 5885.

(7) All new compounds were fully characterized spectroscopically and by HRMS and/or combustion analysis.

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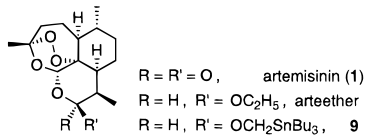
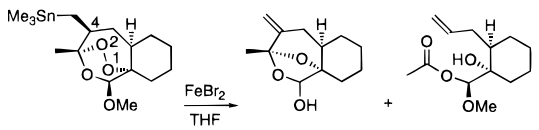


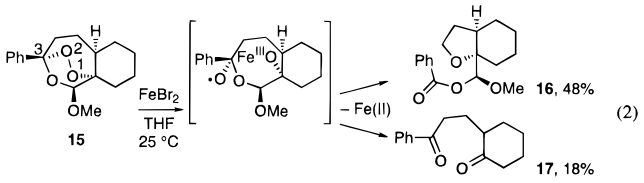
Further experimental support for these mechanistic conclusions comes from the ferrous bromide induced transformation of $C_{4\beta}$-stannyl analog **8** into two major non-tin-containing olefinic products (eq 1). Although formation of non-tin-containing terminal olefin **8b** might result from a concerted radical fragmentation[8] initiated by electron donation from Fe(II) to oxygen-1 in trioxane **8**, formation of exocyclic olefin **8a**, isolated in 15% yield, must arise from β-scission of $Me_3Sn^{\bullet}$ from its C_4-radical precursor.[9] Thus, at least 15% of the Fe(II) reduction of trioxane **8** proceeds *via* the antimalarially crucial pathway involving C_4-radical **2a** (Scheme 1A).[10]



(1)

C_3-substituted analogs **12** and **13**, in which a 1,5-hydrogen atom shift from the secondary C_4-radical intermediate **3a** (Scheme 1B) could occur to form tertiary radical **3b** (from analog **12**) or even considerably more stable[11] secondary benzylic radical **3b** (from analog **13**), both have comparable and considerable antimalarial activity. Thus, competing subsequent 1,5-hydrogen atom shifts[12] apparently are not fast enough to intercept the key C_4-radical intermediates before β-scission of Fe(III)−O$^{\bullet}$ can occur. The antimalarial activities of analogs **12** and **13**, being much higher than those of C_3-methyl and C_3-ethyl analogs[13] **10** and **11** in which no subsequent 1,5-H shift is possible (Table 1), may be due to the higher lipophilicity of analogs **12** and **13** and therefore possibly also to their better transport in biological systems. Both C_3-substituted analogs **12** and **13** react with ferrous bromide and excess HMDB in THF to form hexamethylbenzene, a rearrangement characteristic of a high-valent iron−oxo intermediate.[6] In a control reaction, HMDB in THF was not rearranged when (*t*-BuO)$_2$ was reduced by $FeBr_2$.

To facilitate β-scission of Fe(III)−O$^{\bullet}$ from the C_4-radical intermediate, leading to formation of a C_3-C_4 carbon−carbon double bond,[9b] a structural feature that would stabilize such a new olefinic bond was incorporated. C_3-vinyl analog **14**,[7] in which the new olefinic bond can be stabilized by conjugation with the unsaturated C_3-substituent, is considerably more antimalarially active than the corresponding C_3-ethyl analog **11**.[13b] Ferrous bromide induced reduction of peroxide **14** causes rearrangement of HMDB into hexamethylbenzene.[6] Although 3-phenyltrioxane **15**[13] also has high antimalarial activity, its reduction by $FeBr_2$ unexpectedly does not generate high-valent Fe═O (*i.e.*, no rearangement of HMDB) but does generate an antimalarial product (diketone **17**). The two major products of $FeBr_2$ reduction of 3-phenyltrioxane **15** are ring-contracted tetrahydrofuran **16** and 1,5-diketone **17**, formed plausibly *via* electron transfer to oxygen-1 of trioxane **15** (eq 2).[7] Because some dicarbonyl compounds are known to alkylate proteins,[14] as does artemisinin,[15] the *in vitro* antimalarial activity of 1,5-diketone **17** was determined (IC_{50} = 330 ng/mL, 1400 nM). *This is the first time that any diketone has been reported to have antimalarial activity*;[16] the low antimalarial activity of diketone **17** may be due to its relative difficulty in reaching the malaria parasite inside the red blood cell. Thus, phenyltrioxane **15** may be acting in part as a prodrug for release of 1,5-diketone **17** inside the malaria parasite.[1f]



(2)

In conclusion, the results summarized in eq 1 show that when a group like Me_3Sn, being a better radical leaving group[9] than Fe(III)−O$^{\bullet}$, is situated as in trioxane **8**, then generation of Fe(III)−O$^{\bullet}$ is precluded (*cf.* Scheme 1A). The absence of antimalarial activity of tin-containing compounds **6** and **8**, in contrast to the considerable antimalarial activity of structurally similar compounds[5] **4**, **5**, and **7**, provides the first evidence supporting the central role of a biologically relevant[17] high-valent iron−oxo species in the mode of action of antimalarial *analogs* of the natural trioxane artemisinin.[6] Also, the first example is provided of any 1,5-diketone having antimalarial activity.[18,19]

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Supporting Information Available: Spectra of **6**, **8**, **8a**, **8b**, **9**, **12**−**14**, **16**, and **17** (20 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.

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(10) For example, $FeBr_2$-induced reduction of the potent antimalarial artemisinin leads to only 10−15% of its C_4-hydroxylated product *via* the crucial C_4-radical intermediate.[6]

(11) Approximate homolytic C−H bond dissociation energies (kcal/mol) are as follows: primary, 98; secondary, 94.5; tertiary, 91; $PhCH_2$−H, 85; see: Solomons, T. W. G. *Organic Chemistry*, 5th ed.; John Wiley & Sons: New York, 1992, p 266.

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(17) For some recent studies, see: (a) Dexter, A. F.; Hager, L. P. *J. Am. Chem. Soc.* **1995**, *117*, 817. (b) Minisci, F.; Fontant, F.; Araneo, S.; Recupero, F.; Banfi, S.; Quici, S. *J. Am. Chem. Soc.* **1995**, *117*, 226. (c) Tian, Z.-Q.; Richards, J. L.; Traylor, T. G. *J. Am. Chem. Soc.* **1995**, *117*, 21.

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(19) For a recent ionic mechanism discussion of artemisinin activation by non-heme iron, see: Haynes, R. K.; Vonwiller, S. C. *Tetrahedron Lett.* **1996**, *37*, 257.