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The Biomimetic Iron-Mediated Degradation of Arteflene (Ro-42-1611),an Endoperoxide Antimalarial: Implications for the Mechanism of Antimalarial Activity

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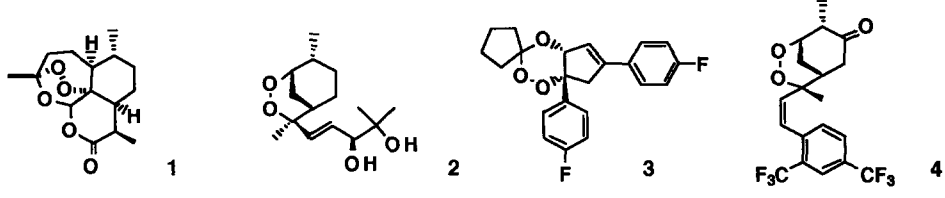
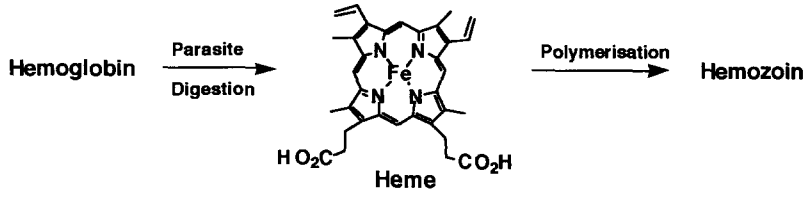
Abstract: Arteflene **4** reacts with $FeCl_2.4H_2O$ in acetonitrile or with hemin / N-acetylcysteine (heme Fe(II)) in acetonitrile to produce the diol **5** and the enone **6**. Treatment of arteflene with Zn/AcOH, a model of NADH dehydrogenase, results in the formation of the diol **5** in 80% yield. The formation of the enone **6** indicates that arteflene fragments to a non-stabilised carbon-centred radical. This radical intermediate is proposed to mediate the potent antimalarial activity of **4**.

The discovery that artemsinin **1** and yinghaosu A **2** possess potent antimalarial activity against chloroquine resistant *Plasmodium falciparum* malaria has led to the development of synthetically more accessible compounds of enhanced activity such as the bis (4-fluorophenyl) cyclopenta-1,2,4-trioxane **3**[1] and arteflene **4**.[2] Other simple endoperoxides possess antimalarial activity, at least *in vitro*, and it has been proposed that the peroxide class of drugs share a common mode of action.[3]



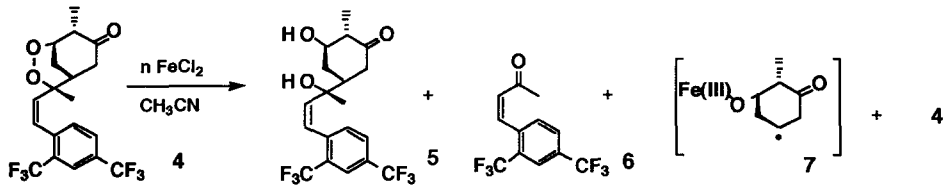
Plasmodium falciparum, in the intraerythrocytic stage of its life cycle, digests hemoglobin, which on proteolysis results in the generation of ferriprotoporphyrin IX (heme). Since "free heme" is toxic to the parasite, it is normally removed by oxidative polymerisation to hemozoin, an insoluble pigment.



In studies with artemisinin, Meshnick and co-workers have isolated a "heme-artemisinin adduct" which has led to the proposal that peroxide antimalarials may act by inhibiting this pathway.[4] Alternatively, heme in the presence of thiols, has been shown to reductively cleave the peroxide bridge of artemsinin. This process results in the formation of several potentially toxic oxygen- and carbon-centred radical intermediates. A number of model studies utilising iron (II) salts and heme iron (II) have been reported for artemisinin with several intermediates being proposed as the ultimate cytotoxic species.[5-8] Recent studies by Jefford et al. have utilised the reagent $FeCl_2.4H_2O$ as a model of heme to demonstrate the rearrangement reactions of artemisinin.[9] In

addition, in order to probe the known formation of deoxoartemsinin *in vivo* , they investigated the use of Zn in acetic acid as a model of dehydrogenase activity. Furthermore, it was proposed in these model studies that peroxides such as yinghaosu **2**, under Fe (II) treatment should produce carbon-centred radical species . In order to test this hypothesis, we have investigated $FeCl_2.4H_2O$ catalysed decomposition of arteflene in CH_3CN /water and compare the results with that of heme iron (II) and hemin iron (III). The aim of the study was to characterise the decomposition products of arteflene, and to identify potential radical intermediates that might mediate the antimalarial activity of this potent antimalarial. The effect of Zn in acetic acid on **4** is also described as a model of dehydrogenase and as a route to obtain standards for metabolism studies.

Treatment of arteflene with 1 equivalent of $FeCl_2.4H_2O$ in CH_3CN led to three products which were separated by chromatography (Scheme 1).[10] These were identified by a combination of LCMS and ^1H NMR as the diol **5** and the enone **6**, together with recovered starting material **4**. Increasing the concentration of $FeCl_2.4H_2O$ led to increased turnover (Table 1). The reactions were also carried out in aqueous media, with little effect on either product ratio or yield. The use of anhydrous $FeCl_2$ in CH_3CN led to lower yields, an observation recently made for Fe(II) catalysed decomposition reactions of cis-fused cyclopenteno-1,2,4-trioxanes.[11]



Scheme1 Fe (II) Catalysed Rearrangement Products of Arteflene

We then examined the reactions of **4** with heme. The conditions employed were identical to those recently employed by Haynes and Vonwiller in their model reactions of artemisinin with heme.[12] Thus one equivalent of hemin Fe(III) chloride together with one equivalent of N-acetylcysteine as Fe (III) reducing agent was employed in aqueous CH_3CN. The reaction was observed to produce the same products but in lower yields (Table 1).

Table 1 Reaction Products of Arteflene with Various Ferrous Salts.

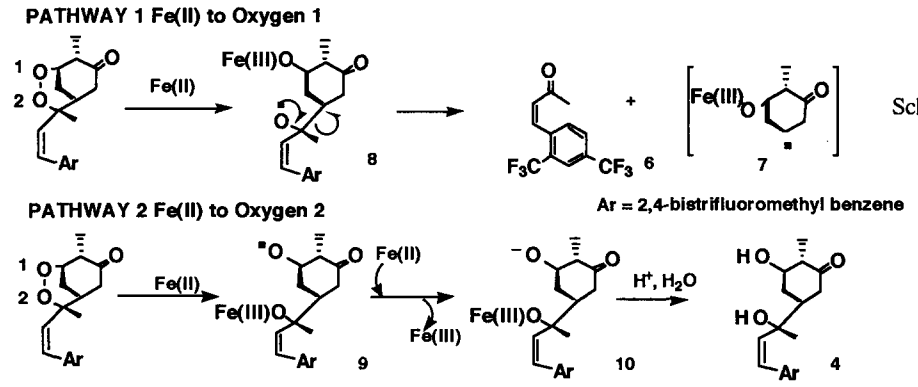
Iron Species (equivalents)	Solvent	Temp.	Reaction Time	Product Composition (%)			
				4	**5**	**6**	Recovery §[13]
$FeCl_2.4H_2O$ (1)	CH_3CN	25°C	1h	60	30	10	90
$FeCl_2.4H_2O$ (2)	CH_3CN	25°C	1h	10	50	40	70
$FeCl_2.4H_2O$ (5)	CH_3CN	35°C	1h	3	55	42	60
$FeCl_2.4H_2O$ (1)	CH_3CN/H_2O	25°C	1h	45	35	20	55
$FeCl_2$.(1)	CH_3CN	25°C	3h	75	15	10	90
Hemin Chloride (1) (N-acetylcysteine)	CH_3CN/H_2O	25°C	1h	74	8	18	85
Hemin Chloride (1)	CH_3CN/H_2O	25°C	1h	95	0	4	90
Hemin Chloride (1)	CH_3CN/H_2O	25°C	24h	90	0	8	80

(§The product composition is the ratio of products isolated. The mass balance for the reaction is made up of polymeric material)

That Fe (II) in heme is a more efficient catalyst than Fe (III) in hemin was confirmed by the lower yield of products obtained when using hemin Fe (III) in the absence of N-acetyl cysteine (26 % versus 4 % conversion). No products clearly resulting from the cyclohexyl fragment **7** were identified but in all the reactions described polymeric material results, presumably from **7**. We then examined the effect of Zn in acetic acid, a reagent that is

known to deoxygenate 1,2,4-trioxanes such as artemisinin. Treatment of arteflene with Zn in AcOH led to the production of the diol **5** as the only product in 80% yield following column chromatography.

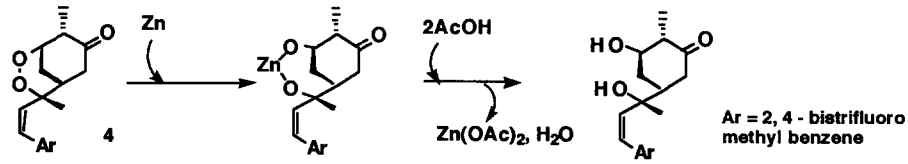
The proposed mechanisms for the formation of the observed products are shown in Scheme 2. Homolytic cleavage of the peroxide bridge by single electron donation from iron (II) (association of Fe(II) with oxygen 1) produces the oxyl radical **8**. This intermediate then collapses to produce the observed enone and the non-stabilised cyclohexyl radical **7**, which under the reaction conditions polymerises. The formation of the diol, a two electron reduction, can be rationalised by association of Fe(II) with oxygen 2 to produce oxyl radical **9** which cannot undergo rearrangement, but can undergo further reduction to the anion **10** which is protonated to give the observed product (Pathway 2, Scheme 3). It is equally feasible that the diol **5** may in fact be derived from similiar reduction of the oxyl radical **8** in pathway 1 by Fe (II).



Scheme 2

Scheme 3 Iron (II) Catalysed Rearrangements of Arteflene

Formation of the diol in the presence of Zn in acetic acid is conveniently rationalised by the oxophilic nature of Zn. The reaction is initiated by donation of a pair of electrons to the peroxide bond as illustrated in Scheme 4. Reaction of this species with two molecules of acetic acid liberates the diol **5** and two molecules of $Zn(OAc)_2$. In this reaction, zinc acts as a source of two electrons, in contrast to the one electron reducing activity of Fe(II).



Scheme 4 Two-Electron Reduction of Arteflene with Zn in Acetic Acid

Each of the products obtained (diol **5** and enone **6**) was tested for antimalarial activity *in vitro*. [14] (Table 2). The enone, a species that can act as a Michael acceptor of thiol containing proteins,*was not toxic in the antiparasitic screen.* However, this may reflect the fact this compound does not gain efficient entry into the parasite and hence cannot access target parasite proteins. Thus, although the decomposition product **6** has low activity and this in turn suggests that the proposed cyclohexyl radical **7** may be the mediator of antimalarial activity, at the present time we cannot rule out the possibility that intracellular generation of **6** leads to parasite death.

In conclusion we propose that the antimalarial activity of arteflene is a result of heme iron (II) mediated one electron reduction of the peoxide bond that ultimately results in the formation of an α,β-unsaturated ketone (enone **6**) and a C-centred radical **7**. This radical presumably kills the parasite by alkylation of vital cellular macromolecules. In addition, it is feasible that **6** might also exert toxicity by protein alkylation of thiol groups.[15] With this knowledge in hand it may now be possible to structurally modify arteflene and its analogues to produce compounds with even greater antimalarial activity.

Table 2 Antimalarial Activity of Arteflene Compared with Diol 5 and Enone 6

Drug	IC_{50} (nM) HB3	IC_{50} (nM) K1
Arteflene **4**	3	10
Diol **5**	Not Active	Not Active
Enone **6**	Not Active	Not Active

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10. **Diol 5**; R_f = 0.2 (20% EtOAc/ hexane, silica); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.88 (1H, s, Ar-H), 7.60-7.80 (2H, m, Ar-H), 7.03 (1H, d, J = 15.40 Hz, vinyl-H), 6.23 (1H, d, J = 15.40 Hz, vinyl-H), 4.21 (1H, d, J = 4.40 Hz, CHOH), 2.45-2.70 (2H, m, CH_2), 2.10-2,41 (3H, m), 1.68-1.80 (1H, m, CH), 1.47 (3H, s, CH_3), 1.15 (3H, d, J = 7.15 Hz, CH_3-CH); EIMS 392 (M^+-18, 100), 375 (49), 333 (36), 283 (62), 263 (69), 219 (33), 153 (34), 110 (82); CIMS 411 (M+ = 1, 4), 393 (M+1-18, 100), 375 (10); Anal. Required C, 55.61 H , 4.91, Found C, 55.50, H, 4.85
Enone 6; R_f = 0.80 (20%EtOAc/ hexane, silica) ^{1}H NMR ($CDCl_3$, 300 MHz) ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.93 (1H, s, Ar-H), 7.77 (1H, d , J = 8.10 Hz, Ar-H), 7.51 (1H, d , J = 7.69 Hz, Ar-H), 7.11 (1H, d, J = 12.36 Hz, vinyl=H), 6.45 (1H, d, J = 12.36 Hz, vinyl-H), 2.11 (3H, s, $COCH_3$); 13 C NMR (191.0, 139.2, 135.7, 131.8, 131.5, 130.0, 128.5, 128.3, 125.1, 123.1, 122.0, 30.9 ; EIMS 283 (M+1, 100), 267 (47), 263 (M+1-HF), 80), 219 (M+1-C_2H_4O, 52); CIMS 565 (2M + 1, 56), 283(M+1, 100), 263 (M+1-HF, 80). Anal. Required C, 51.08 H, 2.86, Found C, 51.5, H, 2.98
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13. Attempts at trapping the cyclohexyl fragment failed. In addition no adduct of heme and arteflene was observed in the reactions involving hemin.
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15. In studies conducted with radiolabelled arteflene it was noted by Meshnick et al. (*Antimicrob. Agents. Chemother.*, **1994**, *38*, 1854-1858) that several specific malarial proteins were alkylated by arteflene. Since the radio-label is in the vinyl function of arteflene it seems reasonable that the protein-arteflene adducts described in this study are in fact derived from reaction of the enone **6** with protein thiol groups.

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