Unified Mechanistic Framework for the Fe(II)-Induced Cleavage of Qinghaosu and Derivatives/Analogues. The First Spin-Trapping Evidence for the Previously Postulated Secondary C-4 Radical

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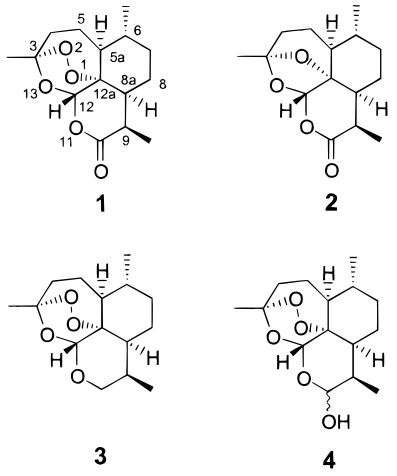
Abstract: Qinghaosu and derivatives were easily reduced by ferrous sulfate in aqueous acetonitrile to give results different from those reported for other reducing systems. The unstable epoxide **7**, a compound that was postulated earlier as a species responsible for the antimalarial activity, now has been isolated and characterized. The earlier speculative secondary C-4 radical has also been trapped with 2-methyl-2-nitrosopropane and thus provides the very first direct evidence for the involvement of radicals in the in vitro cleavage of QHS-type compounds. A unified mechanism featuring interchangeable radical anions and reversible intramolecular radical reactions is proposed for the ferrous ion induced cleavage of the 1,2,4-trioxanes (i.e., QHS and the like). On the basis of this framework, together with consideration of counterion and solvent effects, a large body of divergent experimental outcomes can be satisfactorily rationalized, not only the formation of the main products but also the product ratios as well as their deviation from those obtained under other reaction conditions.

Introduction

Qinghaosu (artemisinin, **1**, QHS) is a novel sesquiterpene endoperoxide isolated[1] in 1971 from Chinese medicinal herb qinghao (*Artemisia annua* L.). Due to their potent antimalarial activity, fast action, and low toxicity, QHS and its derivatives have distinguished themselves as a new generation of antimalarial drugs, especially in the treatment[2] of multi-drug-resistant cases. QHS has a unique carbon framework different from that of all previous antimalarials. Structure−activity studies have shown that the peroxy group is essential for the antimalarial activity. The absence of this peroxy bridge, e.g. in deoxyqinghaosu[3] (deoxyQHS, **2**), leads to complete loss of potency. Deoxoqinghaosu[4] (deoxoQHS, **3**) and dihydroqinghaosu (dihydroQHS, **4**) as well as its derivatives[3] that retain an intact peroxy group, on the other hand, show even higher antimalarial activities than that of QHS itself. These discoveries have stimulated a number of studies on the role of the peroxy group in the biological activity of QHS.



The mode of action of QHS type drugs against *Plasmodium* has been suggested[5] to be at the intraerythrocytic stage. Since the parasitized erythrocytes (red blood cells) are rich in iron, it is natural to conjecture that Fe(II) and Fe(III) might be involved in the antimalarial mechanism. As models for the biochemical course of reaction of **1** in vivo, several groups around the world have conducted investigations[5−10] on the Fe(II)-mediated in vitro cleavage of the peroxy bond since 1990. Some[6−10] used **1** and closely related compounds, while others[5] worked mainly on

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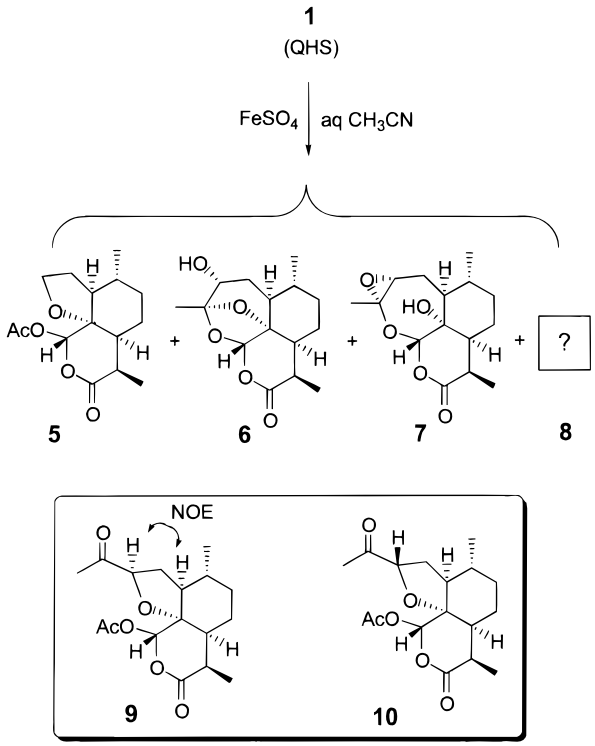
simpler 1,2,4-trioxane systems. The reports on the cleavage have been accumulating at an increasing rate. Mechanistic understanding, however, has not kept pace, partially because the results of different groups are divergent (at least superficially) and partially because the interpretations of the experimental data are not unequivocal (better alternatives exist) and the mechanisms proposed contain unexplained or even questionable steps. Since the knowledge of the in vitro cleavage may play a critical role in elucidation of the parasiticidal mechanism of this new class of drugs, further experimentation as well as scrutiny of previously reported experiments and mechanistic interpretations in the literature is warranted.

Results and Discussion

We have been engaged in a long-term project on QHS chemistry dealing with the structure, reactions, and synthesis of QHS and its derivatives, as well as analogues.[10] In the late 1980s, the publications on DNA damage[11] caused by the Fenton reagent ($Fe(II)/H_2O_2$) caught our attention. The chemical similarity between the Fenton reagent and Fe(II)/QHS and the high concentration of iron in the parasitized red blood cells inspired us to explore the reaction of **1** (and its derivatives) with Fe(II). In this paper, we detail[12] our findings and present the current understanding of the cleavage mechanism as well as its relevance to the parasiticidal activity of QHS-type drugs at the molecular level.

Our cleavage reactions were carried out in aqueous acetonitrile[8] (H_2O/CH_3CN 1:1, pH 4) containing 1 equiv of $FeSO_4$, conditions closer (compared with THF) to the biological ones. In this system at room temperature under a nitrogen atmosphere, **1** could be easily cleaved. Addition of a phosphate buffer (to adjust the medium acidity to pH 6−7) slowed the reaction significantly (probably by affecting the acid-catalyzed rearrangements, vide infra) but did not result in any other changes. Introduction of EDTA or varying the amount of $FeSO_4$ (from catalytic amounts to more than 1 equiv) did not lead to any significant changes in the product composition or yields, either. If the $FeSO_4$ was replaced by $FeCl_3$, however, the reactions simply did not occur at all (cf. ref 8).

Scheme 1



The cleavage of **1** with $FeSO_4$ (Scheme 1) gave two major products, **5** and **6** (in 25% and 67% yields, respectively), which had also been detected in the extract from *Artemisia annua*[13,14] and in the products of pyrolysis[15] and metabolism[16] of QHS. Repeated chromatography of the fractions containing minor components afforded another crystalline product (accounting for ca. 1∼2% yield), which was later identified as epoxide **7**, a compound Posner[6a] has speculated as an intermediate that might be responsible for the parasiticidal activity due to its potent alkylating property. The existence of **7**, however, was assumed[7a] to be "difficult to prove" because of its intrinsic instability.

Together with **7** were isolated some other components of higher polarity as a low-melting mass (**8**). Acetylation of this mixture with acetic anhydride led to a single crystalline acetate **9** (83% yield), whose configuration at C-4 was established according to the NOESY spectroscopy (a cross-peak correlating the H's at C-4 and C-5a was observed). The configuration at C-4 immediately after the formation of the THF ring should be as shown in structure **10**, but under the reaction conditions, the less stable isomer (**10**, $E = 32.26$ kcal/mol, as calculated by MM^+ method using Hyperchem 4.5 program) is converted into the more stable one (**9**, $E = 30.19$ kcal/mol), although so far we do not know whether this inversion occurred before or after the acetylation. It should be emphasized here that no deoxyQHS (**2**) was obtained, although this compound[17] is often found as

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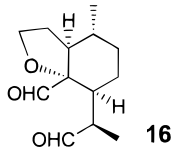
Table 1. Results of Cleavage of **1** and Derivatives with $FeSO_4$ in Aqueous CH_3CN

	compd	products (yields, %)		
1	**1**	**5** (25)	**6** (67)	**7** and **8** (<10)
2	**11**	**12** (37)	**13** (45)	**15b** (4, $\alpha + \beta$)
3	**4a**	**14a** (45)	**15a** (23)	**15b** (25)
4	**4b**	**14b**[a] (39)	**15b** (56)	
5	**4c**	**14c** (46)	**15c** (25)	
6	**4d**	**14d** (59)	**15d** (25)	

[a] Hydrolysis of **14b** led to **16**.



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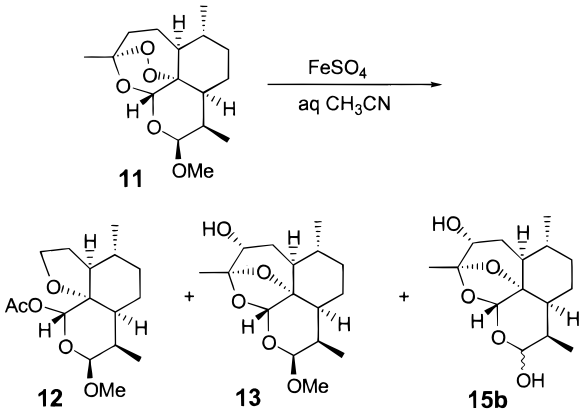
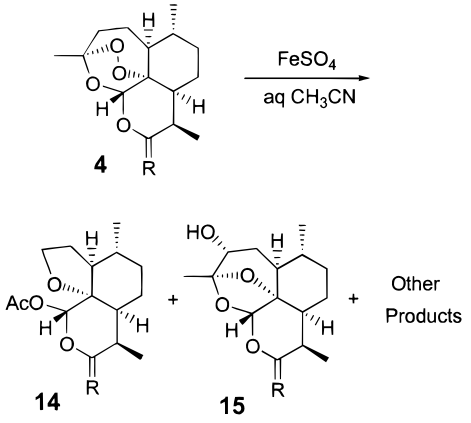
Scheme 2



11 **12** **13** **15b**

Scheme 3



4 **14** **15**

a, R = α-$OCOCH_2CH_2CO_2H$, β-H
b, R = OH, H
c, R = α-H, β-OCH_2Ph
d, R = α-OCOPh, β-H

the predominant product when using many other reducing agents. This is a major difference between the reaction conducted in aqueous medium and that in THF.

Artemether (**11**), a derivative of QHS already on the market as an antimalarial drug, reacted with $FeSO_4$ similarly, giving **12** and **13** in 37% and 45% yields, respectively (Scheme 2). In addition to these two major products, a small amount of the demethyl product (**15b**) was also obtained (ca. 4%). Artesunate (**4a**), another marketed antimalarial drug, and some other derivatives of QHS were also cleaved by $FeSO_4$ to give compounds **14** and **15** (Scheme 3). Again, neither deoxy derivatives of **11** or **4**, nor 4β-hydroxy isomers of **13** or **15** were detected. The results are summarized in Table **1**.

In our preliminary communication,[12] we presented a sketch of the possible mechanism. While it was compatible with the main features of the experimental outcome, many details were unexplained. Having noticed this and similar problems in the work of others, we have reconsidered all of this, taking into account as much relevant information in the literature as possible. We have now worked out a unified mechanistic picture as outlined in Scheme 4, which seems to cover all known facts. For the convenience of discussion, in the following, we shall refer to this picture as "the main mechanism" and the products obtained in the cleavage of QHS with $FeSO_4$ as " the normal products".

It has been agreed by most workers in this field that the Fe(II)-mediated cleavage of 1,2,4-trioxanes begins with a single electron transfer (SET) from a Fe(II) ion to the peroxy bond, resulting in two radical anions (Scheme 4, exemplified by QHS), **1A** (O-1 radical) and **1B** (O-2 radical), each having its own routes to evolve further to give the final products. It has been assumed by others that the two radical anions were irreversibly generated and were not interchangeable as established from the corresponding end product ratio. This presumption leads to a serious problem: Why does modification at an atom (e.g., C-10) remote from the peroxy bond so strongly affect the ratio at which the two radical anions are generated? Up to now, all mechanistic work has only tried to rationalize the formation of the main products obtained in a given run without addressing the deviation in the ratios and the types of the products observed under different conditions or/and with different substrates.

Jefford[5c] et al. have mentioned such a concept of interchangeable radical anions purely for heuristic purposes, neither giving the arguments for its existence nor employing the concept in their interpretation. In rationalizing the divergent product ratios reported by different authors, we find that, by assuming that radical anions **1A,B** are rapidly interchangeable, either through reversible cleavage of the peroxy bond or, more likely, through direct SET between the two oxygen atoms (or an oxy radical substitution at the Fe atom), many "conflicting" results can be easily interpreted.

We have also assumed that the steps after formation of **1A** or **1B** (before the unpaired electron is eliminated from the substrate molecule through either substitution or β-scission) are reversible. This is not often encountered in radical chemistry, presumably because in most cases the reactions take place intermolecularly; the resultant radical is no longer in the same molecule as the starting radical.

Owing to the much higher reactivity of free radicals than oxy anions, the subsequent transformations after generation of the radical anions should be predominated by the radical reactions. Thus, **1A** rearranges by an intramolecular 1,5-hydrogen shift to produce a secondary carbon-centered radical (**17**). This is one of the earliest proposed transformations in the cleavage chemistry of QHS and has been widely accepted by the workers in this field. Recently, however, some authors[5c] question this 1,5-H transfer process on the basis of calculated distances between relevant atoms. The main argument used to reject 1,5-H transfer is that the distance between O-1 and H-4 (i.e., the H at C-4) in the conformation of lowest energy exceeds the critical distance of 2.1 Å. Conformational adjustments, though, could narrow the gap but would never realize the desirable O-1, H-4, and C-4 collinear arrangement. Houk's calculation is also cited as a supporting argument: The 1,5-H transfer requires a boatlike transition state (of high energy) and therefore the radical **17** would be unattainable.

Scheme 4

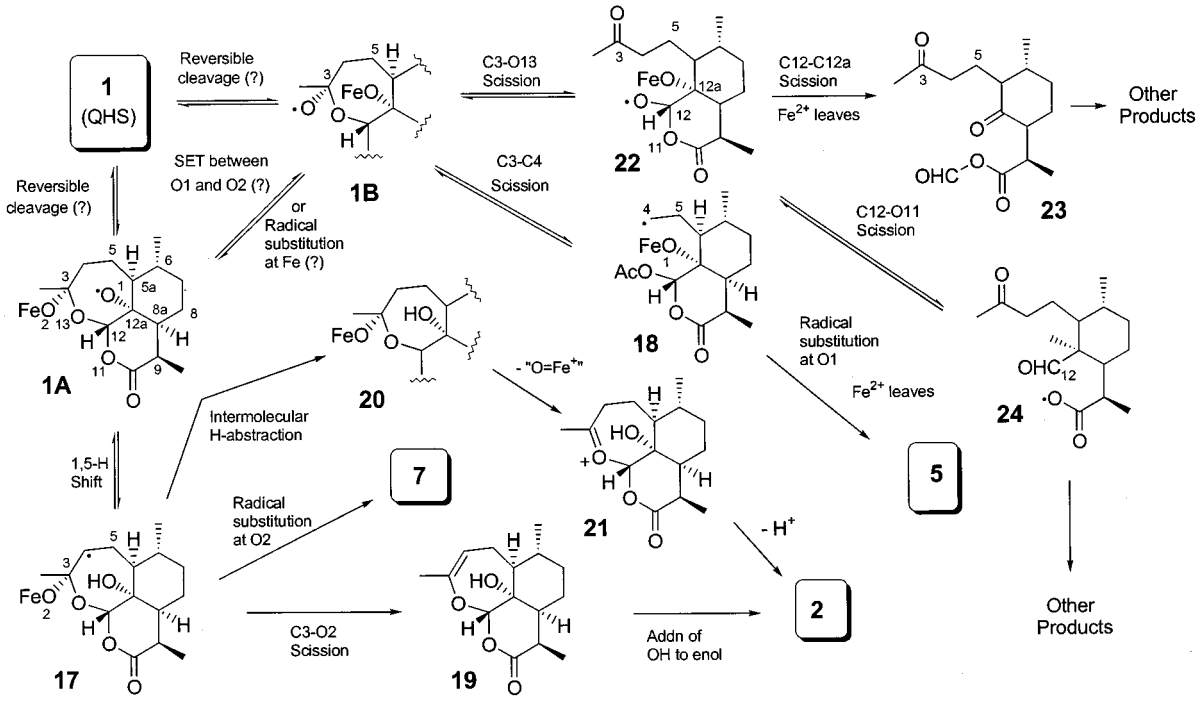


Figure 1. ESR signal recorded in a run in aqueous CH_3CN with **1** as the substrate in the presence of 1 equiv of $FeSO_4$ with MNP as trapping agent (cf. text and the Experimental Section).

We find these arguments are not appropriate for rejecting the 1,5-H transfer. The collinear arrangement has never been a prerequisite for the 1,5-H transfer. Probably no 1,5-H transfers, including those well-established cases of Norrish Type II process, ever occurred via a collinear transition state. Houk's theory (boatlike TS) was derived for open-chain systems. In QHS, the boatlike conformation already exists in the starting entity and only slight twisting of the ring would meet the requirement for the 1,5-H transfer. The energy barrier would not be as high as in an open chain starting species.

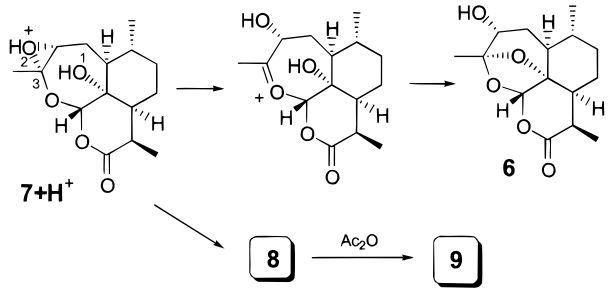
We have also obtained firm experimental evidence for the proposed secondary C-4 radical. In a run with QHS (**1**) as the substrate under the same conditions as aforementioned, we added the spin-trapping agent 2-methyl-2-nitrosopropane (MNP) to the reaction mixture and recorded the ESR spectrum (Figure 1). The detected signal showed additional splitting besides that caused by the coupling to the nitrogen; each line of the triplet was further split into two, indicating that the observed species was *t*-Bu(R_1R_2CH)NO$^{\bullet}$. Thus, the trapped radical was a secondary one (bearing only one proton), which fit best the secondary C-4 radical (**17**). However, there is so far no direct evidence that the trapped radical contains iron. It deserves to be emphasized here that, although such a species[6g] was postulated several years ago and supporting evidence for its involvement in the reaction has been reported, this is very first direct evidence for the existence of a secondary carbon-centered radical in the cleavage reaction.

During the preparation of this manuscript, Robert and Meunier's work[18] appeared in the literature. They isolated and characterized an adduct that contained a moiety of QHS structure corresponding to the primary C-4 radical **18**. Although strictly speaking their results do not provide any direct evidence for the involvement of a radical, it was possible that the adduct was formed via radical addition under the given circumstances.

The secondary C-4 radical (**17**) may have several pathways for further reaction. A β-scission between C-3 and O-2 would give enol **19**, which could be easily converted to deoxyQHS (**2**) by intramolecular addition of the C-12a OH to the enol double bond. During this process, O═Fe(IV) (i.e., O═Fe^{2+}), a species[6] that has been attracting a great deal of attention as possible basis of parasiticidal action, is expected to form. Alternatively, radical substitution at O-2 with Fe^{2+} as the leaving group would lead to epoxide **7**. Due to its intrinsic instability, **7** may be converted to several secondary products by acid (either protonic or Lewis acids) catalyzed rearrangements. Note that the Fe^{2+} is regenerated here, which accounts for the observation that a catalytic amount of Fe^{2+} added in the beginning could work equally well. Finally, the C-4 radical may also abstract a hydrogen intermolecularly (if there exists a good hydrogen donor, such as 1,4-cyclohexadiene, in the reaction mixture) to afford intermediate **20**, which could be easily transformed into **2** via **21**, either via O-13 assisted elimination of O═Fe^{+} or by hydrolysis of the Fe−O bond followed by elimination of water (the process of forming ketal from hemiketal). The observation by Posner and co-workers[6] that the presence of an excess of 1,4-cyclohexadiene led to significantly increased amounts of **2** is most likely due to facilitated intermolecular hydrogen

(18) Robert, A.; Meunier, B. *J. Am. Chem. Soc.* **1997**, *119*, 5968−5969.

Scheme 5



abstraction (and of course the intermolecular hydrogen abstraction by **1A,B**), a process the authors[6a] confusingly termed a "radical trapping experiment". This explanation is much more convincing than the alternative[5c] that the increased amount of **2** was due to transfer hydrogenation with 1,4-cyclohexadiene as the H_2 donor—the absence of a hydrogenation catalyst was overlooked!

Bromide ion from $FeBr_2$ in THF has also been proposed[5c] as a reducing agent, which might be responsible for the formation of **2**. However, the reduction potentials[19] for $Br_2/2Br^-$and QHS do not seem to support this proposal. Haynes and Vonwiller[8b] have also reported that $ZnBr_2$ in diethyl ether could not induce any reaction.

The O-2 radical (**1B**), unlike the O-1 radical which has an α-hydrogen atom (at C-4) available for abstraction, reacts predominantly via *β*-scission. Since elimination of a methyl radical is disfavored, the possible *β*-scissions available to **1B** are those leading to **22** and **18**. The scission of C-3/O-13 bond affords **22**, a species with at least two alternatives at the subsequent step. In the case of QHS (**1**), scission of C-12 and O-11 wins out, as no products derived from **23** were detected. (Note that conversion of **22** into **23** can be regarded as an irreversible process because the radical is eliminated from the molecule). This is an interesting crossroads; which way the reaction goes seems to be dictated by the nature of the substituents at C-10. When a carbonyl is present as in **1**, the cleavage leads to **24**, giving a carboxyl radical, a better leaving group than alkoxide radical due to delocalization of the unpaired electron. The two successive scissions (**1B** to **22** then to **24**) might well occur in a concerted manner. When C-10 is a methyl or substituted methyl group, the scission corresponding to that leading to **23** prevails (vide infra). Another possible route for **1B** to take is the scission of the C-3/C-4 bond. This is also supposed to be a rapid reversible process. Radical substitution at the O-1 (leading to **5**, a step that has been part of Jefford's mechanism[5c] although it was put in a different way without indicating that this is a radical substitution at oxygen), however, is irreversible and likely to be rate-limiting.

The unstable epoxide **7** may have a variety of ways to rearrange in the presence of protonic or Lewis acid catalysts. The formation of **6** (Scheme 5) is probably the simplest. However, it deserves to be pointed out that **6** is most likely formed by O-13 assisted cleavage of the O-2/C-3 bond, followed by attack of O-1 as shown in Scheme 5 (This process is similar to the exchanging of alkoxy groups of a ketal). The other minor components (**8**) are more difficult to conclusively identify because they were obtained as a mixture. However, since they all can be converted to **9** (whose structure has been undoubtedly established) by acetylation, all of these compounds must be derived from **7** with an oxygen attached to C-4 in such a way that the oxygen can leave easily under the acetylation conditions while the OH at C-12a cuts in to form the THF ring.

(19) Reduction potantials: qinghaosu, 0.87 V; dihydroqinghaosu, 1.05 V; arteether, 1.33 V; artemether, 1.50 V. See: Jiang, H.-L.; Chen, K.-X.; Tang, Y.; Cheng, J.-Z.; Li, Y.; Wang, Q.-M.; Ji, R.-Y.; Zhuang, Q.-K. To be published. Cf. also the reduction potentials (taken from *CRC Handbook of Chemistry and Physics*, 73rd ed.; Chemical Rubber Co.: Boca Raton, FL, 1992) for the following: $Br_2/2Br^-$, 1.087 V; Fe^{3+}/Fe^{2+}, 0.771 V; $I_2/2I^-$, 0.536 V; Sm^{3+}/Sm^{2+}, −1.55 V; the latter three are able to reduce the peroxy bond in QHS.

Table 2. Published Product Ratios of Iron-Induced Cleavage of QHS[a]

	reagent	solvent	product ratios **2**	**6**	**5**	remarks	sources
1	$FeBr_2$	THF	6	1	3		Posner[6a]
2	$FeBr_2$	THF/CHD	17	1	4		Posner[6a]
3	$FeCl_2·4H_2O$	CH_3CN	0	1	6		Posner[6a]
4	$FeSO_4$	aq CH_3CN	0	>67	25	**6** plus **7** & **8**	this work
5	$FeCl_2$/imid	CH_3CN	6	16	78		Haynes[8]
6	$FeCl_2·4H_2O$	CH_3CN	(0)	17	78		Jefford[5c]
7	$FeCl_2·4H_2O$/CyH	CH_3CN	(0)	1∼8	84		Jefford[5c]
8	hemin/BnSH	THF	0.2	1	11		Posner[6a]

[a] CHD = 1,4-cyclohexadiene; CyH = cyclohexene; imid = imidazole. The product ratios of Posner were determined by NMR, while others were isolated yields.

To decipher these data, at least two factors must be considered. One is the effect of the counterion, which we believe plays a role in reaction selectivity. $FeCl_2·4H_2O$ seems to be the most active[6a] one. The reaction with this catalyst usually takes only a few minutes. $FeBr_2$ is probably a little less reactive, with which it would take some 10−30 min[6a] to finish the reaction. The activity of $FeSO_4$ is obviously lower; several hours (often overnight) is normally required to consume all of the starting material. $Fe(ClO_4)_2$ has been reported[8b] to be completely inactive. This trend might simply be a consequence of the increasing number of oxygen atoms in the anion, which reduces the probability for Fe^{2+} to deliver one electron to the peroxy oxygen (breaking the peroxy bond) and that for Fe^{3+} to receive one electron from the formal alkoxide (breaking the Fe−OR bond) and thus slow the reactions.

Solvent may also contribute to the divergence of the results. In THF (less polar than CH_3CN), the Fe−OR bond is probably "stronger/tighter" than in CH_3CN (especially the aqueous (aq) one). Breaking of the Fe−OR bond (e.g., in the formation of **5** and **7**) may thus become more difficult and the *β*-scission (breaking the FeO−R bond) becomes more significant. In aq CH_3CN, the oxygen in the Fe−OR bond is probably more like a solvated alkoxide anion, which renders the *β*-scission (formally elimination an O atom radical) very difficult. This explains why the major product in THF (entry 1, Table 2), **2**, is not formed at all in CH_3CN (entries 3, 4, 6, and 7).

In our case ($FeSO_4$/aq CH_3CN, entry 4), the less active form of Fe^{2+} (compared with entry 1) would slow the steps of **17** to **7** and **18** to **5**, while the solvent effect of aq CH_3CN works in the opposite way. Hence the net effects at these two steps are almost null. But, at the step **17** to **19**, the solvent effect is not counterbalanced by the reagent effect; in aq CH_3CN, this path is shut off (all **17** formed from **A** is converted to **7**, and in turn to **6**, etc.). For these reasons, the ratio of **5**/(**2** + **6** + **5**) is more or less the same in both cases, but the ratios of **2**/**6** differ greatly. With $FeCl_2·4H_2O$/CH_3CN (entry 6), the solvent effect

(acceleration of the steps **17** to **7** and **18** to **5**) is probably weaker than that with aq CH_3CN. However, this time, it is not canceled by the reagent (anion) effect; the product distribution pattern changed. The "O-2 product" **5** now has become the major product. Why would the "O-2 route" predominate over the "O-1 route" in this case? A likely reason is that **18** to **5** is the rate-limiting step of the "O-2 route", whereas the 1,5-H shift (which is not affected by the solvent effect) in "O-1 route" may be not as fast as the C-3/C-4 bond breaking/reforming and thus becomes part of the rate-limiting steps.

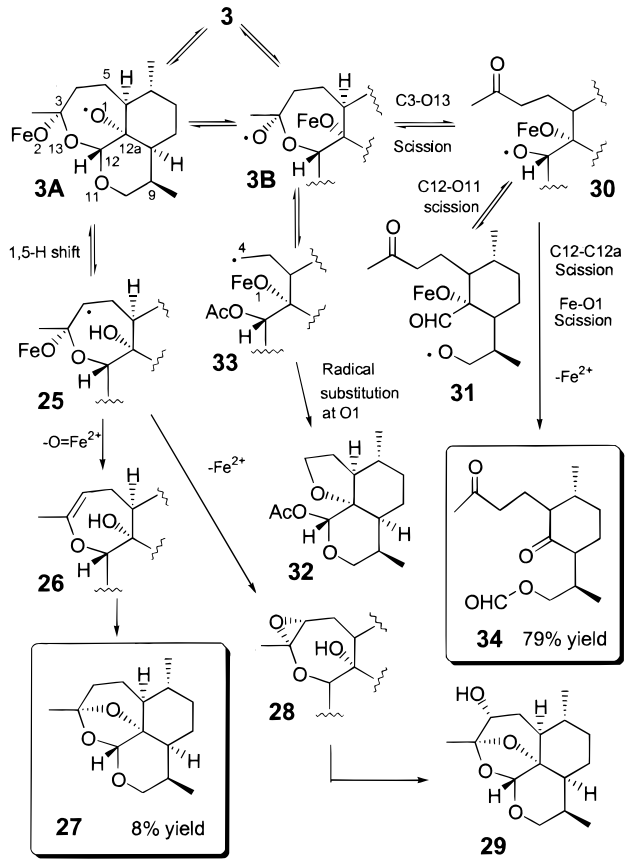
Anhydrous $FeCl_2$ has been found[5b] to be less active than its tetrahydrate. However, Haynes' reaction (entry 5) took only 5 min to complete. This may reflect the effect of imidazole. It seems to be likely that imidazole may form a complex with the iron ion (resembling the situation in heme), at least a small portion of it, and stabilizes both imid-Fe (this formally makes Fe^{2+} a better leaving group) and imid-Fe−O species (this formally makes imid-Fe^{2+}═O a better leaving group in the β-scission) by delocalization of the charge or radical. Again, due to the difference in the rate-limiting steps, the "O-2 route" would be more sensitive to the acceleration than the "O-1 route" and **5** would be the major product. Since both epoxidation and β-scission are accelerated, some **2** would still form even in CH_3CN.

The outcomes[8a] of the reaction catalyzed by hemin seem to be very anomalous. It appears to us that the effects of complexation of the iron ion with porphyrin in hemin are similar to those described above for imidazole. There are, however, some additional complications when using hemin as reagent. For instance, hemin (Fe^{3+}) must be reduced (by, for example, a thiol) to heme (Fe^{2+}) before triggering the main mechanism. The reactions may go rather fast (finished in a few minutes). Therefore, the way of mixing the reactants (i.e., starting with how much heme?) might affect the outcome of the reaction; unlike simple Fe^{3+} species (e.g., $FeCl_3$ alone), hemin may react to some extent on its own as suggested by Haynes[8] before it is reduced to heme and turns on "the main mechanism". The presence of a thiol (good hydrogen donor) may also activate the intermolecular hydrogen abstraction pathway and thus change the product ratio. For instance, owing to the presence of thiol, the radicals involved in the main mechanism (**1A**, **1B**, and **17**) may abstract a hydrogen from the thiol and eventually lead to increased amounts of **2**. Finally, the additional functionalities present in hemin and thiol may contribute to the low recovery (forming, e.g., heme adduct) as well.

The QHS derivatives (Table 1) we have examined fall into two groups, one includes **11** and **4b**, both having a relatively small substituent at C-10. These two compounds gave more "**6**-type" products than "**5**-type" products in the cleavage reaction (similar to QHS). The situation with the other three compounds (**4a**,**c**,**d**), all carrying a larger group at C-10, is reversed, with the "**5**-type" product predominating over the "**6**-type" one. We currently attribute the decreased amount of "**6**-type" products to a slower 1,5-H shift that may result from the conformational changes caused by the large substituents.

Avery and co-workers[7] have conducted a large amount of elegant work in the structure modification of QHS. However, their mechanistic studies did not lead to convincing interpretations. Their mechanisms rely heavily on a less common 1,3-H shift, a process the authors themselves have questioned. We have found that all their results[7a] can be easily explained by our main mechanism. Thus, **3** is cleaved by $FeBr_2$ to afford corresponding "O-1 radical" **3A** and "O-2 radical" **3B** (Scheme 6, note that the configurations at C-12a were incorrectly drawn

Scheme 6

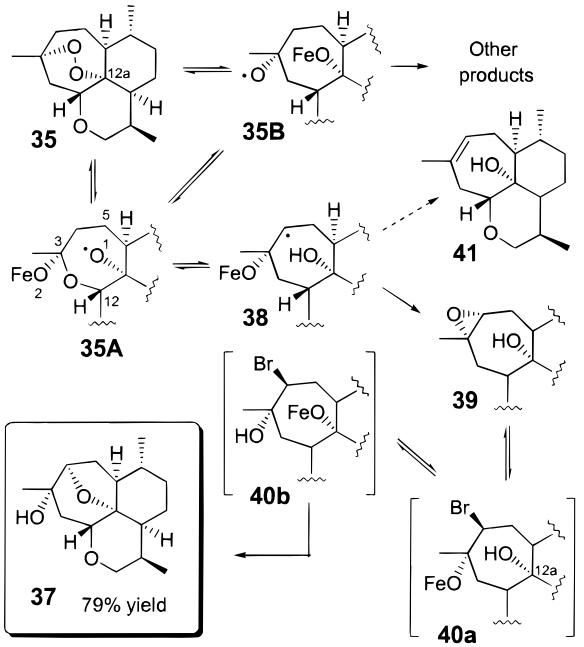


in the literature[7a]) as discussed for QHS. The difference at C-10 changes the relative rates in the "O-2 route" and consequently the main products (compared with QHS). The "O-1 route" is more or less the same as that of QHS. Since the radical substitution at oxygen is relatively slow in THF, the β-scission manifests itself, giving the enol **26** and then **27** (cf. conversion of **19** to **2**) in 8% yield.

The "O-2 route" is entirely different now (different subbranches are now in operation). Unlike in QHS case (Scheme 4) where **22** is mainly converted to **24** (or, conversion of **1B** to **22** then to **24** occurs in a concerted manner due to the relative ease of breaking C-12/O-11 bond), in **3B**, the scission of the C-12/O-11 bond becomes a disfavored process (because alkoxy groups are much poorer radical leaving group compared with carboxy groups, or it can be interpreted as that the reverse, i.e., the addition of the alkoxy radical to the aldehyde, is rather fast) so that the reaction takes another path: breaking the C-12/C-12a bond followed by leaving of Fe^{2+} ion. Again, these three β-scissions may well occur concertedly at an overall rate not only much higher than that for **3B** to **32** (the primary C-4 radical needs time to move to the right position to attack O-1, whereas the three β-scissions may occur without any movements of the atoms) but also remarkably higher than the overall rate for the "O-1 route", so that **34** becomes the predominant product.

The two carba-analogues[7a] (**35** and **36**) of QHS made by Avery et al. gave interesting results in the $FeBr_2$-induced cleavage reactions. The first one (**35**) afforded alcohol **37** as the predominant product (in 79% yield, Scheme 7). After establishment of the structure for **37** by spectroscopic means, they concluded that the radical of **38** did not produce epoxide **39** and attributed the formation of **37** to an uncommon 1,3-H shift. As a matter of fact, this product could well be derived from **39** as proposed in Scheme 7. Thus, the epoxide ring

Scheme 7

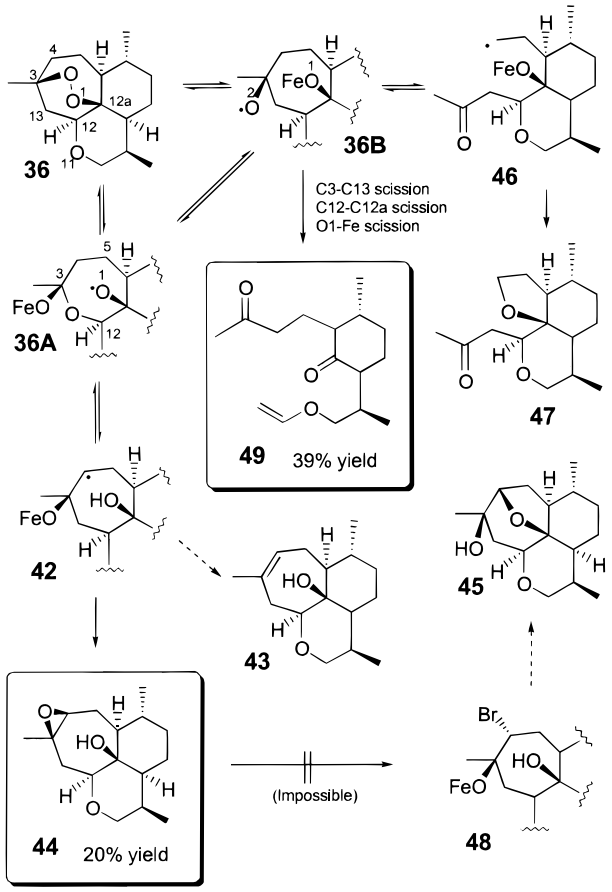


opening by the bromide results in **40a**. Although most of **40a** is expected to be converted back to **39**, some of the **40a** may undergo Fe^{2+} transposition (ligand exchange) to afford **40b**, which would soon lead to **37** due to the favorable position of the C-12a OH for both the ligand exchange and the nucleophilic substitution. The reaction ran for 8 h, which provided enough time for the overall transformation mediated by trace amounts of **40a,b**.

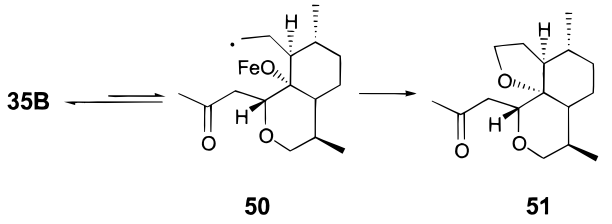
When position 13 is an oxygen, the epoxide opening is dominated by the powerful ketal exchange mechanism as discussed for the **7** to **6** transformation (Scheme 5). Replacement of oxygen with a carbon shuts this path off, providing a chance for the slower reaction caused by the bromide to manifest. This is why two epoxides (**7** vs **39**) gave different products under the same conditions. The other carba-analogue (**36**), differing only at the configurations at C-3, C-12, and C-12a, produced entirely different products (Scheme 8). At first sight this seems to be very strange because these two compounds are so similar to each other. We found the clue by inspection of the models of these two molecules: The inversion of the configurations makes the 1,5-H transfer impossible, unless the seven-membered ring flips to turn the β-hydrogen at C-4 from the quasi-equatorial position to the quasi-axial position. The ring flip certainly takes time, and therefore, the rate for **36A** to **42** is remarkably lower than that for **35A** to **38**. Consequently, the yield of epoxide **44** is much lower than that of **39** (**37**). It deserves to be mentioned here that the back of the epoxy ring in **44** is blocked by the H atoms at C-12 and C-5a. This makes the bromide attack impossible, and hence, no **45** is formed. Without competition of a rather fast "O-1 route", the "O-2 route" now has the chance to operate. Once again, since the scission of the C-12/O-11 bond is a disfavored process (cf. the discussion for conversion of **30** to **31**), the reaction is directed to the elimination of Fe^{2+} from O-1 via three β-scissions (that of C-3/C-13, C-12/C-12a, and Fe/O-1) and finally yields **49**.

It is noted that, in the cleavage of the carba-analogues, there are no products from the β-scission of the secondary C-4 radical (e.g., **38** did not give **41**, Scheme 7) and substitution of the primary C-4 radical at O-1 (e.g., **35B** did not produce **50**, Scheme 9). These phenomena may reveal some not very well

Scheme 8



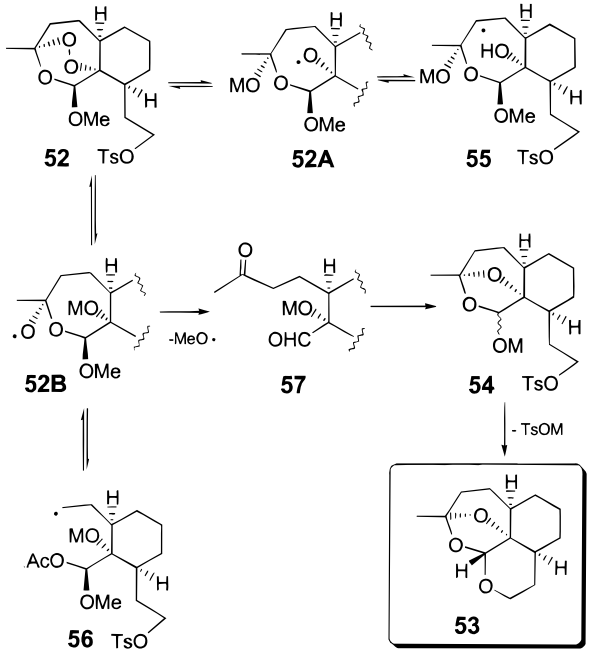
Scheme 9



known aspects of free radical chemistry. Perhaps the elimination reaction barrier for forming an alkene (**41**) is higher than that for corresponding enol (**26**); when the β-scission is slow, most of **38** would of course take another way out to form epoxide **39**. Similarly, the ketone **50** is probably more reactive than ester **18**, so that the generation of the C-4 radical in the case of, e.g., **35B** becomes a disfavored process (the reverse is too fast). This explains why no **51** was formed.

Posner and co-workers[6f] have prepared a simplified analogue, **52**, and examined its cleavage under various conditions (Scheme 10). One of the pathways (the non-iron one-electron reduction path) proposed by them obviously does not fit in with our mechanistic picture. This led us to look into the work. And after closer scrutiny we have found that the discord stems from a difference in interpretation. The main problem with the previously proposed mechanism is the requirement for a highly reactive radical to persist while the more stable anion performs a series of transforms. This appears to be irrational to us. The reason for which the authors chose the anion mechanism instead of the normal radical one was that **53** was not formed during the cleavage reaction. However, the failure of the intramolecular alkylation may have other causes than the absence of alkoxide **54**. The counterion (cation), solvent, and temperature may all

Scheme 10



contribute. A "wrong" cation (other than, e.g., Li^+ or Na^+), an improper solvent, or too low temperature may all lead to such failures. It should be noted that some of their reducing agents (e.g., Zn metal[5c]) may only cause two-electron reduction.

The only thing requiring explanation here is that, if the cleavage is initiated by a single-electron reduction, why **54** would form as the predominant product (this requires excising a methoxy radical, a process that normally does not occur) while other common "O-1 and O-2 products" were not obtained. A possible answer is shown in Scheme 10. We must note that the formation of the common products relies on the facile interchange between the two low-lying energy states of iron (Fe^{3+}/Fe^{2+}). If the Fe^{3+} is replaced by other metal ions (M^{n+}), both the β-scission (elimination of O═M^{n+} from **55**) and radical substitution at oxygen atom (forming epoxide or THF ring accompanied by elimination of $M^{(n-1)+}$) may be drastically retarded.[20] Thus, radicals **56** and **55** would go nowhere except for returning to **52B** and **52A**, respectively. Under these circumstances, the ordinarily very slow, insignificant process of excising alkoxy radical has the opportunity to manifest itself via scission of C-3/O-13 and C-12/OMe (for convenience, we still use QHS numbering here). The intramolecular rearrangement of **57** leads to **54** (formally by the attack of the O-1 alkoxide at ketone carbonyl and subsequent attack of O-2 at the aldehyde). If the ion pairs in **54** are not well solvated ("wrong" cation or poor solvent) or the medium temperature is too low, the reaction would stop at **54** (tosylates are far not as reactive as carbonyls).

Up to this point, we have explained almost all of the known results of the iron-induced cleavage of QHS and derivatives/analogues and shown that behind the diverse outcomes of experiments under different conditions, there exists a relatively simple mechanism framework. We have also shown how the particular mechanisms (i.e., particular sub-branches within the mechanism framework) for a given case are rationalized in terms of the structure of the substrate, the anion, the cation, the solvent, and the temperature. Now we shall turn to its relevance to the molecular-level antimalarial mechanism of QHS-type drugs. It has been generally accepted that, in hemin-induced cleavage of QHS and related compounds, some cytotoxic species is (are) produced that is (are) directly responsible for the antiparasitistic activity. The question is what is it (or what are they)?

One of the suggestions[6] is carbon-centered radicals. Later, O═Fe(IV) and epoxide **7** (as a potential alkylating agent) were also thought[6] to be possible killers. The possible existence of the high-valence iron species O═Fe(IV) initially was probably deduced by logic from the β-scission of secondary C-4 radicals such as **17** and has never been proven beyond all doubt. The evidence available so far includes the rearrangement of hexamethyl Dewar benzene (HDB) to hexamethylbenzene (HB), oxidation of a sulfide to sulfoxide, and oxidation of tetralin to hydroxytetralin; none of them is necessarily bound to a O═Fe(IV) participated reaction. For instance, rearrangement of HDB to HD may be initiated by losing one electron. The receiver of this electron could be, e.g., **1A** (or **1B**); this would lead to increased relative amount of **20** and eventually **2** (product ratio[6a] for **6**:**2**:**5** changed from 1:6:3 to 1:11:3). As for the latter two cases, there is no reason to say the oxidant must be O═Fe(IV). It ought also to be noted that there exists no knowledge about the oxidation power of this so far speculative species. We only know about its counterpart in hemin, which is not necessarily the same. The results from nonhemin model systems have revealed a trend that in aqueous media the β-scission that is expected to generate O═Fe(IV) may not occur at all (since no **2** was formed)! After all, in the parasite, the true species would be oxyheme, not simple O═Fe(IV). Digging very hard in nonhemin model systems for the existence of O═Fe(IV) would not help in understanding the antimalarial mechanism.

The speculative alkylating power of **7** is another thing to be questioned. Posner et al. got the idea that **7** might be an alkylating agent from the easy formation[21] of C−O or C−N bonds by reaction of ketal epoxides (at the carbonyl carbon of the ketals) with alcohols or amines. Those ketal epoxides, however, do not have a favorably positioned built-in OH group to compete with the "outcomers" for C-3. Considering the ease with which it rearranges into other products, we believe that epoxide **7** would make a poor alkylating agent. On the basis of their biological evaluation of **36** and **44** (2/3 of QHS′ activity and null, respectively), Avery et al. have also concluded that the epoxide ring is not an essential part for the antimalarial activity. Their results rule out the possibility for epoxides as alkylating agents by reacting at C-4: in the carba-analogues, the C-3 is far not so reactive as that (carbonyl carbon) in, e.g., **1** and thus does not need to be considered. The C-4 in **39** ought to be much more reactive than in **44**. However, **36** shows higher antimalarial activity than **35** (**44** is inert). Taking all these as a whole, it seems that epoxide **7** is unlikely to be the "killer".

The carbon-centered radicals generated in the cleavage remain the most likely species that are responsible for the parasiticidal activity. As shown in the schemes, there are two types of carbon radicals at C-4: one (primary) is formed from β-scission of O-2 radical (e.g., **18**) and the other (secondary) is produced by 1,5-H transfer (e.g., **17**). Our spin-trapping experiment in aqueous CH_3CN only recorded the latter. This is compatible with the fact that the relative amount of **17** produced in our system is much more than that of **26** (since the combined yields of **6**, **7**, and **9** are much higher than the yield of **5**). Robert and Meunier's results point to the more reactive primary radical **18**,

(20) A stronger reducing agent may reduce the peroxy bond more easily than Fe^{2+}, but later, it may be much more difficult to get back one electron from the oxygen. For example, radical substitution at O−Sm requires Sm^{2+} to leave. This is an $Sm^{3+} \rightarrow Sm^{2+}$ reduction with a reduction potential of −1.55 V, much more difficult than $Fe^{3+} \rightarrow Fe^{2+}$ (0.771 V).

(21) References 13 and 23 in ref 6a.

but their reaction was done with the manganese counterpart of hemin in CH_2Cl_2, where the situation probably differs very much from that in our system. It is possible that the adduct they obtained was formed from an intramolecular radical addition to the porphyrin. The primary C-4 radical has the unpaired electron at the end of the two-carbon side chain that can stretch out to reach the right target carbon in the porphyrin. The secondary one does not have this possibility because of its rigid ring system. This might explain why they did not get the other adduct.

The radical trapping experiments have confirmed the possibility for the highly reactive radicals generated in the cleavage reaction to interact with other molecules. This possibility is a prerequisite for the radicals to be qualified for the lethal agent in the antimalarial mechanism. Considerable amounts of work have already been done to reveal the relationship between the C-4 radicals' stability and the antimalarial activity. The conclusion is not very clear yet because antimalarial activity may be affected by a number of factors other than the reactivity/lifetime of the radical. However, the establishment of a unified mechanism framework for the iron-induced cleavages will certainly facilitate the process of understanding the mode of action of QHS-type drugs at the molecular level and provide a basis for rational drug design efforts.

Summary

In the preceding paragraphs, we have detailed our cleavage reactions of QHS and derivatives catalyzed by $FeSO_4$ in aqueous acetonitrile, including the isolation and characterization of epoxide **7**, whose existence has been proposed earlier but thought to be very difficult to prove. These results play a very important part in analyzing the anion and solvent effects, which have never been addressed in previous studies, on the product distribution pattern. We have also presented the very first direct evidence for the involvement of the earlier proposed secondary C-4 radical. A unified mechanism framework featuring interchangeable radical anions and reversible intramolecular radical reactions has been suggested for the iron-induced cleavage of 1,2,4-trioxanes. It ought to be emphasized that, although some pieces (only the forward steps) of the present mechanism framework have been part of the mechanistic paths proposed by others, their correctness is rather vulnerable to doubt due to the lack of explanation for the disappearance of these paths in some cases and the introduction of uncommon or unlikely mechanisms in rationalization of the unexpected main products.

The mechanism described above allows rationalization of a large body of unexplained experimental observations regarding the formation of individual product as well as the predominance of particular pathways. Thus, the fuzzy picture of the cleavage mechanism caused by the lack of an overall elucidation of the diverse experiments now becomes much better focused. This would help to save further unnecessary efforts on clarifying the in vitro cleavage mechanism itself and direct attention to the relevance of the findings in the model systems to the molecular-level antimalarial mechanism, the rationale of all the work with model systems.

Experimental Section

ESR spectra were recorded at room temperature on a Varian E-112 spectrometer with X-band, field modulation frequency 100 kHz, microwave power 20 mw, response time 0.25 s, center field set 3240 $\times 10^{-1}$ T, and sweep width 10^{-2} T. The magnetic field was determined by an ^{1}H NMR fieldmeter and the microwave frequency by a superhigh frequencymeter. 2-Methyl-2-nitrosopropane (MNP) was purchased from the Sigma Chemical Co. Microanalyses were carried out at the microanalysis laboratories of these Institutes. Flash chromatography was performed on silica gel H (400 mesh).

Typical Procedure for the Reaction of Qinghaosu and Its Derivatives with Ferrous Sulfate: Qinghaosu or its derivative (2 mmol) and $FeSO_4$ (2 mmol) were dissolved in aqueous acetonitrile (50%, v/v, 20 mL), and the mixture was stirred at 37 °C under a nitrogen atmosphere, until the starting material was completely consumed (often overnight) as shown by TLC (petroleum ether/ethyl acetate, 4:1∼2:1 depending on the substrate, visualized with ammonium molybdate). The brown insoluble substance was filtered off, and the acetonitrile was removed under reduced pressure (rotary evaporator). The residue was extracted with ethyl acetate (2 × 10 mL). The combined extracts were washed with brine, dried ($MgSO_4$), and concentrated. Flash column chromatography of the residue (eluting with petroleum ether/ethyl acetate, 4:1∼2:1 depending on the substrate) gave the products.

Spin Trapping Experiment: To the solution of qinghaosu (1 mmol) and ferrous sulfate (1 mmol) in acetonitrile and water (1:1, 10 mL) at 37 °C was added 1 mg of MNP (*t*-BuNO). After 1 h its ESR spectrum was taken. The spectrum (Figure 1) is shown with $A_N = 1.481$ mT, $A_H = 0.301$ mT, and $g = 2.0059$.

Compound **5**: colorless crystal (25% yield), mp 92∼94 °C (hexanes−ethyl acetate) (91−92 °C,[16b] 91−92 °C[13]), $[\alpha]^{20}_D$ +109.2 (*c* 1.0, $CHCl_3$) {$[\alpha]_D$20 +109.2 (*c* 0.109, $CHCl_3$),[16b] $[\alpha]_D$15.5 +93.5 (*c* 1.0 $CHCl_3$)[13]}; IR (KBr) ν_{max} 1760, 1746 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.65 (1H, s), 4.21 (1H, t, $J = 7.6$ Hz), 3.94 (1H, dd, $J = 7.4$, 15.8 Hz), 3.16 (1H, dq, $J = 2.7$, 7.2 Hz), 2.16 (3H, s), 1.8−2.1 (4H, m),1.73 (1H, m), 1.60 (1H, m), 1,47 (1H, m), 1.21 (3H, d, $J = 7.3$ Hz), 1.08 (1H, m), 0.99 (3H, d, $J = 6.3$ Hz); MS(*m*/*z*) 283 ($M^+ + 1$), 165, 151, 137 (100), 109, 43.

Compound **6**: colorless crystal (67% yield), mp 199−201 °C (hexanes−ethyl acetate) (190−192 °C[16b]), $[\alpha]^{20}_D$ −131.1 (*c* 1.0, $CHCl_3$)-(−131.1, (*c* 0.119, $CHCl_3$)[16b]); IR (KBr) ν_{max} 3050, 1730 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.64 (1H, s), 3.63 (1H, d, $J = 7.2$ Hz), 3.21 (1H, dq, $J = 2.5$,7.2 Hz), 2.07 (1H, dt, $J = 4.4$, 13.3 Hz), 1.99 (1H, dd, $J = 1.67$, 9.1 Hz), 1.95 (1H, dq, $J = 3.3$, 13.6 Hz), 1.87 (1H, brs), 1.83 (1H, dq, $J = 3.2$, 13.6 Hz), 1.58 (3H,s), 1.54 (2H, m), 1.28 (1H, m), 1.21 (3H, d, $J = 7.6$ Hz), 1.12 (1H, dq, $J = 3.5$, 13.3 Hz), 1.00 (1H,dq, $J = 3.2$, 13.3 Hz), 0.94 (3H, d, $J = 6.4$ Hz); MS (*m*/*z*) 283 ($M^+ + 1$), 265, 247, 222, 150, 137.

Compound **7:** colorless crystal (1% yield), mp 148−152 °C (petroleum ether−ethyl acetate); $[\alpha]^{19}_D$ +78.5 (*c* 0.14, $CHCl_3$); IR-(KBr) ν_{max} 3500, 1728, 1635, 1144, 1022 cm^{-1}; MS (*m*/*z*) 283 ($M^+ + 1$), 222, 204, 151, 150, 149, 137, 122, 121, 107, 93; ^{1}H NMR (400 MHz, C_6D_6) δ 0.91 (3H, d, $J = 6.4$ Hz), 1.18 (3H, d, $J = 7.4$ Hz), 1.55 (3H, s), 3.17 (1H, m), 3.60 (1H, br), 5.61 (1H, s); ^{13}C NMR (100 MHz, C_6D_6) δ 13.26, 18.93, 21.32, 23.99, 31.12, 33.60, 34.03, 35.22, 40.99, 42.74, 69.90, 83.36, 99.53, 109.54, 170.89. Anal. Calcd for $C_{15}H_{22}O_5$: C, 63.81; H, 7.85. Found: C, 63.73; H, 7.79.

Compounds **8**: colorless solid; mp 85−86 °C; $[\alpha]^{20}_D$ −49.9 (*c* 1.05, $CHCl_3$); IR (KBr) ν_{max} 3320, 2990, 1750, 1720 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.98 (1H, s), 5.98 (1H, d, $J = 13.3$ Hz), 5.60 (1H, d, $J = 13.3$ Hz), 5.54 (1H, s), 4.74 (1H, dd, $J = 4.2$, 10.5 Hz), 4.21 (1H, dd, $J = 2.14$, 7.6 Hz), 4.12 (1H, dd, $J = 3.1$, 6.4 Hz), 3.08 (1H, m), 1.20 (3H, dd, $J = 7.2$ Hz), 1.02 (3H, dd, $J = 6.3$ Hz); MS (*m*/*z*) 283 ($M^+ + 1$), 265 ($M^+ - H_2O$), 237, 207; HRMS found $C_{15}H_{22}O_4$ ($M^+ - H_2O$) 265.1449, $C_{14}H_{21}O_4$ ($M^+ - H_2O - HC{=}O$) 253.1437, calcd 265.1440, 253.1440.

Compound **9** via acetylation of compounds **8**: To a solution of **8** (25 mg, 0.089 mmol) in dry CH_2Cl_2 (0.5 mL) were added acetic anhydride (31 mg, 0.3 mmol), pyridine (40 mg, 0.5 mmol), and a catalytic amount of DMAP. The solution was stirred at 25 °C for several hours and evaporated under reduced pressure. The residue was dissolved with ethyl acetate, washed, and concentrated to give crude product, which was recrystallized from ethyl acetate−petroleum ether to give compound **9** (27 mg, 83% yield): mp 162−164 °C; $[\alpha]^{20}_D$ +55.3 (*c* 0.95, $CHCl_3$); IR (KBr) ν_{max} 1760, 1720 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.66 (1H, s), 4.41 (1H, t, $J = 7.8$ Hz), 3.23 (1H, dq, $J = 5.4$, 7.4 Hz), 2.38 (3H, s), 2.27 (1H, dt, $J = 6.9$ 12.2 Hz), 2.02 (1H, m), 2.00 (3H, s), 1.96 (2H, m), 1.75 (1H, dt, $J = 8.4$, 13.6 Hz), 1.69 (1H, m), 1.66 (1H, m), 1.26 (1H, m), 1.24 (3H, d, $J = 7.3$ Hz), 1.08 (1H,

dq, $J = 4.8$, 13.4 Hz), 0.97 (3H, d, $J = 6.0$ Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 210.37, 171.50, 168.16, 92.05, 85.28, 80.31, 55.11, 46.41, 34.90, 34.50, 30.97, 30.29, 27.25, 24.53, 20.92, 20.29, 12.44; MS (*m*/*z*) 281 ($M^+ - Ac$), 265 ($M^+ - AcO$), 239, 221, 208, 193, 180, 165, 147, 121, 43; HRMS found $C_{17}H_{24}O_6$ (M^+) 324.1552, calcd 324.1572.

Compound **12**: colorless crystal (37% yield), mp 96−97 °C (hexane); $[\alpha]^{20}_D$ +117.4 (*c* 0.87, $CHCl_3$); IR (KBr) ν_{max} 1760, 1365, 1230, 1090, 1020, 930, 500 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (3H, d, $J =$ 7.5 Hz), 0.91 (3H, d, $J = 6.5$ Hz), 2.11 (3H, s), 3.40 (3H, s), 3.90 (1H, m), 4.25 (1H, m), 4.61 (1H, d, $J = 4.2$ Hz), 6.22 (1H, s). Anal. Calcd for $C_{16}H_{26}O_5$: C, 64.40; H, 8.78. Found: C, 64.64; H, 8.95.

Compound **13**: colorless needle crystal (44% yield), mp 65−67 °C (hexane); $[\alpha]^{20}_D$ +18.1 (*c* 1.30, $CHCl_3$); IR (KBr) ν_{max} 3500, 2920, 1465, 1386, 1228, 1026, 930, 870 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (3H, d, $J = 6.4$ Hz), 0.91 (3H, d, $J = 7.5$ Hz), 1.55 (3H, s), 2.44 (1H, m), 3.38 (3H, s), 3.56 (1H, m, $J = 3.0$ Hz), 4.63 (1H, d, $J =$ 4.2 Hz), 5.25 (1H, s). Anal. Calcd for $C_{16}H_{26}O_5$: C, 64.40; H, 8.78. Found: C, 64.77; H, 8.94.

Compound **15b** (from **11**): colorless crystal (4% yield), mp 141−143 °C (petroleum ether−ethyl acetate); $[\alpha]^{20}_D$ −68.7 (*c* 1.32, $CHCl_3$); IR (KBr) ν_{max} 3470, 1454, 1383 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88, 0.89 (3H, dd, $J = 6.2$, 6.2 Hz), 0.96, 1.00 (3H, dd, $J = 7.5$, 7.2 Hz), 1.54 and 1.57 (3H altogether, 2 s), 2.31,2.44 (1H, m), 3.53, 3.55 (1H, m), 4.80, 5.26 (1H, dd, $J = 6.8$, 4.0 Hz), 5.28 and 5.32 (1H altogether, 2 s); MS (*m*/*z*) 267 ($M^+ + 1 - H_2O$), 249, 224, 206, 191, 137, 121. Anal. Calcd for $C_{15}H_{24}O_5$: C, 63.38; H, 8.51. Found: C, 63.47; H, 8.65.

Compound **14a**: amorphous solid (45% yield), mp 69−71 °C; $[\alpha]^{20}_D$ +23.9 (*c* 1.34, $CHCl_3$); IR (KBr) ν_{max} 1761, 1734, 1714 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.78 (3H, d, $J = 7.0$ Hz), 0.86 (3H, d, $J = 6.1$ Hz), 2.10 (3H, s), 2.64 (4H, m), 3.89 (1H, q, $J = 8.2$ Hz), 4.21 (1H, t, $J = 8.2$), 5.82 (1H d, $J = 9.7$ Hz), 6.14 (1H, s); ESI-MS (*m*/*z*) 430 ($M^+ + 2Na$), 407 ($M^+ + Na$).

Compound **15a**: colorless crystal (23% yield); mp 139−141 °C; $[\alpha]^{20}_D$ −139.1 (*c* 1.16, $CHCl_3$); IR (KBr) ν_{max} 3290, 1740, 1703 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.91 (3H, d, $J = 6.4$ Hz), 0.99 (3H, d, $J = 7.4$ Hz), 1.59 (3H, s), 2.55 (1H, m), 2.68 (4H, m), 3.54 (1H, m), 5.27 (1H, s), 5.79 (1H d, $J = 6.4$ Hz); MS (*m*/*z*) 266 ($M^+ - (CH_2COH)_2$), 206, 162, 147.

Compound **15b** (from **4**): colorless crystal (56% yield), mp 149−150 °C, $[\alpha]^{20}_D$ −73.4 (*c* 1.07, $CHCl_3$); identified by comparison with compound **15b** from **11**.

Compound **16**: colorless crystal (39% yield), mp 107−109 °C; $[\alpha]^{20}_D$ +36.5 (*c* 0.60, $CHCl_3$); IR (KBr) ν_{max} 3440, 1734, 1724 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.95 (3H, d, $J = 6.3$ Hz), 1.17 (3H, d, $J = 7.1$ Hz), 2.41 (1H, m), 3.97 (1H, q, $J = 8.5$ Hz), 4.12 (1H, dt, $J = 8.5$, 2.0 Hz), 9.56 (1H, d, $J = 2.4$ Hz), 9.93 (1H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 13.13, 20.40, 27.00, 27.38, 30.50, 35.20, 46.96, 48.92, 56.73, 67.22, 88.53, 203.84, 205.54; MS (*m*/*z*) 225 ($M^+ + 1$), 207, 195, 137.

Compound **14c**: colorless crystal (47% yield), mp 86−88 °C (petroleum ether−ethyl acetate); $[\alpha]^{19}_D$ +99.1 (*c* 0.19, $CHCl_3$); IR (KBr) ν_{max} 3400, 1760, 1450, 1360, 1200, 1080, 1000 cm^{-1}; MS (*m*/*z*) 373 ($M^+ - 1$), 315, 165, 139, 138, 137, 96, 91, 55; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (6H, d, $J = 6.8$ Hz), 2.14 (3H, s), 3.90 (1H, q, $J = 7.9$ Hz), 4.26 (1H, t, $J = 7.9$ Hz), 4.44 (1H, d, $J = 12.1$ Hz), 4.79 (1H, d, $J = 3.9$ Hz), 4.93 (1H, d, $J = 12.1$ Hz), 6.32 (1H, s), 7.33 (5H, m). Anal. Calcd for $C_{22}H_{30}O_5$: C, 70.56; H, 8.08. Found: C, 70.51; H, 8.11.

Compound **15c**: colorless crystal (25% yield), mp 102−104 °C (petroleum ether−ethyl acetate) $[\alpha]^{19}_D$ −53.6 (*c* 0.19, $CHCl_3$); IR (KBr) ν_{max} 3500, 1660, 1060 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85 (3H, d, $J = 6.3$ Hz), 0.95 (3H, d, $J = 7.4$ Hz), 1.51 (3H, s), 2.46 (1H, m), 3.55 (1H, d, $J = 2.2$ Hz), 4.49 (1H, d, $J = 12.4$ Hz), 4.83 (2H, m), 5.26 (1H, s), 7.30 (5H, m). Anal. Calcd for $C_{22}H_{30}O_5$: C, 70.56; H, 8.08. Found: C, 71.30; H, 8.06.

Compound **14d**: colorless crystal (59% yield), mp 160−163 °C (petroleum ether−ethyl acetate) $[\alpha]^{19}_D$ +20.4 (*c* 0.74, $CHCl_3$); IR (KBr) ν_{max} 1750, 1720, 1592 cm^{-1}; ^{1}H NMR (100 MHz, $CDCl_3$) δ 0.91 (3H, d, $J = 7.3$ Hz), 0.92 (3H, d, $J = 4.2$ Hz), 2.08 (3H, s), 3.93 (1H, q, $J =$ 8.1 Hz), 4.30 (1H, t, $J = 7.5$ Hz), 6.08 (1H, d, $J = 9.5$ Hz), 6.30 (1H, s), 7.45 (3H, m), 8.08 (2H, m). Anal. Calcd for $C_{22}H_{28}O_6$: C, 68.02; H, 7.27. Found: C, 67.82; H, 7.30.

Compound **15d**: amorphous solid (25% yield), mp 97−98 °C (petroleum ether−ethyl acetate) $[\alpha]^{19}_D$ −56.9 (*c* 0.28, $CHCl_3$); IR (KBr) ν_{max} 3527, 1713, 1281, 1013, 818, 714 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (3H, d, $J = 6.3$ Hz), 1.04 (3H, d, $J = 7.1$ Hz), 1.55 (3H, s) 2.75 (1H, m), 3.52 (1H, m) 5.33 (1H, s), 5.98 (1H, d, $J = 6.9$ Hz), 7.43 (2H, t, $J = 7.7$ Hz), 7.56 (1H, t, $J = 7.4$ Hz), 8.08 (2H, d, $J = 7.1$ Hz). Anal. Calcd for $C_{22}H_{28}O_6$: C, 68.02; H, 7.27. Found: C, 67.74; H, 7.30.

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Supporting Information Available: Reproductions of MS for **7**, ^{1}H NMR for **5, 6, 7, 8, 9, 12, 13, 15b, 14a, 14c, 14d, 15a, 15c, 15d,** and **16,** NOESY and COSY spectra of compound **9**, ^{13}C NMR of compounds **7, 9,** and **16,** and the ESR spectrum (22 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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