New Insights into the Degradation of Qinghaosu (Artemisinin) Mediated by Non-Heme-Iron Chelates, and Their Relevance to the Antimalarial Mechanism

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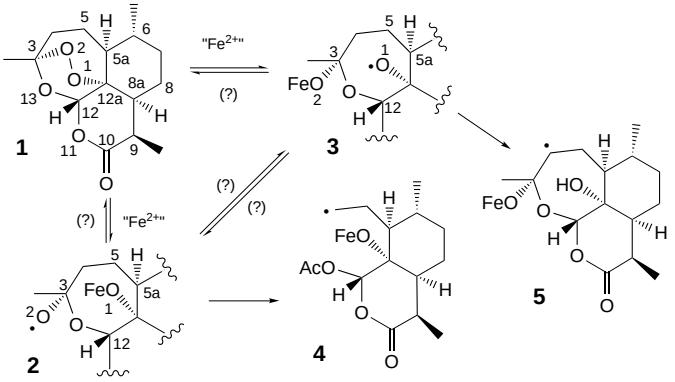
Probing the degradation of qinghaosu (**1**) induced by cysteine-iron chelates by varying the acidity of the reaction medium led to interesting insights into the cleavage of **1**, which help to elucidate the antimalarial action of the 1,2,4-trioxanes.

With the wide-spread distribution of multi-drug-resistant malaria cases and malaria's reappearance in Europe [1][2], the trioxane-type drugs exemplified by qinghaosu (QHS; **1**) have attracted much attention [3][4] because of their novel mode of action. Since the early 1990s, it is broadly believed that the intraparasitic free heme is responsible for the cleavage preceding the parasiticidal action, because of its unique occurrence and high reactivity. However, this notion has recently been challenged by the observation [5] that in the presence of cysteine, even traces of non-heme iron may cleave QHS rapidly.

Scheme 1. *The Two C-Centered Radicals* **4** *and* **5**, *Generated from QHS* (**1**). For clarity, some ligands at Fe^{III} are not shown.



As a model for the intracellular species containing Fe–S type redox centers, the cysteine-iron chelate has provided remarkable new insights into the parasiticidal action

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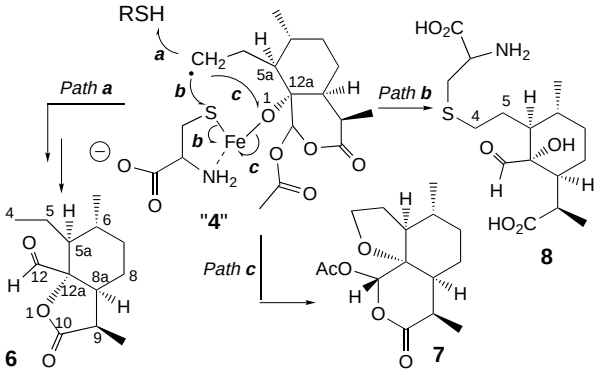
of QHS. For instance, the observed covalent bonding between the QHS-derived radical and the S-atom provides a possible (and practically the only) molecular-level explanation for the irreversible alkylation of non-heme proteins associated with the cleavage of QHS. Furthermore, the 'intramolecular' alkylation of the S-ligand strongly suggests that the two C-centered radicals **4** and **5** (see *Scheme 1*) contribute differently to the antimalarial action, with **4** being the most likely 'killing' species. In this contribution, we wish to communicate some further insights gained from the cysteine-iron model, which would not only help to elucidate the parasiticidal action but also facilitate the rational design of future generations of trioxane-based antimalarials.

As illustrated in *Schemes 2* and *3*, there exist several ways [6] for **4** and **5** to evolve further; some of the paths (*e.g.*, *Path* ***c*** in *Scheme 2*) could never damage the intracellular biomolecules, while others were suggested to be responsible for the lethal effects of QHS. It thus becomes imperative to know how the C-centered radicals choose among these possible pathways. To probe the elusive relations between the 'O-1' and 'O-2' routes (*i.e.*, *via* O(1) radical **3** and *via* O(2) radical **2**, resp.; see *Scheme 1*) as well as those between the H-abstractions and radical substitutions, we examined the effects of added base on the cleavage in the cysteine-iron model system.

Scheme 2. *Some Reaction Paths for the Primary Radical* **4**



Scheme 3. *Some Reaction Paths for the Secondary Radical* **5**

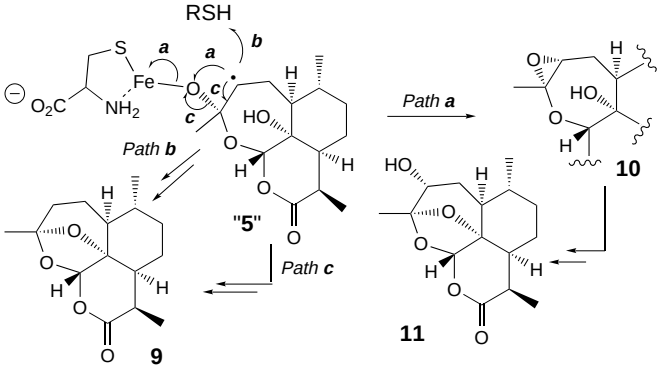


Table 1 shows that, with the increase in the initial amount of added base, more **6**, **8**, **9**, and cystine were formed. The formation of **7** and **11** (*via* **10**), however, was suppressed. Grouping these end products according to the initial radical **2** or **3** reveals that a larger excess of initial base resulted in an increase in reaction *via* the 'O-2' route since the total 'O-2' products, *i.e.*, **6**–**8**, all from the O(2) radical **2**, increased monotonically when the initial base amount was increased. Such a shift of the balance between two routes associated with the increase in the basicity of the reaction medium may reflect a faster elimination of **2** compared with **3**, providing supporting evidence for the postulate of interchangeable [7] (*cf.* [6] above) radical anions. This is based on the following considerations: Oxidation of the cysteine SH releases a proton by the transformation $RSH + Fe^{III} \rightarrow RS^{\bullet} + Fe^{II} + H^{+}$, which makes the reaction medium increasingly acidic. The formation of cystein-iron chelates (more powerful reducing agents than the free Fe^{II} ion), however, requires relatively high basicity. According to *Jameson et al.* [8], the predominant complex (in H_2O) at pH 2.7–3.9, 5.5–8, and 8.8–11.2 is $[FeL]^{+}$, [Fe(OH)L], and $[Fe(OH)L_2]^{2-}$, respectively, where L stands for cysteine. Therefore, more ligands are expected to attach to the Fe^{2+} ion at relatively higher pHs. Addition of a larger excess of base at the beginning could relatively better maintain the basicity of the reaction medium and consequently a higher concentration of the complex(es) (giving a more-intense blue-purple color) containing more ligands. Introduction of additional ligand(s) to a lower-order complex would inevitably increase the steric crowding around the Fe-core and thus make the reducing species bulkier. In another [9] context, we have shown that increasing the steric hindrance at C(9) of QHS (**1**) led to greatly reduced overall cleavage rates, with the 'O-2' route being practically shut off, which indicated that the attack at O(1) is more sensitive to steric crowding. Similarly, a bulkier reducing agent would be less likely to attack O(1) than O(2). Consequently, more **3** would have formed and reaction *via* the 'O-1' route would have increased (rather than decreased as observed), if **2** and **3** were not interchangeable.

It is even more interesting to note that the ratios for the H-abstraction to the radical substitution for each route (*i.e.* the 'O-1' or 'O-2' route) also varied with regular trends

Table 1. *The Effects of Excess Base on the Product Distribution*[a])

Excess base [mol-%]	0	5	10	20
Cystine [mmol]	0.12	0.15	0.19	0.20
6 [mmol]	0.073	0.078	0.085	0.111
7 [mmol]	0.339	0.224	0.219	0.155
8[b]) [mmol]	*ca.* 0	0.130	0.129	0.182
9 [mmol]	0.012	0.013	0.014	0.020
11 [mmol]	0.100	0.055	0.053	0.032
"O-1"[c])	0.112	0.068	0.067	0.052
"O-2"[d])	0.412	0.431	0.434	0.448

[a]) The reactions were carried out in parallel at 38–40° (bath) under N_2 in MeCN/H_2O 1:1 (*v*/*v*) (10 ml) starting with 0.50 mmol of QHS, 1.00 mmol of cysteine hydrochloride monohydrate, 5.0 ml of MeCN, 5.0 ml of doubly distilled deaerated H_2O, $5.0 \cdot 10^{-4}$ mmol of Fe^{II}/Fe^{III} ion, and the indicated amount of Et_3N. The amount (in mmol) of the products (except **8**) was estimated from the amount of isolated **6** and the molar ratios measured from the integrals for the acetal (or aldehyde) ^{1}H-signals in the ^{1}H-NMR spectra of the crude product mixtures (QHS was not detected). [b]) Estimated from the missing mmol. [c]) (**9** + **11**). [d]) (**6**–**8**).

(*Table 2*). For example, increasing the amount of base added led to an increase in the amount of **6** (*i.e.*, **6**/(**6**–**8**)) as well as that of **8** and a decrease in the weight of **7** in the total 'O-2' products. Similarly, the amount of **9** in the total 'O-1' products increased with increasing the initial excess base. These trends reflect the influence of the increased steric crowding at the Fe-core caused by higher basicity on the relative rates of the corresponding reaction paths. The H-abstraction from the cysteine SH by **4** (*Path* **a** in *Scheme 2*) would be least affected, because the primary radical is not so close to the Fe chelate. *Path* **c**, however, would be affected much more due to the repulsion between H–C(5a), H–C(9), *etc.* and the bulky chelate in the conformation required for the back-side radical attack. That is why the amount of **7** in the total 'O-2' products remarkably decreased. Formation of **8** does not require the chelate moiety to approach the lactone ring as in *Path* **c**. Therefore, the rate would not be lowered so much as in *Path* **c**. There may also be a statistically favorable factor: in the bulkier chelate, *i.e.*, $[Fe(OH)L_2]^{2-}$, there are two S-atoms available for the primary radical to attack. Secondary radicals are more sensitive to steric hindrance than primary ones. It is, hence, not surprising that the substitutions with **5** (*e.g.*, *Path* **a** in *Scheme 3*) would be significantly suppressed when the reducing agent became bulkier. Note that no 'intramolecular' attack at the S-atom by the secondary radical in **5** was observed.

Table 2. *The Effects of Excess Base on the Ratio Abstraction/Substitution*

Excess base [mol-%]	0	5	10	20
6/(**6**–**8**) [%]	18	18	20	25
7/(**6**–**8**) [%]	82	52	51	35
8/(**6**–**8**) [%]	~0	30	30	41
9/(**9**+**11**) [%]	11	19	21	38
11/(**9**+**11**) [%]	89	81	79	62

Through these simple experiments, some valuable insights into the parasiticidal action of QHS are obtained: *1*) Random intermolecular attacks by the C-centered radicals are not likely to be responsible for the parasiticidal activities of QHS (**1**), when one takes into account the quenching by the abundant intracellular glutathione (GSH, a good H-donor similar to cysteine in this work). Besides, there exists some built-in 'self-quenching' mechanism[2]) (*e.g.*, *Path* **c** in *Scheme 2*) that may compete even more keenly than GSH with the intermolecular damaging processes, especially when the cleavage is mediated by small reductants. *2*) When cleaved by intracellular Fe–S type redox centers containing multi-S-ligands (*i.e.*, bulkier chelates), the 'intramolecular' alkylation of the S-ligands can be quite effective; the inactivation of the redox center due to alkylation is thus very possible. *3*) Contrary to the broadly accepted notion, the secondary C-centered radical **5** probably does not contribute to the antimalarial activity at all, because of its inability to deliver 'intramolecular' attacks.

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[2]) Although these reactions are well-known, no one seems to have realized that they constitute radical self-quenching devices and may be responsible for the extraordinarily low toxicity of QHS to host cells.

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