Cleavage of Qinghaosu (Artemisinin) Induced by Non-Iron Transition-Metal Ions in the Presence of Excess Cysteine

by **Yikang Wu*** and **He-Hua Liu**

State Key Laboratory of Bio-organic and Natural Products Chemistry,
Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences,
354 Fenglin Road, Shanghai 200032, China
(e-mail: yikangwu@pub.sioc.ac.cn)

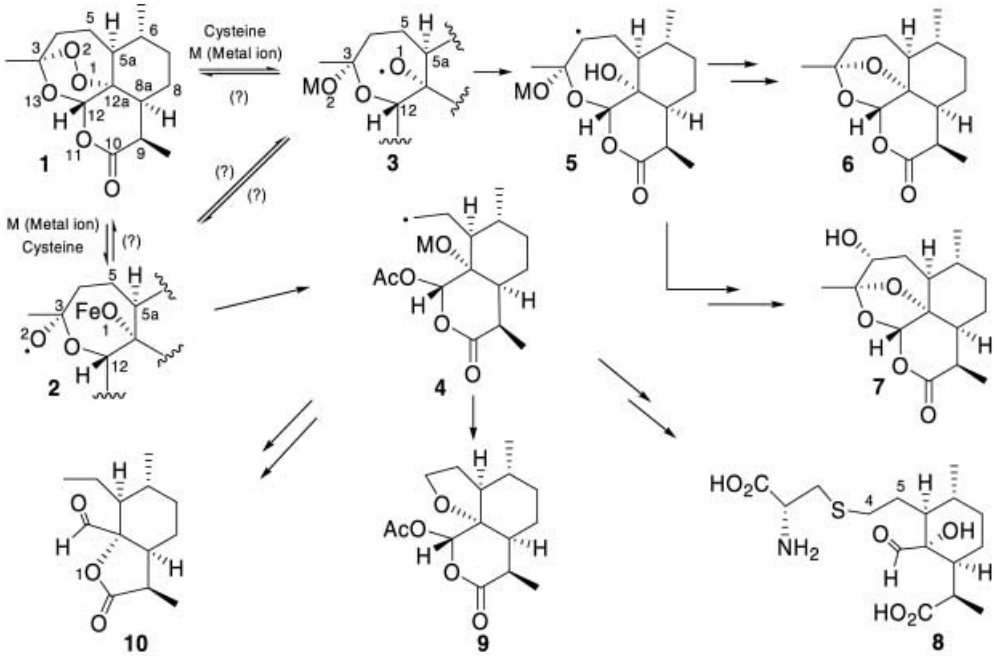
In the presence of an excess of cysteine, a catalytic amount of a non-iron transition-metal ion (Co^{2+}, Cu^{2+}, Ni^{2+}, Ti^{4+}, and Mn^{2+}) may also induce cleavage of qinghaosu (artemisinin; **1**) to give those end products previously reported for Fe^{2+}-mediated degradation.

The mode of antimalarial action of qinghaosu (QHS; **1**) type compounds (1,2,4-trioxanes) has been an enduring subject of research [1] since the late 1970s as a consequence of wide-spreading [2] of multidrug-resistant malaria cases. It was noticed in the 1970s that the peroxy bridge in QHS was essential for its antimalarial activity. However, the potential reason for the importance of the peroxy bridge remained entirely unclear until the early 1990s, when *Posner* and *Oh* [3] showed, through a simplified analog, that ferrous ion could induce cleavage of such compounds and, thus, generate radicals. The radical-generating ability was also demonstrated by many others [4] to be common to all 1,2,4-trioxanes, which, along with the well-established biological notion that radicals may cause damage to cell components, promoted the concept that QHS kills the malaria parasite through the formation of transient radicals.

As a prerequisite for *in vivo* generation of radicals from QHS, there must exist some one-electron reducing species inside the intra-erythrocytic malaria parasite. Until now, much attention [1a] has been attracted to the intraparasitic free heme and hemozoin (oxidized and polymerized heme) because they are present in substantial quantities. Alternatively, those intraparasitic redox enzymes/functional proteins might [5] also cleave QHS and, thus, could be potential targets of QHS. In that context, the central transition-metal ion(s) of the redox centers may not necessarily be limited to Fe^{2+}, because many known metalloenzymes [6] do have non-iron metals at their active centers. It is, therefore, very interesting to know whether transition-metal ions other than Fe^{2+} may also induce a similar cleavage of QHS and what the outcome would be.

The cleavage reactions were carried out at 35° for 12 h under N_2 MeCN/H_2O 1:1 (*v*/*v*) containing QHS (0.50 mmol), cysteine hydrochloride (1.00 mmol), Et_3N, and added metal ion ($4.0 \cdot 10^{-4}$ mmol). The main results are summarized in *Table 1*. Remarkable differences were indeed observed with different metal ions under otherwise identical conditions. One of the most obvious differences lay in the rates. With Co^{2+} or Cu^{2+} (added as $CoCl_2$ or $CuSO_4$, resp.), the reaction proceeded almost as fast as with Fe^{2+}. At the end of the 12 h reaction, there was no QHS (**1**) left. The reaction with Ti^{4+}

Scheme



(added as $TiCl_4$), however, was substantially slower (*ca.* 14% of **1** remained unchanged). The cleavage-induced by Ni^{2+} or Mn^{2+} (added as $NiSO_4$ or $MnSO_4$, resp.) was rather sluggish and incomplete, with a large amount of QHS recovered unchanged at the end of the 12-h reaction.

To obtain the redox profiles of the metal chelates under the cleavage reaction conditions, we also recorded cyclic voltammograms (CV) (*Fig. 1*) in the absence of QHS. By comparison with the cyclic voltammogram of cysteine (not shown) under the same conditions (except without adding any metal ion and QHS), it appeared that the curves in the left-hand region of each CV (+1.2 to +0.6 V) were associated with the redox of cysteine itself. The curves in the −0.4 to −1.0 V region should be related to the redox of the metal ions (chelates). The CVs for the Ni^{2+} and Mn^{2+} chelates were quite similar within this region, with no distinct peaks at all. This might explain the difficult degradation of QHS when these two metal ions were involved under the given conditions. The cyclic voltammogram of the Co^{2+} system was probably the closest one to the system with the Fe^{2+} ion. Therefore, the comparable rates of the Co^{2+} and Fe^{2+} systems seemed also to be reasonable. Cu^{2+} Chelate(s) did not show obvious peaks in the −0.4 to −1.0 V region. However, there were indeed clearly defined peaks in the lower trace in the +0.4 V to 0 V region (corresponding to the electron release from the chelates), which should be enough to reduce QHS (+0.87 V [8]). It seemed that the ease with which the cleavage reaction took place was not related to the redox potentials. Possible involvement of inner-sphere electron transfer from the transition-metal ion was previously proposed for a similar phenomenon [9].

Table 1. *Results of Cleavage* (*cf. Scheme*) *of QHS* (**1**) *Induced by Various Transition-Metal Ions* [a])

	Products [mmol]					
	1	**6**	**7**	**8**[b])	**9**	**10**
Fe^{2+} [c])	0	0.014	0.053	0.129	0.219	0.085
Co^{2+}	*ca.* 0	*ca.* 0	0.0388	0.122	0.260	0.0791
Ti^{4+}	0.070	*ca.* 0	0.0367	0.118	0.226	0.0498
Cu^{2+}	*ca.* 0	0.00628	0.0310	0.188	0.207	0.068
Ni^{2+}	0.333	*ca.* 0	*ca.* 0	0.105	0.020	0.0411
Mn^{2+} [d])	0.384	*ca.* 0	*ca.* 0	0.110	0.00443	0.0164

[a]) The reactions were carried out at 35° for 12 h under N_2 in MeCN/H_2O 1:1 (*v*/*v*; 10 ml) starting with 0.50 mmol of **1**, 1.00 mmol of cysteine hydrochloride, 150 µl of Et_3N, and $4.0 \cdot 10^{-4}$ mmol of the indicated metal ion (added as $CoCl_2$, $TiCl_4$, $CuSO_4$, $NiSO_4$, and $MnSO_4$, resp.). The amounts of the products (except **8**) were estimated from the molar ratios measured by means of the integrals of the acetal (or aldehyde) signals in the ^{1}H-NMR spectra of the crude product mixtures. [b]) Estimated from the missing amount in mmol (which agreed well with reversed-phase HPLC isolation results). [c]) Taken from [5b], for comparison. [d]) Mn^{2+} has previously been used to replace Fe^{2+} as the central ion of a simplified heme analog in cleavage of QHS, entirely for experimental convenience, see [7]. However, neither the product distribution for the cleavage reaction nor the potential biological significance of the replacement of Fe^{2+} with Mn^{2+} was given.

The differences between the metal ions were also observed in the product distribution. The general trends were similar to that observed with Fe^{2+}, with most products formed from **1** *via* the O(2) radical route (*via* **2** and **4**). The O(1) products (*via* **3** and **5**) were essentially **7** (accounting for only 7–8% of the total yields) when using Co^{2+} or Ti^{4+} as the central ion. Essentially no **6** was detected (except in the run with Cu^{2+}) (see *Scheme*).

The relative proportions of the O(2) products **8**–**10** also varied with the central ion (see *Table 2*). Formation of **8** was always significant. When Ni^{2+} and Mn^{2+} ions were involved, the highest proportion of **8** in the total O(2) products was recorded. Use of Cu^{2+} as the central ion also led to *ca.* 41% of **8** in the total O(2) products. These data indicated that the *S*-alkylation also took place readily when non-iron transition-metal ions were employed as the central ion in the chelates (see *Scheme*). By analogy, if the cleavage is induced by an *in vivo* S–M (M = transition-metal ion) type redox center rather than the simple cysteine chelates, a substantial portion of **4** would also undergo similar attacks and irreversibly inactivate the redox center.

Table 2. *The O(2) Product Ratios of Cleavage of QHS* (**1**) *Induced by Various Transition-Metal Ions* [a])

Added metal ions	Product ratios [%]		
	8/(**8**+**9**+**10**)	**9**/(**8**+**9**+**10**)	**10**/(**8**+**9**+**10**)
Fe^{2+}	29.8	50.6	19.6
Co^{2+}	26.6	56.4	17.2
Ti^{4+}	30.0	57.4	12.6
Cu^{2+}	40.6	44.7	14.7
Ni^{2+}	63.2	12.0	24.7
Mn^{2+}	84.1	3.4	12.5

[a]) For further information, see *Footnote a* in *Table 1*.

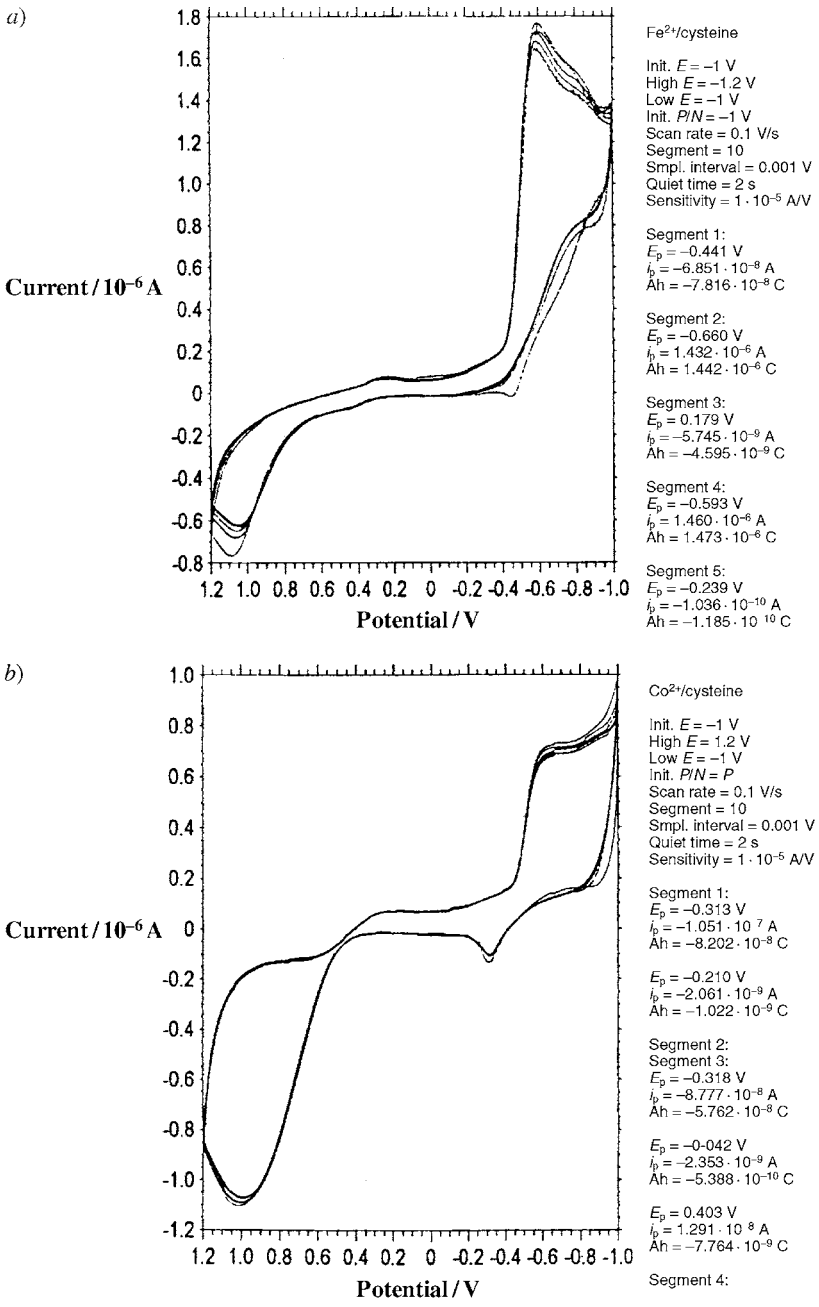
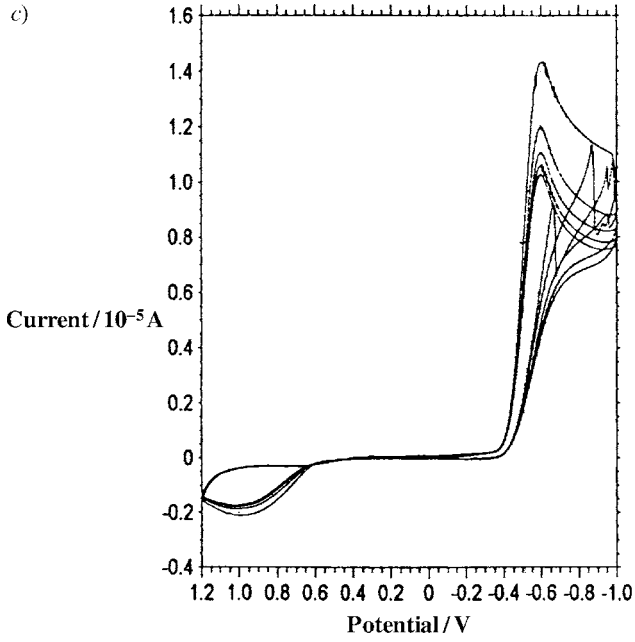


Figure. *Cyclic voltammograms of the solutions of cysteine hydrochloride* (1.00 mmol) *and* Et_3N (150 µl) *in* $MeCN/H_2O$ *1:1* (*v*/*v*) *containing* $4.0 \cdot 10^{-4}$ *mmol of* a) $Fe_2(SO_4)_3$, b) $CoCl_2$, c) $TiCl_4$, d) $CuSO_4$, e) $NiSO_4$, *and* f) $MnSO_4$

c)



Ti^{4+}/cysteine

Init. $E = -1$ V
High $E = 1.2$ V
Low $E = -1$ V
Init. $P/N = P$
Scan rate = 0.1 V/s
Segment = 10
Smpl. interval = 0.001 V
Quiet time = 2 s
Sensitivity = $1 \cdot 10^{-5}$ A/V

Segment 1:
$E_p = 0.217$ V
$i_p = -1.135 \cdot 10^{-8}$ A
Ah = $-7.076 \cdot 10^{-9}$ C

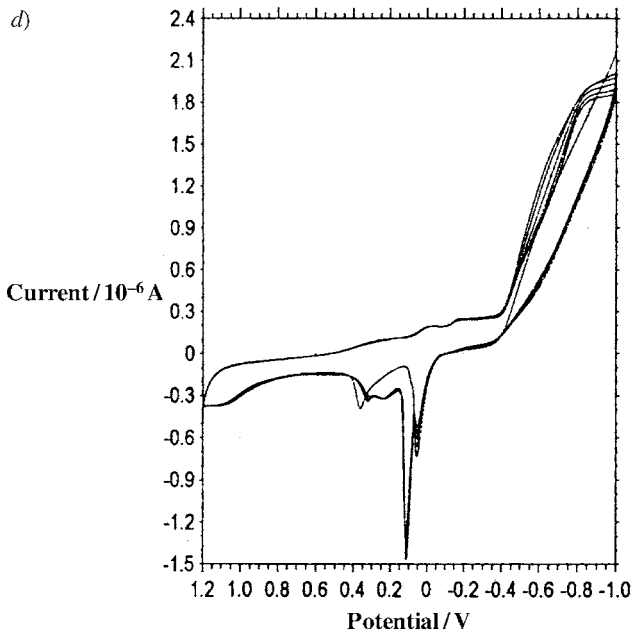
Segment 2:
$E_p = -0.622$ V
$i_p = 1.323 \cdot 10^{-5}$ A
Ah = $1.692 \cdot 10^{-5}$ C

Segment 3:
$E_p = 0.348$ V
$i_p = -1.401 \cdot 10^{-8}$ A
Ah = $-1.725 \cdot 10^{-8}$ C

Segment 4:
$E_p = -0.595$ V
$i_p = 1.115 \cdot 10^{-5}$ A
Ah = $1.128 \cdot 10^{-5}$ C

Segment 5:
$E_p = -0.234$ V
$i_p = -1.510 \cdot 10^{-8}$ A
Ah = $-1.723 \cdot 10^{-8}$ C

d)



Cu^{2}-/cysteine

Init. $E = -1$ V
High $E = 1.2$ V
Low $E = -1$ V
Init. $P/N = P$
Scan rate = 0.1 V/s
Segment = 10
Smpl. interval = 0.001 V
Quiet time = 2 s
Sensitivity = $1 \cdot 10^{-6}$ A/V

Segment 1:
$E_p = 0.057$ V
$i_p = -6.947 \cdot 10^{-7}$ A
Ah = $-3.267 \cdot 10^{-7}$ C

$E_p = -0.363$ V
$i_p = 2.983 \cdot 10^{-7}$ A
Ah = $-2.385 \cdot 10^{-7}$ C

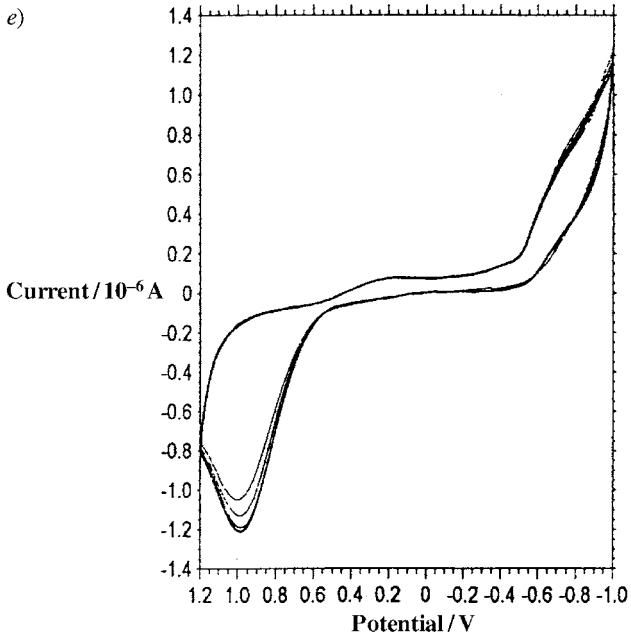
Segment 2:
Segment 3:
$E_p = 0.114$ V
$i_p = -1.400 \cdot 10^{-6}$ A
Ah = $-7.623 \cdot 10^{-7}$ C

Segment 4:
Segment 5:
$E_p = 0.115$ V
$i_p = -1.332 \cdot 10^{-6}$ A
Ah = $-7.216 \cdot 10^{-7}$ C

Segment 6:
$E_p = -0.032$ V
$i_p = -5.946 \cdot 10^{-8}$ A
Ah = $4.887 \cdot 10^{-8}$ C

Figure (cont.)

e)



Ni^{2+}/cysteine

Init. $E = -1$ V
High $E = 1.2$ V
Low $E = -1$ V
Init. $P/N = P$
Scan rate = 0.1 V/s
Segment = 10
Smpl. interval = 0.001 V
Quiet time = 2 s
Sensitivity = $1 \cdot 10^{-5}$ A/V

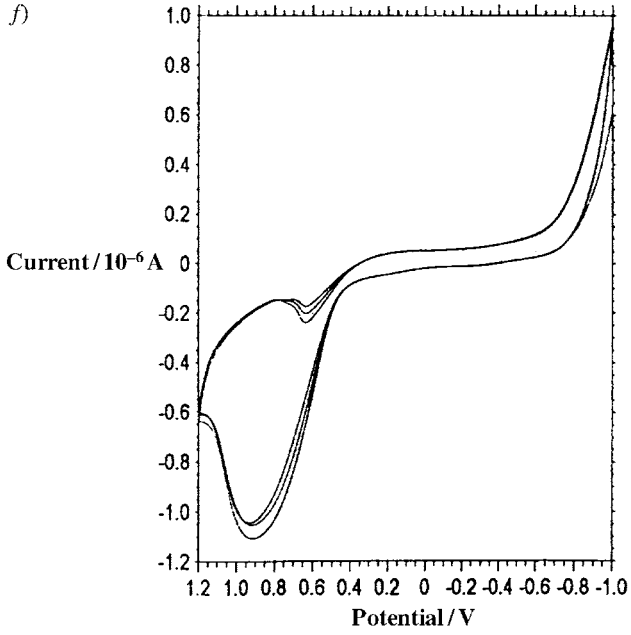
Segment 1:
Segment 2:
Segment 3:
$E_p = 0.120$ V
$i_p = -1.452 \cdot 10^{-8}$ A
Ah = $-1.984 \cdot 10^{-8}$ C

Segment 4:
Segment 5:
$E_p = 0.279$ V
$i_p = -1.988 \cdot 10^{-8}$ A
Ah = $-2.910 \cdot 10^{-8}$ C

Segment 6:
Segment 7:
$E_p = 0.046$ V
$i_p = -1.099 \cdot 10^{-8}$ A
Ah = $-9.949 \cdot 10^{-9}$ C

Segment 8:
Segment 9:
$E_p = 0.234$ V
$i_p = -1.675 \cdot 10^{-8}$ A
Ah = $-2.216 \cdot 10^{-8}$ C

f)



Mn^{2+}/cysteine

Init. $E = -1$ V
High $E = 1.2$ V
Low $E = -1$ V
Init. $P/N = P$
Scan rate = 0.1 V/s
Segment = 10
Smpl. interval = 0.001 V
Quiet time = 2 s
Sensitivity = $1 \cdot 10^{-6}$ A/V

Segment 1:
Segment 2:
Segment 3:
Segment 4:
Segment 5:
Segment 6:
Segment 7:
Segment 8:
Segment 9:
Segment 10:

Figure (cont.)

Compared with the formation of **8** and **9**, hydrogen abstraction (**10**) appeared to be much less favorable in most cases. This is consistent with the fact that in the absence of an excellent hydrogen donor (*e.g.*, SH of cysteine), the hydrogen abstraction has never been observed.

Hypotheses play a very important role in the study of the antimalarial mechanism of QHS. The objectivity of a hypothesis, therefore, inevitably has a strong influence on the investigation. By putting the problem in a broader perspective, we may avoid omitting critical factors in the hypothesis in the first place and, hence, help us focus future endeavors on the most-promising directions.

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