Chemical and electro-chemical reduction of qinghaosu (artemisinin)

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Received (in Cambridge, UK) 30th August 2000, Accepted 27th October 2000
First published as an Advance Article on the web 30th November 2000

1,2,4-Trioxane is the essential segment of the new antimalarial agent qinghaosu **1**, and hence its reduction is an important plausible process related to the bio-activity mode. An overview of its reduction and a careful examination of some reducing systems are presented herewith. Electrochemical reduction is a two-electron reduction, which is confirmed by the isolation of product deoxyqinghaosu. However, in the presence of a catalytic amount of $Fe^{II/III}$ electrochemical reduction yields mainly the single-electron-reduction products, which are identified with the products from reduction of qinghaosu with one equivalent ferrous sulfate or a catalytical amount of $Fe^{II/III}$ and excess of other reducing agents, such as ascorbic acid, or cysteine. These results mean that qinghaosu is reduced by ferrous ion and the resulting ferric ion is then reduced on the electrode to regenerate ferrous ion. In addition, qinghaosu could be reduced to deoxyqinghao by iodide, but not by bromide, and also could be reduced by the ascorbic acid–$CuSO_4$ system to give free-radical reaction products.

Qinghaosu[1] (artemisinin, **1**, Fig. 1) isolated from Chinese herb qinghao (*Artemisia annua* L.) belongs to a new generation of antimalarial agents. Its derivatives, such as artemether[2] (**2**) and artesunnate[3] (**3**), have been shown to be powerful weapons to fight the malaria parasite, especially the multidrug-resistant strains. Early structure–activity studies[2] have already revealed that the characteristic peroxy functionality in these compounds is essential for their antimalarial activity. Therefore, exploring the reduction of such peroxides could be an approach to uncovering the antimalarial mechanism of this type of compound. Since the period of structure determination in the 1970s a series of studies on the chemical reduction have been performed and then, in the early 1990s, the reduction of qinghaosu and its derivatives or analogues with ferrous ion, a process possibly occurring in malaria-infected red cells, attracted attention from several laboratories. More recently some reports about electrochemical reduction of qinghaosu have also appeared in the literature, but no attempt was made to clarify the details of this chemical process. Herewith we would like to give an overview on all these reductions, and present some new results, especially on the electrochemical reduction and a comparison between both electrochemical and chemical reduction.

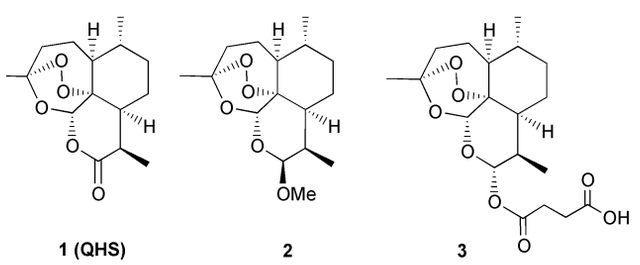


Fig. 1

Results and discussion

Hydrogenation of qinghaosu in the presence of palladium on carbon yields deoxyqinghaosu[1] **4**, an inactive compound containing one fewer oxygen atom (Scheme 1). This reduction is proposed to proceed *via* a dihydroxy intermediate **5**, which may be obtained immediately after work-up and then cyclized to compound **4** on standing. Reduction[4] with zinc–acetic acid also yielded this compound. Hydride reducing agents, on the other hand, reduce preferentially the lactone in qinghaosu. Thus, reduction of qinghaosu with sodium borohydride[1] in methanol at 0 °C gives dihydroqinhaosu **6**, and reduction with sodium borohydride–boron trifluoride–diethyl ether[5] gives deoxoqinghaosu **7**. The more powerful reducing agent lithium aluminium hydride may reduce the lactone, peroxide and then the unmasked hemiketal and acetal to give the exhaustively reduced product **8** and several partial reduction products.[6,7] Recently we found that qinghaosu can also be reduced to deoxyqinghaosu by iodide, a classic reagent for the qualitative analysis of peroxide, but not by bromide. Triphenylphosphine has been used as the reagent for the semiquantitative analysis of peroxide during the structure determination of qinghaosu.[1] Recent re-examination of this reduction showed that this was a quite complicated reaction, but deoxyqinghaosu could still be isolated among the product mixture in 23% yield.

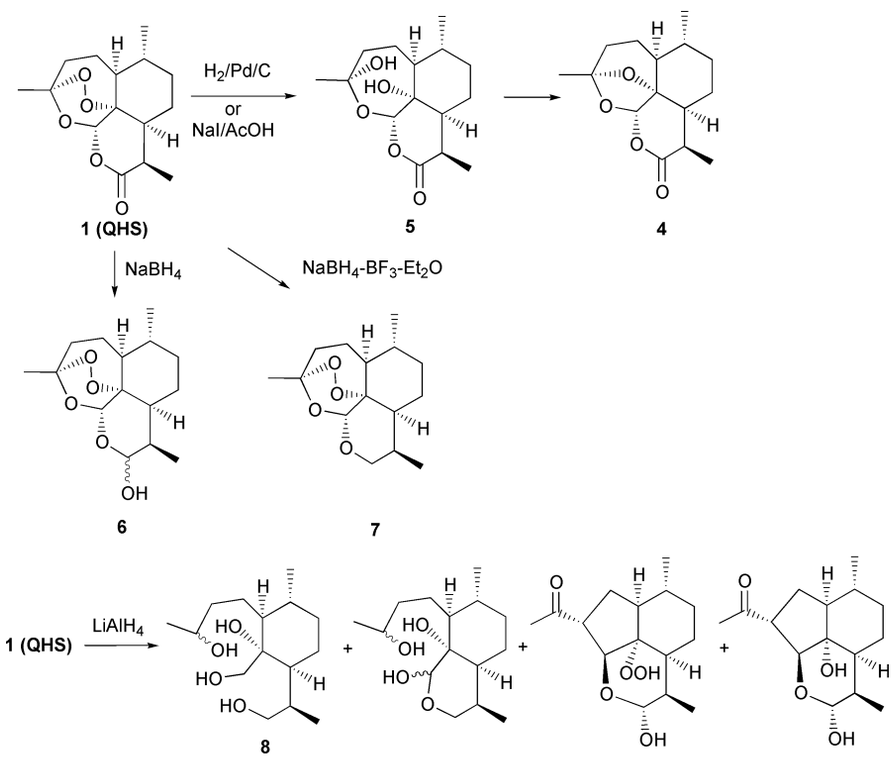
Considering the high concentration of iron in the parasitized erythrocytes (red blood cells), several laboratories have engaged in a study of the reductive degradation of qinghaosu and its derivatives with ferrous ion since the early 1990s.[8] It was found that qinghaosu could be degraded mainly to tetrahydrofuran compound **9** and 3-hydroxydeoxyqinghaosu **10**. A mechanism *via* a primary and a secondary carbon-centred free radical was proposed through careful isolation and identification of small amounts of other reaction products, **11** and **12**. The intermediacy of both carbon-centred free radicals were also confirmed by an EPR experiment.[8c,9] Thereafter we also found that, in the presence of cysteine, qinghaosu could be degraded by even catalytical amounts of ferrous or ferric ion.[10] The isolation of compound **13** and adduct **14**, formed from the primary carbon-centred radical by abstraction of a proton or coupling of cysteine and then by a rearrangement, respectively, confirmed convincingly the proposed free-radical mechanism (Scheme 2).

DOI: 10.1039/b007056o

Table 1 Chemical and electrochemical reduction of QHS[a]

Run	1 (mmol)	Conversion (%)	Total yield of products and their (%) proportions						
			Total yield	**4**	**9**	**10**	**12**	**13**	**15**
1-1	1	27	26	100	[b]	[b]	[b]	[b]	[b]
1-2	1	>98		23					
2-1-1	1	90	90	1.2	41.3	9.8	31.5	1.3	14.9
2-1-2	1	>98	98	3.8	26.6	10.3	45.3	[b]	13.8
2-2-1[c]	1	20.5	20	2.4	7.1	16.6	26.1	0.5	47.4
2-2-2	1	35.5	35.5	16.7	19.7	13.9	11.9	[b]	37.5
2-3	2.5	98	98	0.8	63.3	30.4	3.5	[b]	2.2
2-4	2.5	67	67	12.3	20.7	36.8	26.7	[b]	4.2
3-1	1	7	5.6	100	[b]	[b]	[b]	[b]	[b]
3-2	1	98	98	6	59	7	6	[b]	22
3-3[c]	1	15	≈10	0.5	3.6	29.3	19.5	[b]	47.2

[a] **1-1**, Reduction with NaI; **1–2**, Reduction with Ph_3P; **2-1-1**, Reduction with Vc–$FeCl_3$; **2-1-2**, Reduction with Vc–0.1 equiv. $FeCl_3$; **2-2-1**, Reduction with Vc–$CuSO_4 \cdot 5H_2O$; **2-2-2**, Reduction with Vc–0.1 equiv. $CuSO_4 \cdot 5H_2O$; **2-3**, Reduction with Vc–EDTA–Fe^{III}; **2-4**, Reduction with Vc–haemin. **3-1**, Electrochemical reduction; **3-2**, Electrochemical reduction in the presence of EDTA–Fe^{III}; **3-3**, Electrochemical reduction in the presence of haemin. [b] Could not be separated. [c] The ratio of products was determined by HPLC.

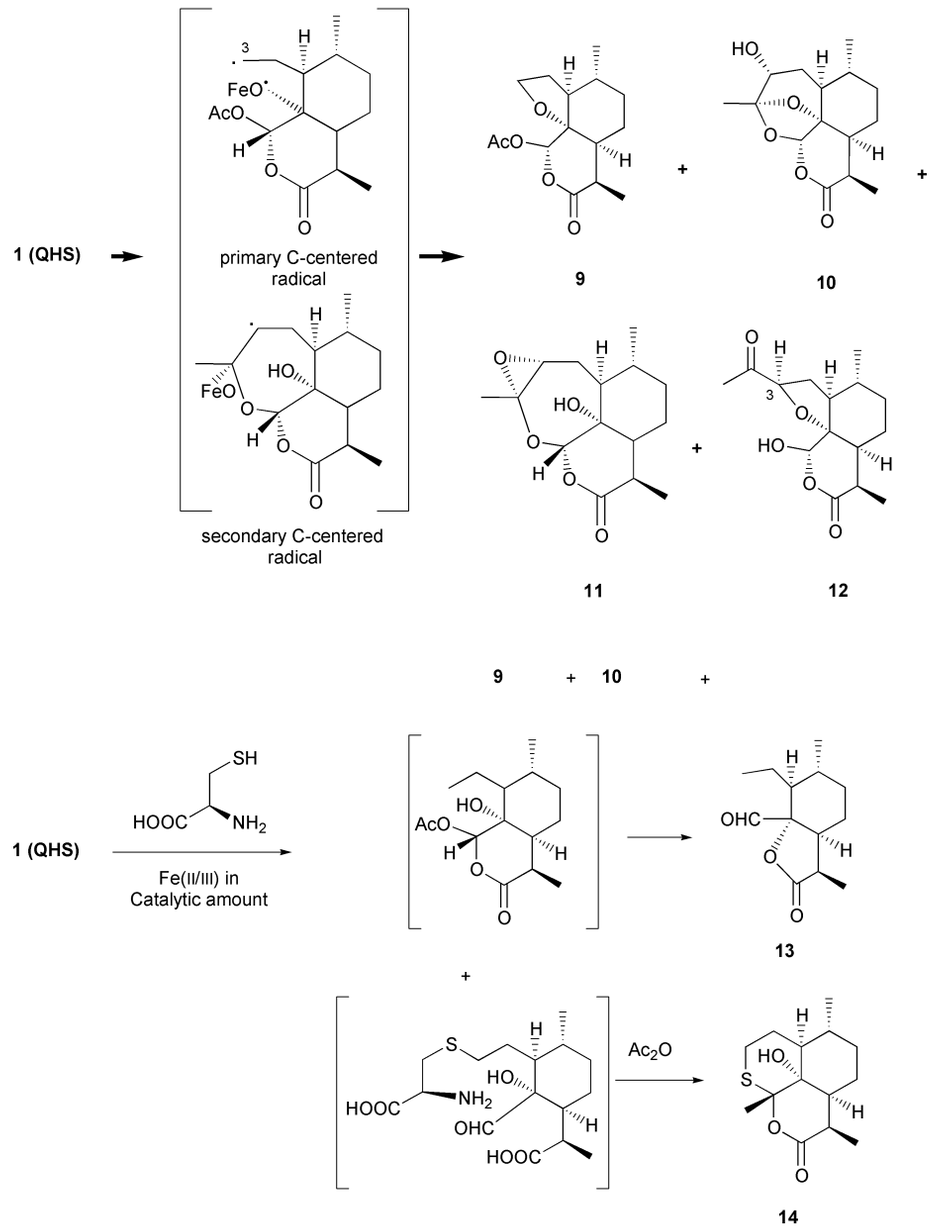


Scheme 1

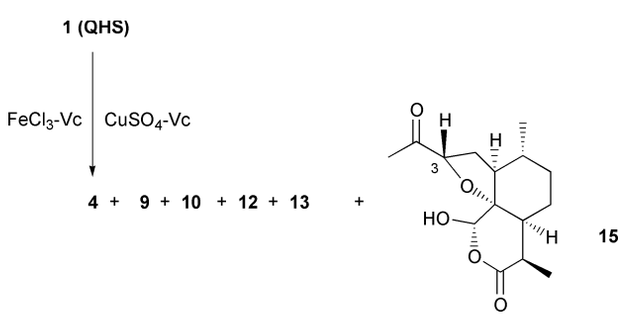
Along with a study of the chemical reaction mechanism there is a very interesting observation that should be mentioned, namely that the reactivity of these 1,2,4-trioxanes towards ferrous ion is parallel to their *in vivo* antimalarial activity.[11]

In continuing our exploration of the Fe-catalyzed degradation of qinghaosu and comparing it with the electrochemical reduction (*vide infra*), several other reduction systems were recently examined. At first the Fe-catalyzed degradation was performed with ferric chloride in the presence of excess of ascorbic acid (Vc). This reaction proceeded smoothly as in the reaction with stoicheiometrical amount of ferrous sulfate, and compounds **9**, **10**, and **12** were obtained. Besides these compounds traces of compound **13** and deoxyqinghaosu **4**, as well as compound **15**, a new C-3 epimer of compound **12**, were also isolated and identified (Scheme 3). We have predicted the formation of compound **15** in the reaction of qinghaosu and $FeSO_4$, but under that reaction condition we failed to isolate it.[8c] In a further experiment it was shown that ascorbic acid–Fe^{3+}–EDTA and ascorbic acid–haemin systems also reduced qinghaosu to give predominantly these free-radical reduction products. The difference was that the reduction was much more rapid in the presence of the Fe^{3+}–EDTA complex than that in the presence of haemin. In another experiment cupric sulfate, instead of ferric chloride, was used for the reductive degradation. The Cu-catalyzed degradation took place to give all the free-radical reaction products as in the case of Fe-catalyzed degradation, but it was not so efficient as the latter. All these experiments also showed that in the presence of other reducing agents such as ascorbic acid a catalytical amount of $Fe^{II/III}$ or $Cu^{I/II}$ was enough for the free-radical degradation of qinghaosu.

Since 1992 a series of studies on the electrochemical reduction[12] of qinghaosu and its derivatives on the electrode in the presence of a ferric salt, ferric complex or in the absence of any other reducing agents have been carried out. The reduction potential of qinghaosu in 20% aq. acetonitrile with Ag/AgCl as reference electrode was found to be −1.08 V and an irreversible two-electron reducing process was detected. In the presence of haemin or EDTA–Fe^{3+} [12c] the reduction potential of qinghaosu fell to −0.48 V or −0.49 V, respectively. However, no attempts



Scheme 2

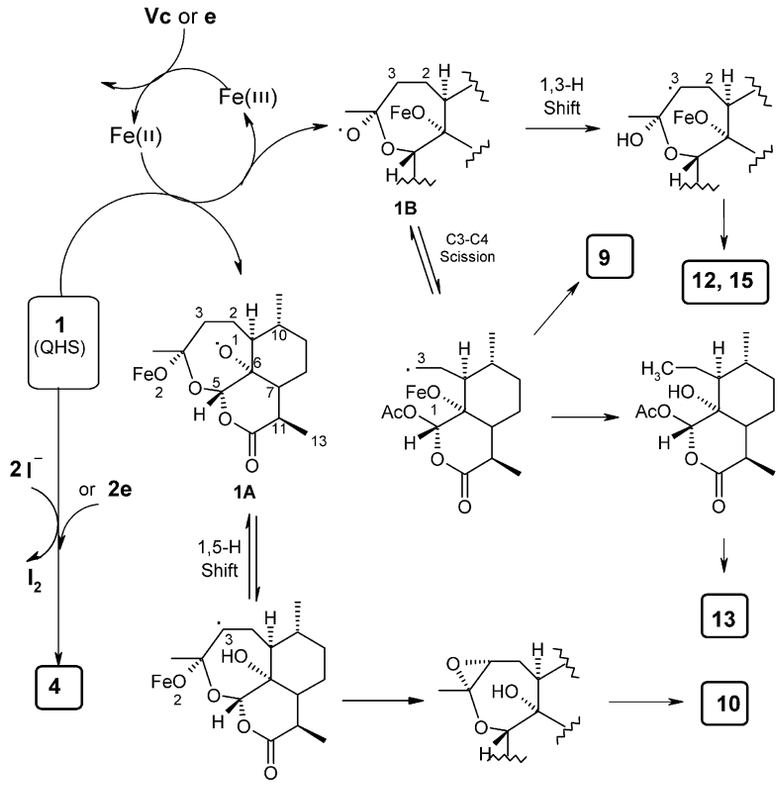


Scheme 3

have been made to isolate and characterize the products of the electrochemical reduction to date.

To explore the electrochemical reduction and to compare it with chemical reduction, reduction of qinghaosu was conducted under different electrochemical reaction conditions in our laboratory. Thus electrochemical reduction of qinghaosu was performed in aq. acetonitrile–phosphate buffer. After electrolyzing at 2 V for 4 h, only deoxyqinghaosu **4** was obtained in 5% yield besides recovered qinghaosu. However, in the presence of EDTA–Fe^{3+} qinghaosu was electrochemically reduced rapidly to give predominantly compounds **9**, **10**, **12** and **15**, the same as those obtained from chemical reduction of qinghaosu by Vc–$FeCl_3$. In the presence of haemin, qinghaosu was reduced slowly and similar products, albeit in different proportions, were detected. The new results are summarized in Table 1.

The results listed in Table 1 show that the electrochemical reduction of QHS in the absence of any additives proceeded very slowly and only gave a two-electron-reduction product. This situation is similar to the reduction with sodium iodide in THF–acetic acid. In the presence of a catalytical amount of a ferric compound the similar electrochemical reduction of QHS proceeded much more quickly and gave predominantly the single-electron reduction products, which means that in these cases QHS is reduced by ferrous ion and the resultant ferric ion is then reduced on the electrode. Similar results were also obtained when ascorbic acid was used as the reducing agent for the ferric ion instead of electricity. It is not clear yet whether the single electron reduction takes place in solution or on the surface of the electrode. However, the reduction of QHS in the presence of EDTA–Fe^{III} is faster than that in the presence of haemin no matter whether electrode or ascorbic acid is used to generate and regenerate Fe^{II} species. It suggests that whether



Scheme 4

regeneration of Fe^{II} species occurs on electrode or in solution is not a key point. Referring to our proposed unified mechanism framework for the Fe^{II}-induced cleavage of QHS, all the reductions of QHS described above could be summarized as in Scheme 4.

Experimental

Chemicals and general methods

A standard solution of haemin (from Shanghai Institute of Biochemistry, Chinese Academy of Sciences) was prepared immediately prior to each experiment by first dissolving haemin in several drops of 0.1 M NaOH, followed by diluting this solution with phosphate buffer (25 mM, pH 6.864) and finally bringing it to pH 7.0 with 0.1 M HCl. The 0.05 mol L^{-1} EDTA–Fe^{III} was prepared by mixing 0.1 mol L^{-1} EDTA with 0.1 mol L^{-1} $FeCl_3$. In the electroreduction experiments, a three-electrode cell system with a glass carbon or platinum wire as working electrode, a platinum wire auxiliary electrode, and a KCl-saturated Ag/AgCl reference electrode was employed. HPLC: ALLMA C_{18} (4.6 × 250 mm, id 5 μm), Mobile phase: CH_3OH–water 80 : 20 (v/v), flow rate 1.0 mL min^{-1}. Detection wavelength: 210 nm.

Mps were measured on a ZMD-2 melting apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 MC autopol polarimeter and are given in units of 10^{-1} deg cm^2 g^{-1}. IR spectra were obtained on a Perkin-Elmer 983 spectrophotometer. ^1H NMR spectra were taken on an AMX-300 or Inova-600 instrument. Mass spectra were measured on an HP 5989A spectrometer.

1-1. Reduction with sodium iodide

A mixture of 282 mg (1 mmol) of QHS, 375 mg (2.5 mmol) of sodium iodide and 0.5 mL of acetic acid in 10 mL of THF was stirred under nitrogen at room temperature overnight. After removal of most of the THF under reduced pressure, the reaction mixture was diluted with EtOAc, washed successively with saturated aq. Na_2SO_3, water and brine, and then dried over Na_2SO_4. Removal of solvent gave a residue, which was subjected to silica gel column chromatography to afford 70 mg (26%) of deoxyqinghaosu **4** and 205 mg of recovered QHS.

1-2. Reduction with triphenylphosphine

A mixture of 282 mg (1 mmol) of QHS and 524 mg (2.0 mmol) of triphenylphosphine in 10 mL of toluene was refluxed under nitrogen for 6 h. To the cold reaction mixture was added 0.5 mL of acetic acid and the stirring was continued for 2 h. TLC of the reaction mixture showed a very complicated pattern, and only deoxyqinghaosu **4** was detectable. After removal of toluene under reduced pressure the obtained residue was diluted with ethyl acetate, washed with water, and dried over $MgSO_4$. Removal of the solvent and chromatography on silica gel gave 60 mg (22.6%) of deoxyqinghaosu **4**.

2-1-1. Reduction with Vc-$FeCl_3$

To 282 mg (1 mmol) of QHS in 100 mL of 1 : 1 aq. acetonitrile–water were added 1 mmol of ascorbic acid and 1 mmol of $FeCl_3$. The mixture was stirred at room temperature under a nitrogen atmosphere for 4 h. Acetonitrile was removed under reduced pressure (rotary evaporator), and the residue was extracted with ethyl acetate (2 × 10 mL). The combined extracts were washed with water and dried over $MgSO_4$. After evaporation of the solvent, the residue was chromatographed on a silica gel column to give 28 mg of recovered QHS, 3 mg of compound **4** (1.2%), 105 mg of compound **9** (41.3%), 25 mg of compound **10** (9.8%), 80 mg of compound **12** (31.5%), 3 mg of compound **13**[8] (1.3%), and 38 mg of a new compound **15** (14.9%) in 90% total yield (weight-%).

Compound **15**: mp 125–127 °C; $[a]_D^{20}$ −19.9 (*c* 0.8, $CHCl_3$); IR (KBr) ν_{max} 3509, 2981, 2877, 1746, 1706 cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ 0.99 (3H, d, *J* 6.1 Hz), 1.19 (3H, d, *J* 7.4 Hz), 2.32 (3H, s), 3.33 (1H, m), 4.44 (1H, d, *J* 7.9 Hz), 5.52 (1H, s); MS (*m*/*z*) 283 (M + 1), 265, 207 ($C_{15}H_{22}O_5$ requires C: 63.81, H: 7.85. Found: C: 63.69, H: 7.72%).

All other known compounds were identified by ^{1}H NMR (600 MHz) spectroscopy, MS, TLC, and HPLC.

2-1-2. Reduction with Vc–0.1 equiv. $FeCl_3$

The procedure was similar to **2-1-1**, but 2 mmol of ascorbic acid and 0.1 mmol of $FeCl_3$ were used. Work-up gave no recovered QHS, 10 mg of compound **4** (3.8%), 75 mg of compound **9** (26.6%), 29 mg of compound **10** (10.3%), 127 mg of compound **12** (45.3%), and 39 mg of compound **15** (13.8%) in 98% total yield.

2-2-1. Reduction with Vc–$CuSO_4 \cdot 5H_2O$

The reduction was performed as **2-1-1** except for using Vc–$CuSO_4 \cdot 5H_2O$ instead of $FeCl_3$. 224 mg of QHS was recovered and the products were obtained in 20% total yield. The proportions of these products were determined by RP-HPLC: **4** (2.4%), **9** (7.1%), **10** (16.6%), **12** (26.1%), **13** (0.5%), and **15** (47.4%).

2-2-2. Reduction with Vc–0.1 equiv. $CuSO_4 \cdot 5H_2O$

The reduction was performed as **2-2-1** except for using 2 mmol of Vc and 0.1 mmol of $CuSO_4 \cdot 5H_2O$ instead of 1 mmol of both reagents. 182 mg of QHS was recovered and the products were obtained in 35.5% total yield. The proportions of these products were determined by column chromatography: 16 mg of compound **4** (16.7%), 20 mg of compound **9** (19.7%), 14 mg of compound **10** (13.9%), 12 mg of compound **12** (11.9%), and 38 mg of compound **15** (37.5%).

2-3. Reduction with Vc–EDTA–Fe^{III}

To 705 mg (2.5 mmol) of QHS in 100 mL of 1:1 aq. acetonitrile–phosphate buffer (25 mM, pH 6.864) were added 5 mmol of ascorbic acid and 0.25 mmol of EDTA–Fe^{III}. The mixture was stirred at 25 °C under a nitrogen atmosphere for 4 h. Acetonitrile was removed under reduced pressure (rotary evaporator). The residue was extracted with ethyl acetate (3 × 10 mL). The combined extracts were washed with water and dried over $MgSO_4$. After evaporation of the solvent, the residue was chromatographed on silica gel to give 11 mg of recovered QHS and 5 mg of compound **4** (0.8%), 437 mg of compound **9** (63.3%), 210 mg of compound **10** (30.4%), 24 mg of compound **12** (3.5%), and 15 mg of compound **15** (2.2%) in 98% total yield (weight-%). These products were identified with authoritative samples by ^{1}H NMR (300 or 600 MHz) sprctroscopy MS, TLC, HPLC.

2-4. Reduction with Vc–haemin

The reduction was performed as **2-3** except for using Vc–haemin instead of Vc–EDTA–Fe^{III}. Together with 230 mg of recovered QHS, 55 mg of compound **4** (12.3%), 98 mg of compound **9** (20.7%), 174 mg of compound **10** (36.8%), 126 mg of compound **12** (26.7%), and 20 mg of compound **15** (4.2%) were obtained by column chromatography on silica gel in 67% total yield (weight-%). These products were identified with authoritative samples by ^{1}H NMR (300 or 600 MHz) spectroscopy, MS, TLC, HPLC.

3. General procedure of electrochemical reduction

To 282 mg (1 mmol) of QHS in 100 mL of 1:1 aq. acetonitrile–phosphate buffer (25 mM, pH = 6.864) was added haemin (0.1 mmol) or 0.2 mL of 50 mM EDTA–Fe^{III} (0.1 mmol), or nothing for the control run. The mixtures were stirred at room temperature under a nitrogen atmosphere and electrolyzed at 2.0 V for 4 h. Acetonitrile was removed under reduced pressure (rotary evaporator). The residue was extracted with ethyl acetate (2 × 10 mL). The combined extracts were washed with water and dried over anhydrous $MgSO_4$. After removal of the solvent, the residue was chromatographed on silica gel or analyzed by HPLC.

3-1. Electrochemical reduction of QHS without additive. Besides 262 mg of unreduced QHS, 15 mg of deoxyqinghaosu (**4**, yield 5.6%) was obtained, which was identified by ^{1}H NMR (300 or 600 MHz) spectroscopy, MS, TLC, and HPLC.

3-2. Electrochemical reduction of QHS in the presence of EDTA–Fe^{III}. <5 mg of unreduced QHS (<2%), 16 mg of compound **4** (6%), 163 mg of compound **9** (59%), 19 mg of compound **10** (7%), 17 mg of compound **12** (6%), and 61 mg of compound **15** (22%) were separated by silica gel column chromatography in 98% total yield (weight-%). Compounds **4**, **9**, **10** and **12** were identified with authoritative samples by ^{1}H NMR (300 or 600 MHz) spectroscopy, MS, TLC, HPLC.

3-3. Electrochemical reduction of QHS in the presence of haemin. 240 mg of QHS was recovered. The proportions of these obtained products were determined by RP-HPLC: **4** (0.5%), **9** (3.6%), **10** (29.3%), **12** (19.5%), and **15** (47.2%).

Acknowledgements

This work was supported by the National Natural Science Foundation of China (29572075, 09561423, 29832020, 39870899), the Chinese Academy of Sciences (KJ951-A1-504), and the Ministry of Science and Technology of China (970211006-6). We thank Dr Yikang Wu for help improving the manuscript.

References

1 J.-M. Liu, M.-Y. Ni, J.-F. Fan, Y.-Y. Tu, Z.-H. Wu, Y.-L. Wu and W.-S. Chou, *Huaxue Xuebao*, 1979, **37**, 129 (*Chem. Abstr.*, 1980, **92**, 94594w).
2 Y. Li, P.-L. Yu, Y.-X. Chen, L.-Q. Li, Y.-Z. Gai, D.-S. Wang and Y.-P. Zheng, *Yaoxue Xuebao*, 1981, **16**, 429 (*Chem. Abstr.*, 1983, **97**, 92245n).
3 X. Liu, *Yaoxue Tongbao*, 1980, **15**, 183.
4 Y. Li, P.-L. Yu, Y.-X. Chen, J.-L. Zhang and Y.-L. Wu, *Kexue Tongbao (Engl.)*, 1986, **31**, 1038 (*Chem. Abstr.*, 1987, **107**, 23154v).
5 M. Jung, X. Li, D. A. Bostos, H. N. ElSohly and J. D. McChesney, *Tetrahedron Lett.*, 1989, **30**, 973.
6 Y.-L. Wu and J.-L. Zhang, *Youji Huaxue*, 1986, **6**, 153 (*Chem. Abstr.*, 1986, **105**, 191426n).
7 L.-K. Sy, S.-M. Hui, K.-K. Cheung and G.-D. Brown, *Tetrahedron*, 1997, **53**, 7493.
8 (*a*) J. N. Cumming, P. Polypradith and G. H. Posner, *Adv. Pharmacology*, 1997, **37**, 253; (*b*) G. H. Posner, S. B. Park, L. Gonzalez, D.-S. Wang, J. N. Cumming, D. Klinedinst, T. A. Shapiro and M. D. Balci, *J. Am. Chem. Soc.*, 1996, **118**, 3537; (*c*) W.-M. Wu, Y.-K. Wu, Y.-L. Wu, Z.-J. Yao, C.-M. Zhou, Y. Li and F. Shan, *J. Am. Chem. Soc.*, 1998, **120**, 3316 and references cited therein; W.-M. Wu, Y.-K. Wu, Y.-L. Wu, Z.-J. Yao, C.-M. Zhou, Y. Li and F. Shan, *J. Am. Chem. Soc.*, 1998, **120**, 13002 and references cited therein.
9 A. R. Butler, B. C. Gilbert, P. Hulme, L. R. Irvine, L. Renton and A. C. Whitwood, *Free Radical Res.*, 1998, **28**, 471.
10 Y.-K. Wu, Z.-Y. Yue and Y.-L. Wu, *Angew. Chem., Int. Ed.*, 1999, **38**, 2580.
11 D.-Y. Wang, Y.-K. Wu, Y.-L. Wu, Y. Li and F. Shan, *J. Chem. Soc., Perkin Trans. 1*, 1999, 1827.
12 (*a*) Y. Chen, C.-X. He, S.-M. Zhu and H.-Y. Chen, *J. Electrochem. Soc.*, 1997, **144**, 1891; (*b*) Y. Chen, S.-M. Zhu, H.-Y. Chen and Y. Li, *Huaxue Xuebao*, 1997, **55**, 921 (*Chem. Abstr.*, 1997, **127**, 311409n); (*c*) Y. Chen, S.-M. Zhu and H.-Y. Chen, *Huaxue Xuebao*, 1998, **56**, 925 (*Chem. Abstr.*, 1998, **129**, 316389g); (*d*) Y. Chen, J. M. Zheng, S. M. Zhu and H. Y. Chen, *Electrochim. Acta*, 1999, **44**, 2345; (*e*) F. Zhang, D. K. Gosser Jr. and S. R. Meshnick, *Biochem. Pharmacol.*, 1992, **43**, 1805; (*f*) H.-L. Jiang, K.-X. Chen, Y. Tang, J.-Z. Chen, Y. Li, Q.-M. Wang, R.-Y. Ji and Q.-K. Zhuang, *Indian J. Chem. Sect. B*, 1997, **36**, 154; (*g*) Electrochemical reduction of other bicyclic endoperoxides, ascaridole and dihydroascaridole, see: M. S. Workentin and R. L. Donkers, *J. Am. Chem. Soc.*, 1998, **120**, 2664.