Ferrous ion induced cleavage of the peroxy bond in qinghaosu and its derivatives and the DNA damage associated with this process

Wen-Min Wu,[a] Zhu-Jun Yao,[a] Yu-Lin Wu,*[a] Kun Jiang,[a] Yan-Fang Wang,[a] Hai-Bao Chen,*[a] Feng Shan[b] and Ying Li*[b]

[a] *State Key Laboratory of Bio-organic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, China*

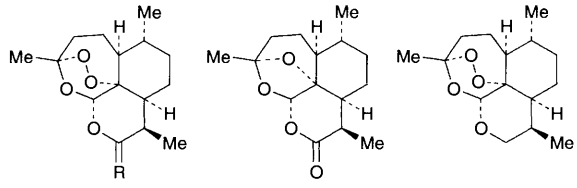
[b] *Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China*

In the presence of iron(II) the peroxy bridge of the drug qinghaosu and its derivatives are cleaved to give two major rearrangement products; DNA damage due to this free radical process is observed and may be responsible for the antimalarial activity of these compounds.

Qinghaosu (artemisinin; **1**), a sesquiterpene endoperoxide, was isolated from the Chinese medicinal herb qinghao (*Artemisia annua* L.) in 1971.[1] Its remarkable antimalarial activity and low toxicity have made it an important addition to the range of antimalarial drugs, especially for the treatment of multidrug-resistant cases.[2] Structurally qinghaosu is entirely different from all previous antimalarial drugs. Studies of the structure–activity relationships have shown that the peroxy group is necessary for the antimalarial activity. The absence of this peroxy bridge, *e.g.* in deoxyqinghaosu **7**,[3] leads to complete inertness in pharmacological screening. Deoxoqinghaosu **8**[4] and dihydroqinghaosu **2** as well as its derivaties[3] that retain an intact peroxy group in the molecule have shown even greater antimalarial activity than qinghaosu itself. These discoveries have inspired a number of studies on the role of the peroxy group in the biological activity of qinghaosu.

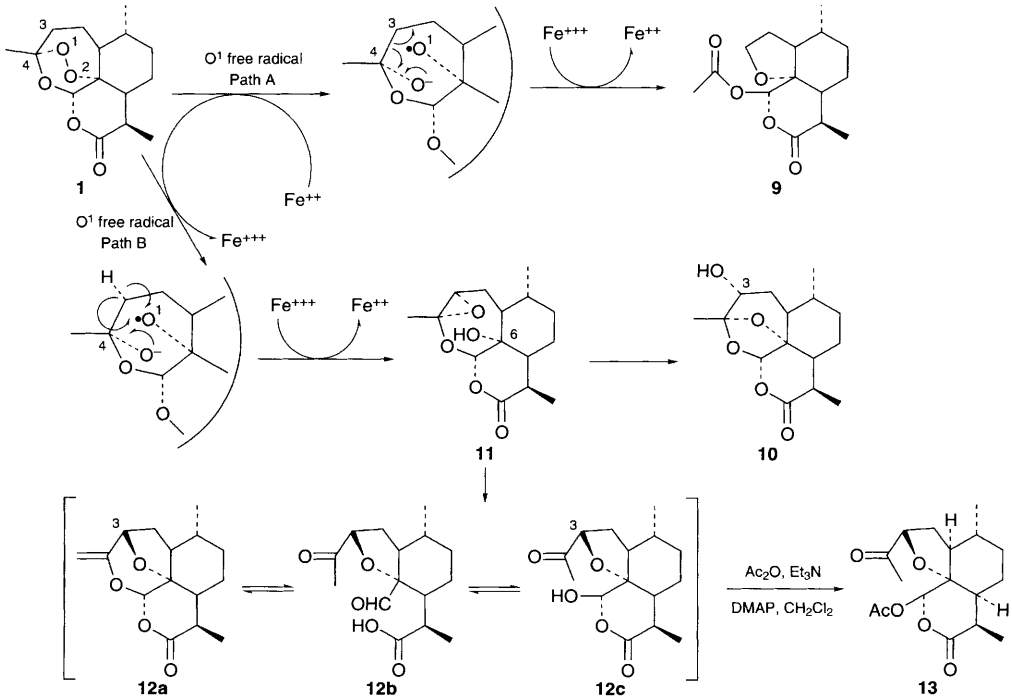
From studies with model systems, Jefford *et al.*[5] suggested that a 1,2,4-trioxane peroxy group might form a complex with the Fe^{II} of haem with subsequent single electron transfer to give a radical anion which, in turn, afforded a toxic ferryl iron–oxene intermediate. Recently, Posner *et al.*[6] proposed a different mode of action for a trioxane antimalarial drug in which a high valence, non-haem iron-oxo species, Fe^{IV}=O, is formed during activation of the trioxane by Fe^{II}. This was proposed to be responsible for the oxidative damage to biological macromolecule. More recently Haynes and Vonwiller[7] reported future studies of the behaviour of qinghaosu in the presence of Fe^{II} and Fe^{III}.

While engaged in a long-term project on qinghaosu, we have been systematically studying the structure, reaction and synthesis of qinghaosu, its derivatives as well as analogues.[8] During this process, literature on DNA damage[9] caused by the Fenton



1 R = O (Quinghaosu)
2 R = H, OH
3 R = α-H, β-OMe
4 R = α-OCOCH$_2$CH$_2$CO$_2$H, β-H
5 R = α-H, β-OCH$_2$Ph
6 R = α-OCOPh, β-H

7 **8**



Scheme 1

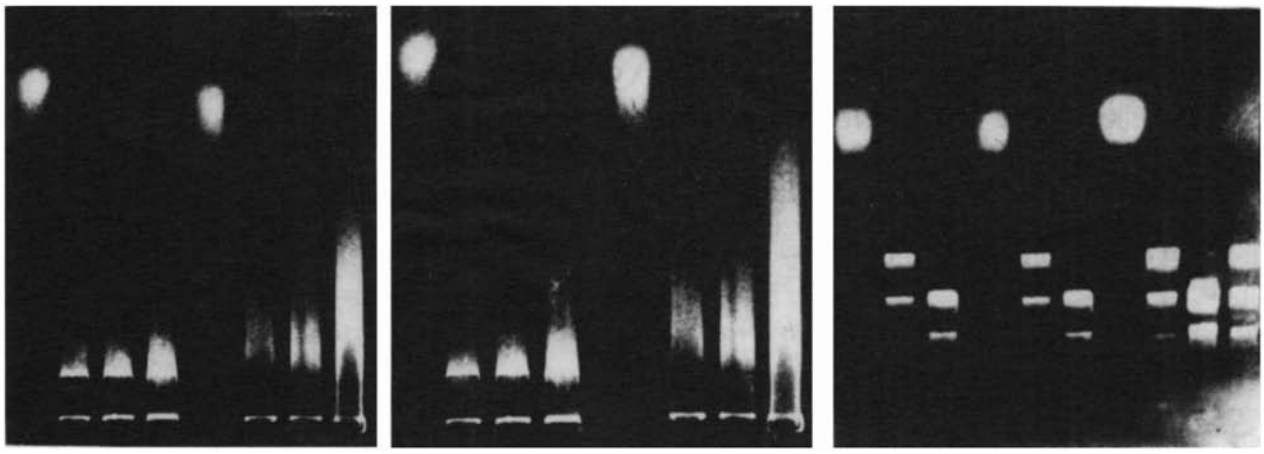


Fig. 1 DNA interaction with qinghaosu–Fe^{II} ion. Lane 1, DNA pUC18 control; lane 2, pUC18 +Fe^{++} (10 mM); lane 3, pUC18 + qinghaosu (10 mM); lane 4, pUC18 + Fe^{++} (10 mM) + qinghaosu (10 mM); lane 5, pUC18 + Fe^{++} (5 mM); lane 6, pUC18 + qinghaosu (5 mM); lane 7, pUC18 + Fe^{++} (5 mM) + qinghaosu (5 mM); lane 8, pUC18 + Fe^{++} (5 mM); lane 9, pUC18 + artemether (5 mM); lane 10, pUC18 + Fe^{++} (5 mM) + artemether (5 mM); lane 11, calf thymus DNA control; lane 12, calf thymus DNA + Fe^{++} (10 mM); lane 13, calf thymus DNA + qinghaosu (10 mM); lane 14, calf thymus DNA + Fe^{++} (10 mM) + qinghaosu (10 mM); lane 15, salmon DNA control; lane 16, salmon DNA + Fe^{++} (10 mM); lane 17, salmon DNA + qinghaosu (10 mM); lane 18, salmon DNA + Fe^{++} (10 mM) + qinghaosu (10 mM); lane 19, calf thymus DNA control; lane 20, calf thymus DNA + Fe^{++} (10 mM); lane 21, calf thymus DNA + artemether (10 mM); lane 22, calf thymus DNA + Fe^{++} (10mM) + artemether (10 mM); lane 23, salmon DNA control; lane 24, salmon DNA + Fe^{++} (10 mM); lane 25, salmon DNA + artemether (10 mM); lane 26, salmon DNA + Fe^{++} (10 mM) + artemether (10 mM).

oxidant (Fe^{++}/H_2O_2) came to our atention. The chemical similarity between the Fenton reagent and Fe^{II}/qinghaosu and the high concentration of Fe^{II} in the red blood cells infected by malarial parasites encouraged us to launch a program to explore the reaction of qinghaosu with Fe^{II} and the possible DNA damage caused by this system. Here we report our findings.

At room temperature, qinghaosu reacted smoothly with 1 equiv. of ferrous sulfate in aqueous acetonitrile (1 : 1, pH 4) under nitrogen. Addition of a phosphate buffer (to adjust the medium acidity to pH 6–7) did not result in any change, except that the reaction was substantially slower. Two major products were obtained as crystalline solids identified as **9** (25%) and **10** (67%). These products were also detected in the extract from *Artemisia annua*[10,11] and in the products of pyrolysis and metabolism of qinghaosu.[12,13] Another crystalline product (recovered in *ca.* 2% yield) was identified as epoxide **11** by NMR spectroscopy and MS. The remaining high polarity products were obtained as a low-melting point mass. The characteristic spectroscopic peaks implied the presence of methyl ketone, a secondary alcohol, terminal enolic methylene group and an aldehyde. Acetylation of this complex mixture with acetic anhydride led to the formation of a crystalline acetate **13** in essentially quantitative yield. The structure and stereochemistry of compound **13** were later unequivocally established by 2D NMR spectroscopy. With these products isolated and the structures established, we were able to envisage the mechanistic picture as shown in Scheme 1. Similar results were also obtained from artemether **3** and derivatives **4**, **5** and **6**. The details will be reported in a separate full paper later.

Additional evidence for our mechanistic proposal comes from the observations that no diastereoisomer of compounds **9**, **10**, **11** or **13** was detected and no reaction occurred if Fe^{III}, rather than Fe^{II}, was added at the start. We also examined the effect of introducing ethylenediaminetetraacetic acid (EDTA) and of varying the relative concentration of the Fe^{II}. No sensible changes in product composition or yield were observed. What should be noted is that a catalytic amount of Fe^{II} is enough to induce the homolytic cleavage of peroxy bonds, an essential part of our mechanistic proposal.

Having established the reaction pathways shown in Scheme 1, we turned to an examination of the DNA cleaving properties of this free radical redox process. Incubation of pUC18 supercoiled DNA with qinghaosu–ferrous sulfate (1 : 1) at concentrations greater than 0.25 mM in an aqueous acetonitrile buffer (pH 6.5) at 37 °C for 24 h resulted in exhaustive cleavage of DNA (forming smaller segments) and disappearance of all the form I, II and III DNA. Use of artemether instead of qinghaosu also produced lesions in supercoiled DNA, but with lower efficacy. Apart from pUC18 supercoiled DNA, calf thymus DNA or salmon DNA could also be cleaved with the same qinghaosu–ferrous sulfate system. In this case, both **9** and **10** were detected as the major metabolites. Some analytic results from electrophoresis experiments are shown in Fig. 1.

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