Pergamon

Tetrahedron Letters, Vol. 37, No. 2, pp. 257-260, 1996

0040-4039/96 $15.00 + 0.00

0040-4039(95)02142-6

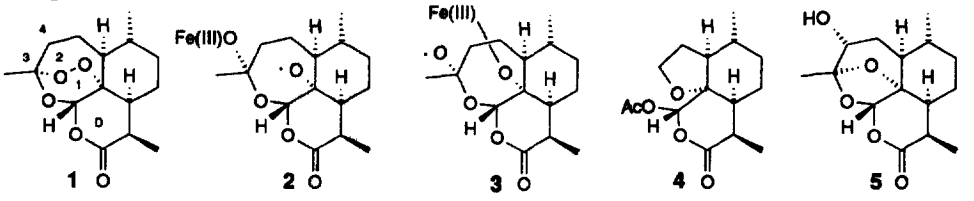
The Behaviour of Qinghaosu (Artemisinin) in the Presence of Non-Heme Iron(II) and (III).

Richard K. Haynes[*,a] **and Simone C. Vonwiller**[*,b]

a. Department of Chemistry, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong.
b. The Department of Organic Chemistry, The University of Sydney, NSW, 2006, Australia.

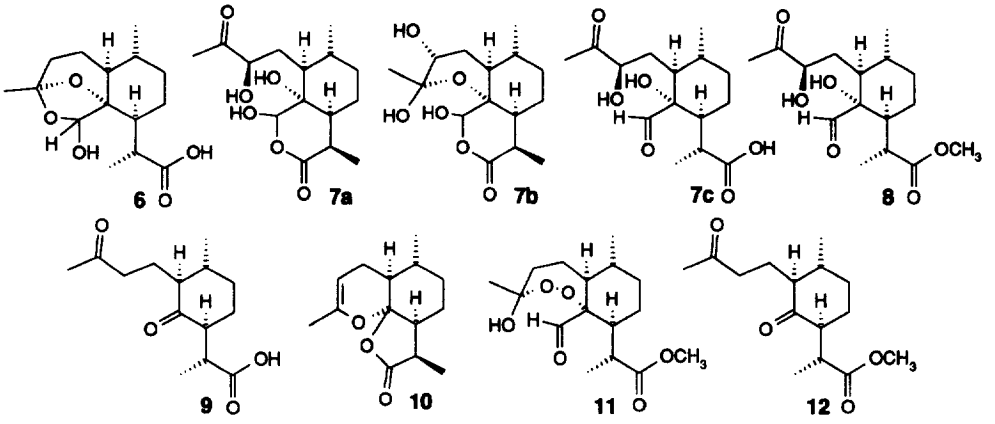
Abstract:. In aprotic solvents with $FeCl_3$, $FeCl_3$/*N*-Acetyl cysteine or $FeCl_2$, qinghaosu (artemisinin) undergoes rearrangement to give the tetrahydrofuran acetate, 4-hydroxydesoxoqinghaosu or the enol lactone as the major product depending on the amount of catalyst used and the polarity of the reaction medium.

In the preceding paper, the formation of products from the interaction of QHS **1** with hemin is interpreted in terms of reductive ring opening of the peroxide bridge to provide alkoxy radicals **2** and **3** which lead *inter alia* to desoxoQHS and tetrahydrofuran **4**. On one occasion, arteannuin D **5** (to 2%) is also isolated.[1] Considerable significance has been attached by others to formation of **4** and **5** from QHS and $FeBr_2$ *in THF*, in relation to biological activity; the central tenet of the proposal being that reductive cleavage of the peroxide bridge leads to carbon-centred radicals which provide both **4** and **5**.[2-5] In order to probe conditions required for formation of **4** and **5**, we have studied the behaviour of QHS in the presence of heme[1] and non-heme iron, and herewith report our results with non-heme iron.



1 **2** **3** **4** **5**

Whereas QHS reacts slowly in the presence of hemin [Fe(III)] to give low yields of the precursor **6** to desoxoQHS under aprotic conditions,[1] the $FeCl_3$-induced reaction proceeds much more rapidly to give the tetrahydrofuran **4** and arteannuin D **5**. Thus, QHS in MeCN containing pyridine (1 eq) with $FeCl_3$ (1 eq) during 1.5 days afforded **4** (85%) and **5** (8%). With $FeCl_3$ diethérate (0.2 eq) and HOAc (1 eq) in CH_2Cl_2, QHS was converted into **4** (12%), **5** (40%) and its three ring opened forms **7a**, **7b**, and **7c** (34%)[6] within 7 h.[7] In the presence of O_2 the conversion was significantly slower, requiring over 50 h to go to near completion. When QHS was treated with $FeCl_3$ (0.1 eq) in ether under N_2, compounds **4** (52%) and **5** (8%) and the diketo acid **9** (34%) were formed after 1.5 h. However, with $FeCl_3$ (1 eq) in ether under N_2, the tetrahydrofuran **4** (18%), arteannuin D **5** (4%), and the enol lactone **10** (68%) were formed after only 30 min. Under such conditions, the

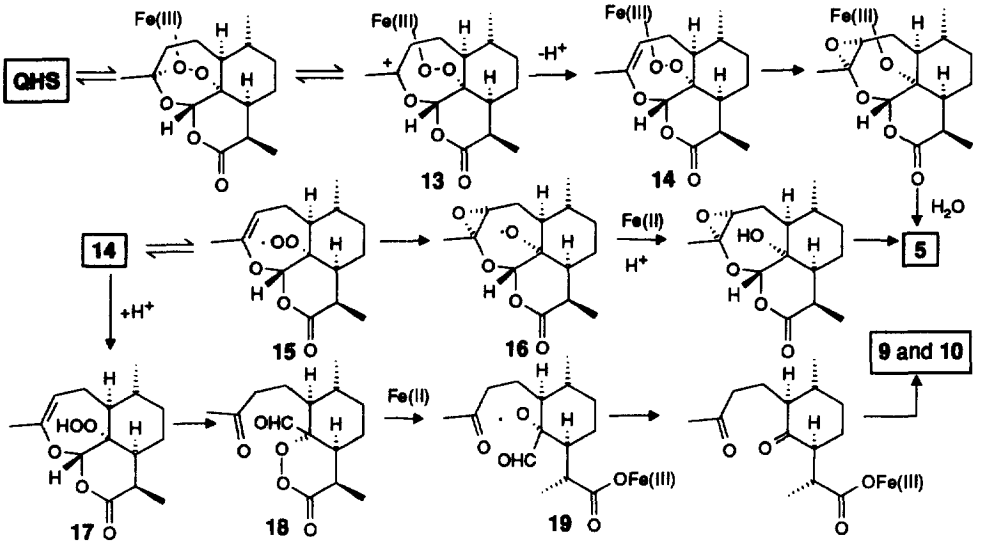


diketo acid **9** is converted smoothly into enol lactone **10**. With $FeCl_3$ (0.2 eq) in the presence of acetic acid (1 eq) in ether, the product distribution from QHS changed to the tetrahydrofuran **4** (37%), arteannuin D **5** (5%), the diketo acid **9** (12%) and the enol lactone **10** (36%). Thus, by changing the solvent polarity and the amount of catalyst used it is possible to selectively enhance formation of the tetrahydrofuran **4**, arteannuin D **5** or the enol lactone **10**, all of which have been characterised previously as products of the thermal decomposition of QHS.[8] QHS was inert in the presence of $Fe(ClO_4)_3$ or $Fe(acac)_3$ under the above conditions.

In parallel with the hemin reactions, thiols enhanced the transformations of QHS in the presence of $FeCl_3$. Addition of $FeCl_3$ (1 eq) to QHS, *N*-acetyl cysteine (1 eq) and imidazole (1 eq) in MeCN resulted in a transient purple blue colour[9] and rapid conversion (5 min) into **4** (79%) and **5** (16%). $Fe(OSO_2CF_3)_3$ also produced a purple colour with QHS/*N*-acetyl cysteine/imidazole in MeCN but the reaction was slower to give after 18 h clean conversion into **4** (41%) and **5** (50 %). $Fe(ClO_4)_3$ in the presence of thiol did not affect QHS. $FeCl_2$ (1 eq) in the presence of imidazole (1 eq) in MeCN induced rapid (5 min.) and quantitative conversion of QHS into **4**, **5** and the desoxoQHS precursor **6** in a ratio of 78:16:6. $Fe(ClO_4)_2.6H_2O$ in MeCN both under O_2 or Ar and with or without added thiol, LiCl in THF, $ZnBr_2$ in ether, triflic acid in CH_2Cl_2, and HCl in ether all had no effect. The peroxy hemiacetal methyl ester **11** with $FeCl_3$ (1 eq) in ether cleanly gave the diketo ester **12** over 3.5 h as compared to 1.5 h required to consume QHS. Similarly, the use of $FeCl_3$ (1 eq)/*N*-acetyl cysteine (1eq)/imidazole (1eq) in MeCN led only to the diketo ester **12** and *N*-acetyl cystine from **11**.

Thus, while a pathway involving iron(II) under Fenton conditions[10] is responsible for formation of tetrahydrofuran **4**, a pathway distinct to the direct reduction of the peroxide bridge must account for the formation of compounds **5** and **9** (which leads to **10**). The first step is considered to be *Lewis acid complexation to the peroxide bridge* at O-2 with ligand displacement from iron, which induces reversible opening of the peracetal to the stabilised cation **13** (Scheme 1). Cation **13** is well placed to provide enol ether **14** which gives arteannuin D **5** via intramolecular epoxidation; this epoxidation may proceed hetereolytically from **14** or via extrusion of Fe(II) to provide the peroxy radical **15**.[11-13] The latter accounts for the unique effectiveness of redox Lewis acids in inducing this transformation, and the sensitivity of the reaction to O_2. Formation of **9** from QHS formally requires an equivalent of water for opening of the lactone ring, albeit a possibility which in the ether solvent cannot be rigorously excluded. As the conversion of ester peroxy hemiacetal **11** into ester diketone **12** in the presence of $FeCl_3$ in ether most obviously proceeds via free hydroperoxide and the derived peroxy radical,[1,12,13] formation of **9** from QHS may proceed initially via protolysis of enol ether **14** to free hydroperoxide **17** which with water provide the free acid corresponding to **11**. However, given that dihydroQHS rearranges via the open

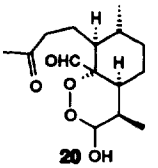
hydroperoxide to compound **20** under acid catalysis,[14] the alternative pathway involving rearrangement of **17**, and which does not require water, appears more likely. Thus, formation of acyl peroxide **18** is followed by reductive cleavage to alkoxy radical **19**, which via β-scission produces **9** and **10**. Finally, the most apparent route to the desoxoQHS precursor **6** is via direct reduction of hydroperoxide **17** with Fe(II).

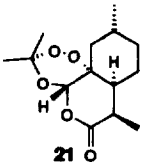


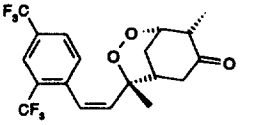
Scheme 1

In this and the preceding paper we have shown that QHS displays multifaceted reactivity in the presence of iron(II) and iron(III) and the outcome is sensitive to the reaction conditions. Overall the results do not enable us to assign the biologically active species produced from QHS, but taken together with current literature data, they do suggest that the C-4 radical leading to formation of tetrahydrofuran **4**, and consequently the reductive mode of ring opening of the peroxide bridge may not be the prime basis for biological activity. Formation of a C-4-centred radical in the 5-*seco*-artemisinin derivative **21** is definitely disfavoured, yet this compound displays activity against *P. falciparum* (W-2) of 1.46 ng mL^{-1} *in vitro*.[15] Compound **20** is also active,[14] yet in common with our compound **11**, is unlikely to produce C-centred radicals. The remarkable observation is made that attachment of substituents at C-4, or of large 'radical stabilizing' substituents at C-3 of artemisinin suppresses antimalarial activity.[2-4] Avery has demonstrated that large substituents at C-3 suppress activity, irrespective of their electronic qualities.[15] However, *substituents at either C-3 or C-4 will prevent access of reagents to the peroxide bridge*, particularly where complexation precedes activation. The well-known sensitivity of conformational changes of QHS derivatives brought about by substitution in the D-ring also argues for a precomplexation effect, rather than a direct, reductive cleavage of the peroxide. Thus, the proposal as encapsulated in the Scheme that QHS behaves as a masked source of free hydroperoxide must be considered as a basis for biological activity. Consequently, it follows that formation of desoxoQHS under physiological conditions also reflects the biological activity. All active peroxidic antimalarials possess groups, either alkoxy, aryl or alkyl capable of stabilizing the positive charge induced by heterolytic ring opening, a property which indeed is manifest in tertiary peroxides in general.[16] The Roche arteflene **21**[17] is a most interesting case in

providing a second possibility for ring opening by retro-Michael reaction. Clearly, then, the selection of optimum peroxidic antimalarials should take into account this alternative mode of peroxide cleavage.







Acknowledgment. We thank the Australian Research Council for financial support of this work. SCV gratefully acknowledges the award of a Queen Elizabeth II Fellowship (1992-1997).

References

1. Haynes, R. K.; Vonwiller, S. C. *Tetrahedron Lett.* **1995**, *36*, preceding paper
2. Posner, G. H.; Oh, C. H.; Wang, D.; Gerena, L.; Milhous, W. K.; Meshnick, S. R.; Asawamahasadka, W. *J. Med. Chem.* **1994**, *37*, 1256; .
3. Posner, G. H.; Cumming, J. N.; Ploypradith, P.; Oh, C.- H. *J. Am. Chem. Soc.* **1995**, *117*, 5885.
4. Posner, G. H.; Wang, D.; Cumming, J. N.; Oh, C. H.; French, A. N., Bodley, A. L.; Shapiro, T. A. *J. Med. Chem.* **1995**, *38*, 2273.
5. A proposal of β-scission in synthetic 1,2,4-trioxanes is based on incorrectly assigned products: Jefford, C. W.; Favarger, F.; Vicente, M. G. H.; Jacquier, Y. *Helv. Chim. Acta* **1995**, *78*, 452. We thank Professor J. Boukouvalas for pertinent comments.
6. Treatment of the mixture with diazomethane led cleanly and quantitatively to a single methyl ester **8**. ^{1}H NMR δ 0.94 (3H, d, J = 6.2 Hz, 6-CH_3), 1.32 (3H, d, J = 7.0 Hz, 9-CH_3), 3.66 (3H, s, OCH_3), 9.95 (1H, s, CHO); ^{13}C NMR δ 17.0, 20.3, 26.9, 27.6, 29.2, 30.9, 34.9, 42.5, 48.4, 51.6, 57.2, 83.6 (C4), 89.6 (C12a), 176.0 ($COOCH_3$), 204.4 (CHO), 208.8 (C3) (carbon and hydrogen atoms are numbered according to the QHS skeleton).
7. The ratio of **5** + **7** : **4** varied from 2 : 1 to as much as 23 : 1.
8. Compounds **4**, **5** and **10** form in 12%, 10%, and 4% yield respectively on heating QHS at 190 °C: Lin, A. J.; Klayman, D. L.; Hoch, J. M.; Silverton, J. V.; George, C. F. *J. Org. Chem.* **1985**, *50*, 4505.
9. This is believed to be due to a ferrous complex bearing a thiyl radical ligand: Hamed, M. Y.; Silver, J.; Wilson, M. T. *Inorg. Chim. Acta* **1983**, *78*, 1.
10. See, however, Sugimoto, H.; Sawyer, D. T. *J. Am. Chem. Soc.* **1984**, *106*, 4283; Sawyer, D. T.; Kang, C.; Llobet, A.; Redman, C. *J. Am. Chem. Soc.* **1993**, *115*, 5817.
11. Traylor, T. G.; Ciccone, J. P. *J. Am. Chem. Soc.* **1989**, *111*, 8413; Traylor, T. G.; Xu, F. *J. Am. Chem. Soc.* **1990**, *112*, 178.
12. Labeque, R.; Marnett, L. J. *Biochemistry* **1988**, *27*, 7060 and references therein.
13. Gardner, H. W.; Jursinic, P. A. *Biochim. Biophys. Acta* **1981**, *665*, 100; Dix, T. A.; Marnett, L. J. *J. Biol. Chem.* **1985**, *260*, 5351.
14. Baker, J. K.; McChesney, J. D.; Chi, H. T. *Pharm. Res.* **1993**, *10*, 662.
15. A very large amount of elegant synthetic work has been carried out by Avery and coworkers which provide most useful leads on activities of QHS derivatives: Avery, M. A.; Bupp, J. PCT/US91/01832, WO 91/14689, Oct. 1991.
16. Hiatt, R. *Organic Peroxides*, Swern, D., Ed.; Wiley-Interscience: New York, 1972; Vol 3, Ch 1, pp 21-23.
17. Hofheinz, W.; Bürgin, H.; Gocke, E.; Jaquet, C.; Masciadri, R.; Schmid, H.; Stohler, H.; Urwyler, H. *Trop. Med. Parasitol.* **1994**, *45*, Supplement III, 261.

(*Received in UK* 9 *October* 1995; *revised* 9 *November* 1995; *accepted* 10 *November* 1995)