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

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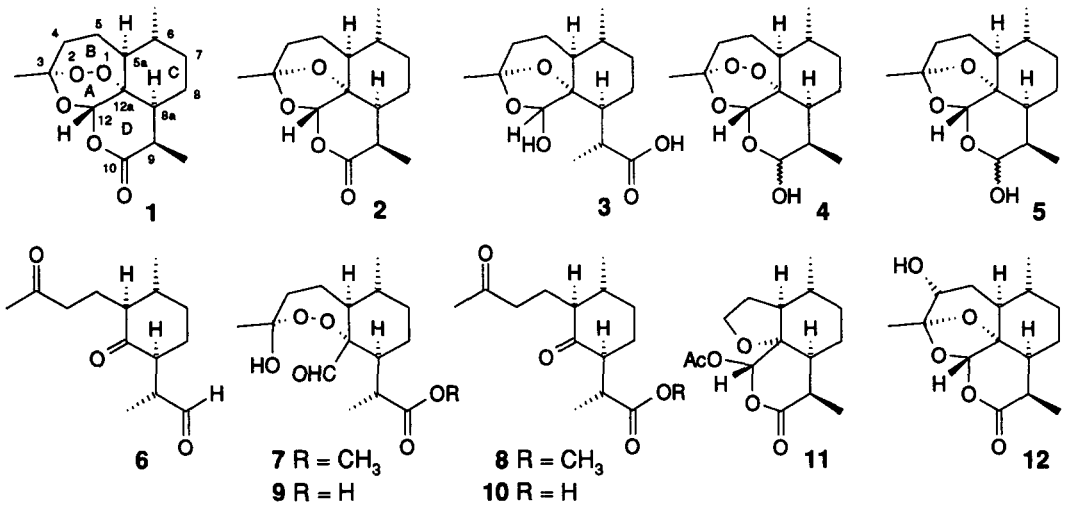
Abstract: With hemin [chloroprotoporphyrin IX iron(III)] or hemin/cysteine in aqueous MeCN, oxygen loss from the peroxide bridge of qinghaosu takes place to give a precursor to desoxoqinghaosu, a known malaria-inactive metabolite, in low yield. Ring-opened forms of qinghaosu such as the free hydroperoxide or peroxyhemiacetal react with hemin and heme to give predominantly the diketone resulting from oxygen loss from the peroxide bridge followed by deformylation.

Qinghaosu (artemisinin) **1** and its derivatives, now in routine use for treatment of severe malaria, are currently believed to act by an oxidative mechanism.[1] Other simple endoperoxides also possess antimalarial activity at least *in vitro*[2] and thus the question is why the QHS compounds with their elaborate functionality are so effective. DesoxoQHS **2** has been identified as a metabolite, but it is not established if its formation is associated with the mode of action. Meshnick and coworkers provide evidence which appears to implicate an adduct formed between QHS and hemin produced by breakdown of hemoglobin by the parasite in the erythrocyte.[3,4] The proposal is made that it is heme iron(II) which reduces QHS, with the reduction of hemin iron(III) to heme iron(II) being induced by an exogenous, possibly thiol-based, electron source.[5] Recent work with either QHS or synthetic trioxanes have as a consequence focussed on iron(II) and commence with the premise that reductive cleavage of the peroxide linkage leads to carbon-centred radicals which are held to be the biologically active species.[6] Nevertheless, hemin iron(III), either in hemin itself, or in hemozoin,[7] may also be a target for QHS interaction and thus a proper delineation of the role of QHS should include an evaluation of the behaviour of QHS in the presence of both iron(II) and iron(III). In this and the following papers, we describe results of such studies.

Aqueous media were used in order to mimic physiological conditions. With hemin (1 eq) in MeCN-H_2O (1:1 containing phosphate buffer at pH 7.8) at room temperature over 10 days, QHS was converted into the desoxoQHS precursor **3**[8] in only 23% yield.[9] No other products from QHS alone were detected, although examination of the reaction mixture by reverse phase tlc indicates that a heme-derived species, less polar than heme itself, is produced in the QHS-hemin reaction mixtures. DihydroQHS **4** was consumed within 3 days to give a 1:1 mixture of the desoxodihydroQHS precursor **5** and the diketoaldehyde **6**.[8] Recovery of material was poor (8% combined yield) and no other products from the dihydroQHS alone were observed; again an apparent adduct is formed with the hemin. With QHS and hemin in the presence of cysteine (1 eq) or hydroquinone (1

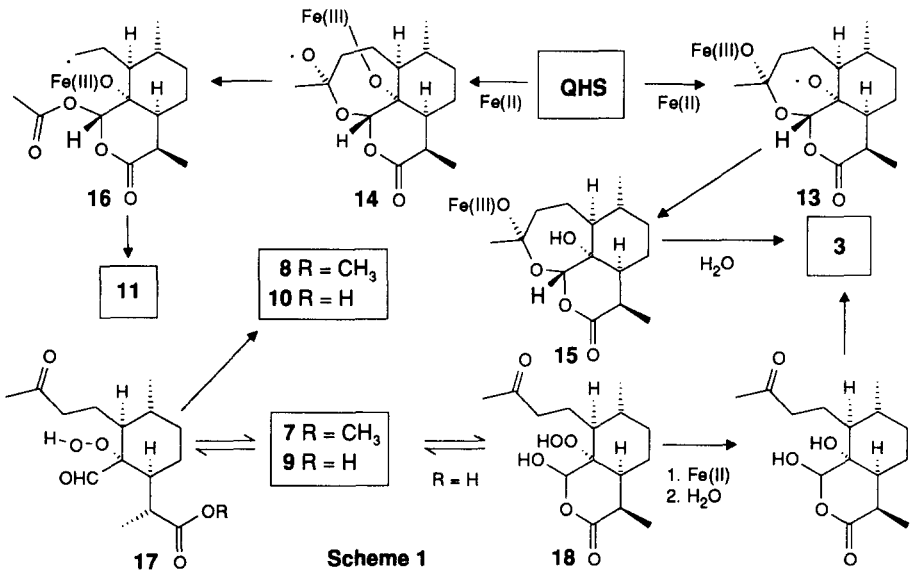


eq) a rapid reaction took place to give **3** within 10 min. but again in only 20-30% yield. The outcome was the same for reactions conducted under Ar or O_2. Under the same conditions under N_2, dihydroQHS **4** was also rapidly consumed. However, only traces of products **5** and **6** were recovered. When QHS was treated with catalytic hemin (0.1 eq) and cysteine (1 eq), the reaction did not proceed to completion and afforded recovered QHS (36%) and the desoxoQHS precursor **3** (6%).

Disulfide formation is observed in the latter cases, suggesting that the thiol reduces the hemin.[10] Products resulting from thiol transfer to QHS in the presence of hemin were never found; attempts to trap thiyl radicals[11] from the thiols with methyl linoleate were unsuccessful. QHS alone had no effect on the thiol. Transient observation of *p*-benzoquinone by TLC in the QHS-hemin-hydroquinone systems suggests that hydroquinone also reduces the hemin. Thus it does indeed appear that heme iron(II) is a reactive species in all these reactions. However, the inability of hemin to sustain a catalytic cycle in the presence of excess thiol indicates that it is irreversibly consumed in the reaction.

In the reactions involving QHS, the desoxoQHS precursor **3** was the only isolable product but this was obtained in only 20 -30% yield. The observation by reverse phase tlc of an adduct formed between QHS and hemin supports Meshnick's observation that QHS forms a covalent adduct with hemin.[4] In contrast, the peroxyhemiacetal methyl ester **7**[8,12] with hemin *or* hemin/cysteine in aqueous MeCN at pH 7.8, rapidly gave *in quantitative yield* the diketo ester **8**.[8] *Thus, adduct formation of 7 with hemin does not occur.* Significantly, when the free acid **9**[8,12] was treated under the same conditions the desoxoQHS precursor **3** (20%) was obtained in addition to the diketo acid **10** (42%).[8] Control experiments indicate the reactions are due solely to hemin or heme. QHS (1 eq) with tetraphenylporphine iron(III) chloride (TPPFeCl, 1 eq) under non-aqueous conditions with *N*-acetyl cysteine (1 eq) in CH_2Cl_2 gave not compound **3**, but rather the tetrahydrofuran **11** as the major product (74% isolated yield) together with arteannuin D **12** after 45 min; *adduct formation between TPPFeCl and QHS is not observed.* Posner and coworkers reported that QHS with hemin/benzenethiol in THF gives **2**, **11**, and **12** in a ratio of 2:90:8 (70%).[13] However, when QHS was treated with hemin (1 eq) and *N*-acetyl cysteine (1 eq) in THF under N_2 a slow reaction took place over 7 h to give compounds **2** (6%), **3** (2%), **11** (5%) and **12** (2%). Again, recovery of material was poor and tlc showed formation of the hemin-QHS adduct, unrecorded by Posner in his system.[13]

The inability of peroxy hemiacetals **7** or **9**, or of **3**, to form adducts with hemin indicates that the tetracyclic structure of QHS and the peroxide unit are required for its formation. Fenton-type cleavage of the peroxide bridge affords radicals **13** and **14**. The first via reduction by Fe(II) and subsequent protonation, or via intermolecular hydrogen abstraction from a hydrogen donor gives alcohol **15** and thence **3**. Radical **14** may undergo β-scission to the primary carbon-centred radical **16** which then forms tetrahydrofuran **11**.[14] Radical **16** may react with susceptible alkenes, for example those in hemin, to form adducts, *thus diverting it away from the tetrahydrofuran-forming pathway.*[15] This is consistent with the observations that only trace amounts of **11** are observed in the presence of hemin,[16] and that when TTPFeCl is used as the heme iron source adduct formation does not take place and **11** is the *major* product. For peroxy hemiacetals **7** and **9**, these processes become insignificant due to the flexibility of the molecule and they react as free hydroperoxides. By analogy with closely related cases, the α-formyl hydroperoxides **17** rapidly decompose in the presence of Fe(II) or Fe(III) via alkoxy[17] or peroxy[18] radicals to **8** and **10**. When a free carboxylic acid group, as in **9** is capable of forming a hemiacetal, *viz* **18**, the formyl group is protected, thereby enabling reduction of the hydroperoxide to take place.[19]



Scheme 1

While the formation of compounds **2**, **3** and **11** can be attributed to the reductive cleavage of the peroxide bridge of QHS and subsequent transformations, the nature of the biologically active intermediate still awaits clarification. Although a great deal of importance has been placed on the significance of the formation of the very *minor* product, namely arteannuin D **12**, under aprotic conditions in terms of carbon-centred radicals,[13,14] our studies of the behaviour of QHS with non-heme iron(II) and (III) in non-aqueous media cause us to consider an alternative viewpoint. The results of these studies are presented in the following paper.

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8. For convenience, carbon and hydrogen atoms for all compounds are numbered according to the QHS skeleton **1**. **3**: ^{1}H NMR (200 MHz) δ 0.89 (3H, d, *J* = 5.9 Hz, 6-CH_3), 1.26 (3H, d, *J* = 7.0 Hz, 9-CH_3), 1.53 (3H, s, 3-CH_3), 3.35 (1H, dq, *J* = 7.0, 4.2 Hz, H9), 5.45 (1H, s, H12); **5**: ^{1}H NMR (400 MHz) (*denotes minor diastereomer) δ *0.895 (3H, d, *J* = 5.8 Hz, 6-CH_3), 0.90 (3H, d, *J* = 5.5 Hz, 6-CH_3), *0.97 (3H, d, *J* = 7.5 Hz, 9-CH_3), 1.01 (3H, d, *J* = 7.5 Hz, 9-CH_3), *1.52 (3H, s, 3-CH_3), 1.54 (3H, s, 3-CH_3), 4.77 (1H, dd, *J* = 6.7, 6.7 Hz, H10), *5.31 (1H, dd, *J* = 5.2, 5.2 Hz, H10), 5.34 (1H, s, H12), *5.36 (1H, s, H12); **6**: ^{1}H NMR (400 MHz) δ 1.10 (3H, d, *J* = 6.0 Hz, 6-CH_3), 1.17 (3H, d, *J* = 7.4 Hz, 9-CH_3), 2.13 (3H, s, 3-CH_3), 9.74 (1H, d, *J* = 0.9 Hz, CHO); **7**: ^{1}H NMR (200 MHz) δ 0.89 (3H, d, *J* = 6.5 Hz, 6-CH_3), 1.22 (3H, d, *J* = 7.2 Hz, 9-CH_3), 1.28 (3H, s, 3-CH_3), 3.19 (1H, dq, *J* = 7.2, 3.3 Hz, H9), 3.38 (1H, br s, OH), 3.65 (3H, s, OCH_3), 9.91 (1H, d, *J* = 2.6 Hz, CHO); ^{13}C NMR (50 MHz) δ 17.9, 20.4, 22.3, 24.0, 25.2, 31.9, 35.4, 37.7, 41.2, 49.9, 51.5, 59.6 (OCH_3), 94.1 (C12a), 105.9 (C3), 175.3 ($COOCH_3$), 200.4 (CHO); **8**: ^{1}H NMR (200 MHz) δ 1.07 (3H, d, *J* =5.7 Hz, 6-CH_3), 1.18 (3H, d, *J* = 6.6 Hz, 9-CH_3), 2.12 (3H, s, 3-CH_3), 2.77 (1H, dq, *J* = 7, 7Hz, H9), 3.67 (3H, s, OCH_3); ^{13}C NMR (50 MHz) δ 15.1, 20.1, 20.5, 29.89, 31.0, 34.4, 39.3, 40.2, 41.2, 51.6, 53.5, 56.8 (OCH_3), 176.1 ($COOCH_3$), 209.1 (C3), 211.6 C12a); **9**: ^{1}H NMR (200 MHz) δ 0.90 (3H, d, *J* = 6.5 Hz, 6-CH_3), 1.15 (3H, d, *J* = 7.1 Hz, 9-CH_3), 1.28 (3H, s, 3-CH_3), 3.0 - 3.3 (1H, m, H9), 9.93 (1H, br s, CHO); **10**: ^{1}H NMR (200 MHz) (*denotes a minor diastereomer) δ *0.95 (3H, d, *J* = 6.4 Hz, 6-CH_3), 1.10 (3H, d, *J* = 5.8 Hz, 6-CH_3), *1.11 (3H, d, *J* = 7.3 Hz, 9-CH_3), 1.21 (3H, d, *J* = 6.9 Hz, 9-CH_3), 2.13 (3H, s, 3-CH_3), *2.21 (3H, s, 3-CH_3), 2.87 (1H, dq, *J* = 6.7, 5.3 Hz, H9), *3.37 (1H, dq, *J* = 7.2, 5.3 Hz, H9).
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19. At pH 10, conditions under which nucleophilic ring opening of QHS by hydroxide is likely to occur to expose the free formyl group, **3** and **10** are formed in a 30:70 ratio from QHS in the presence of hemin; at pH 7.8, **3** is the only product.

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