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Ring Opening of Artemisinin (Qinghaosu) and Dihydroartemisinin and Interception of the Open Hydroperoxides with Formation of *N*-Oxides – A Chemical Model for Antimalarial Mode of Action

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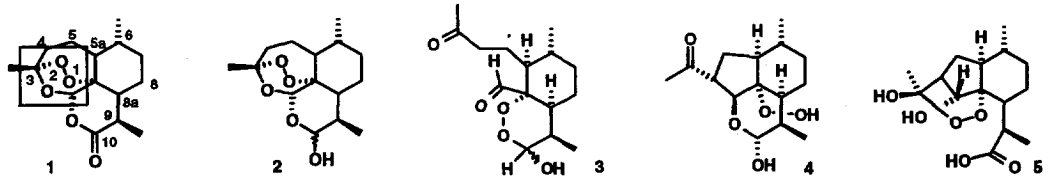
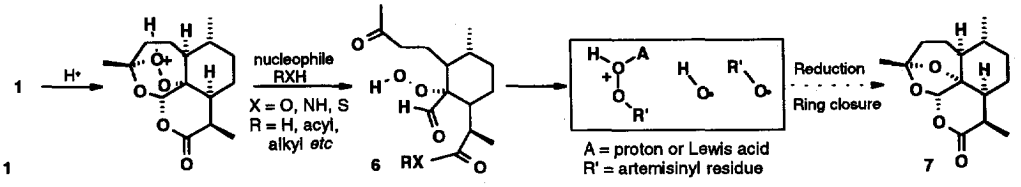
Abstract: In CH_2Cl_2 in the presence of benzylamine, artemisinin transfers an oxygen atom from the intermediate open hydroperoxide to tertiary amines to form *N*-oxides and *N*-benzyl-11-azadesoxyartemisinin. Base-catalyzed side reactions interfere with the corresponding reaction involving dihydroartemisinin. The reactions serve as a model for biological activity in which the act of binding to a protein liberates hydroperoxide.

The efficacy of artemisinin **1** (qinghaosu) and derivatives based on dihydroartemisinin **2** for treatment of malaria is now established.[1-3] Not only do these compounds have exceptional activity against the malaria parasite, but are also active against other protozoans,[3] and individual cell lines.[4] Thus, the 1,2,4-trioxane pharmacophore within artemisinin confers activity against diverse biological entities.



Both **1** and **2**, the active metabolite of the derivatives, possess low bioavailability and are rapidly eliminated.[1,2] They also undergo facile 'unzipping' to give *inter alia*, products **3** – **5**.[5-7] In line with this, we have posited that activity is due to the trioxane unit acting as a source of hydroperoxide,[8,9] which provides electrophilic oxygenating species, or hydroxyl or alkoxyl radicals via reductive cleavage with *exogenous* iron(II) or other reducing agents (Scheme 1). These species will be capable of hydroxylating biomolecules, or of abstracting hydrogen atoms. Desoxoartemisinin **7** thereby will appear as a metabolite, as in fact is observed in patients treated with artemisinin.[1]



Scheme 1

In pursuing this thesis further, we have focussed on means of unzipping the artemisinin nucleus under mild conditions, and trapping the open hydroperoxide. In this context, we noted that Ziffer and coworkers[10] converted **1** into 11-aza derivatives **9** by treatment with ammonia and primary amines in MeOH followed by treatment (to close the open hydroperoxide **8**, detectable in reaction mixtures by NMR spectroscopy) with silica gel/sulfuric acid in CH_2Cl_2 (Scheme 2). However, *the intriguing aspect was the appearance of the 11-azades-*

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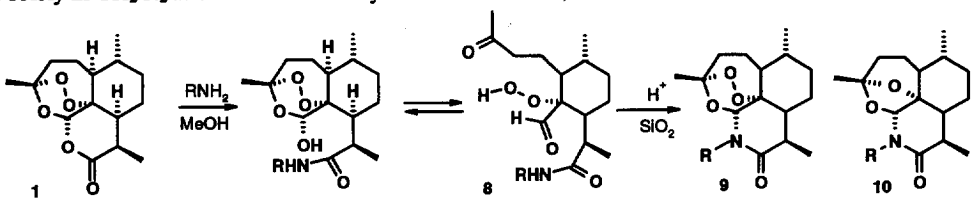
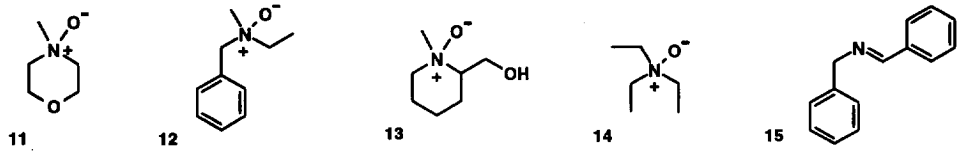
oxo by-product **10**, *whose formation was not explained.* In repeating this work, we found that **1** in MeOH with ammonia, benzylamine and other primary amines followed by removal of solvent, and treatment of the residue in CH_2Cl_2 with silica gel/H_2SO_4 or *p*-TsOH gave the desoxo compounds **10** only. However, for reactions conducted solely in CH_2Cl_2 at 0-5 °C followed by the acid treatment, 11-azaartemisinins **9** were obtained in mod-



Scheme 2 **1** **8** **9** **10**

erate yield, together with the desoxo compounds **10** in *ca.* 40% yield. At higher temperatures, the desoxo compounds **10** were the major products. Next, **1** (2 mmol.) was treated with the tertiary amine, *N*-methylmorpholine (NMM, 5 eq.) in CH_2Cl_2. No reaction was observed. *However, use of benzylamine (1 eq) together with NMM (5 eq) at room temperature for 24 h gave the* N*-oxide* **11** *(72% isolated yield) together with the desoxo compound* **10** *(R = Bn).* *N*-Oxides **12** and **13** were also obtained from the corresponding tertiary amines and **1** in the presence of benzylamine. Product identity was rigorously secured by comparison with authentic samples. Analysis of the *N*-oxide **12** from *N*-benzylethylmethylamine by means of NMR spectroscopy admixed with (*S*)-*tert*-butylphenylphosphinothioic acid[11] reveals it to be racemic.



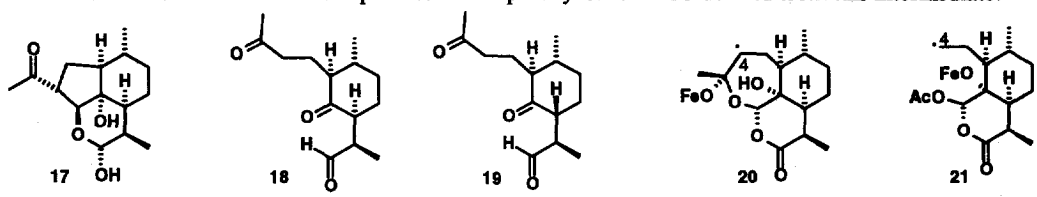
11 **12** **13** **14** **15**

The use of triethylamine was also examined, as formation of *N*-oxide **14** is easily monitored by NMR spectroscopy. As above, whilst **1** (5 mmol) and Et_3N (25 mmol) in CH_2Cl_2 did not react, addition of benzylamine (1 eq) induced formation of the *N*-oxide **14**. The reaction was best conducted in a two phase system (water/CH_2Cl_2) followed by extraction of the *N*-oxide into the water from the organic layer. The very polar *N*-oxide was isolated in 60% yield after evaporation of the aqueous layer, and chromatography of the residue over silica gel in MeOH-EtOAc (1:1). From the organic phase were recovered the azadesoxoartemisinin **10** (R = Bn) as the other major product, unchanged **1** (to 10%) and small quantities of desoxoartemisinin **7**.

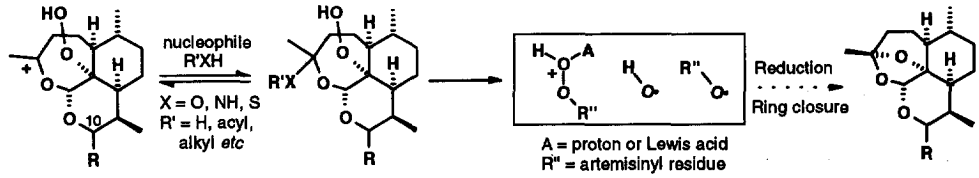
It is apparent also that oxygen transfer to benzylamine takes place, as is noted during treatment of **1** with tertiary aromatic amines (*N*-isopentyl-*N*-methylaniline) and nitrogen heterocycles (quinoline), which did not react. The imine **15**, obviously formed during the acid treatment stage between unreacted benzylamine and benzylimine arising via dehydration of the incipient *N*-benzyl hydroxylamine, was obtained in 29% isolated yield. In addition, it is important to note that **7** does *not* react alone with benzylamine in CH_2Cl_2 to provide **10**; that is, formation of the product **10** is associated with oxygen transfer from the open hydroperoxide **8** followed by closure of the derived alcohol.

Whilst dihydroartemisinin **2** was initially expected to react in the absence of the primary amine nucleophile, no reaction in fact took place between **2** and Et_3N in MeOH or CH_2Cl_2 at room temperature. However, addition of aqueous 1M KOH induced formation of the *N*-oxide **14**, together with the lactol **17**[7] (26%). This compound arises either via oxygen transfer from the intermediate hydroperoxide corresponding to **3** followed by aldolisation and formation of the hemiacetal, or from the hydroperoxide **4**.[7] In MeOH, with Et_3N under reflux, **2** gave desoxoartemisinin **7** (35%) and the aldehydes **18** and **19**.[12] It was not possible to detect the *N*-oxide;

thermal instability[13] may have precluded this. Formation of all compounds is nevertheless diagnostic of the intermediate open hydroperoxide.[9,12] Formation of desoxoartemisinin **7** is easily rationalized through operation of the base-induced Kornblum-de la Mare process on the peroxyhemiacetal **3** derived from this intermediate.[14]



Whilst base-induced reactions interfere with interception of the hydroperoxide from **2**, the ease of interception of hydroperoxide **8** from **1** is remarkable. In general, *N*-oxide formation is facilitated by electrophilic oxygenating agents such as peracids, or hydroperoxides in the presence of Lewis or protic acids.[13,15] Thus, the artemisinin hydroperoxides will become more potent oxygen transfer agents upon protonation, or on complexation with an electrophilic site, as compared to the current situation. In addition, the hydroperoxides have the potential to undergo reductive cleavage to provide *oxygen* radicals. Thus we like to think that the reactions here are indicative of a biological mode of action - the trioxane *binds* to a protein surface and releases hydroperoxide, which then undergoes heterolytic oxygen transfer to nitrogen or sulfur, or provides hydroxyl radicals which hydroxylate susceptible groups, or abstract hydrogen atoms *etc.* (*cf.* Scheme 1).[16] However, in a biological system, the *key* to the general activity of the 1,2,4-trioxane in **1** and **2** is in the *unique electronic ability of the trioxane* to provide hydroperoxide through generation of an oxo-stabilized cation upon *heterolysis* of the C3-O2 bond (Scheme 3).[9b] It must be noted that tertiary peroxides in general *undergo heterolysis on protonation by acid to provide hydroperoxides.*[17] In this sense tertiary peroxides also should possess antimalarial activity, albeit at lower levels, than do 1,2,4-trioxanes, as heterolytic cleavage to provide hydroperoxide, in so far as it relates to stability of the derived tertiary cation, will be less facile. In addition, those peroxides which have the potential of providing hydroperoxides via retro-Michael reaction upon binding, are active antimalarials.[9b,18]



Scheme 3

The antimalarial activity of **1** and **2** is assumed to be due to reductive cleavage of the *intact* peroxide by ferroheme within the parasite to generate C-centred radicals *eg.* **20** and **21**, the reagents supposed to alkylate biomolecules.[2,19,20] Other species - iron-oxo intermediates, carbonyl compounds, epoxides - arising via iron(II)-mediated decomposition of **1**, **2** or of synthetic trioxanes are also held to serve as parasiticidal agents.[20] Generation of C-centred radicals leading to epoxides and carbonyl compounds is common in reductive cleavage of endoperoxides,[21] yet many such peroxides *do not display appreciable anti-malarial activity.*[22] Although adducts arising via reaction of Mn(II) porphyrins with the putative radical **21** derived from **1** have been characterized,[23] *their very formation implies that the radical 21*, if indeed this is generated by ferroheme within the parasite, *will not have a lifetime sufficient for it to migrate away from the heme to react with biomolecules.*[16] Such radicals also react at a diffusion controlled rate with oxygen, and are oxidized by iron(III).[24]

Results of oxygen transfer experiments conducted on **2** and 10-deoxyartemisinin derivatives (*cf.* Scheme 3) in the presence of Lewis and protic acids will be described elsewhere.

Acknowledgments: The Hong Kong Research Grants Council (Grant HKUST 591/95P) and HKUST (RIG.SC03 95/96) are thanked for most generous financial support.

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