Alkylation of Sulfur Ligand in Cysteinate-Iron Chelates by a 1,2,4,5-Tetraoxane†

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Reaction of a 1,2,4,5-tetraoxane with cysteinate-iron in the presence of excess methyl cysteinate led to formation of sulfur-alkylated methyl cysteinate in 33% yield, illustrating a possible mechanism for tetraoxanes' antimalarial action.

Keywords antimalarial, artemisinin, tetraoxanes, free radicals, alkylation

Introduction

Organic peroxides are of great current interest[1] in malaria chemotherapy due to the wide-spreading and ever-increasing cases of multi-drug resistant malaria. Qinghaosu[2][1 also known as artemisinin, abbreviated as QHS (Fig. 1)] was the first compound of this category that showed great promise as a novel weapon to combat the very serious threat of chloroquine-resisting strains then in the 1970's. Although some more effective derivatives[3] of QHS such as artemether and artesunate were later developed into marketed drugs, much efforts[4,5] are still made in seeking structurally simple organic peroxides that remain at least part of the antimalarial potency of QHS. 1,2,4,5-Tetraoxanes such as those[5] developed by Nojima, Vennestrom, McCullough, and Wataya are among those that possess significant antimalarial activity yet are synthetically much more easily accessible than QHS.

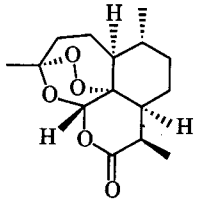

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Fig. 1 Structure of Qinghaosu (**1**).

Although not proven beyond all doubt yet, it is broadly agreed[6] among the investigators in this field that the antimalarial activity of QHS is related to the iron-induced cleavage of the peroxy bond. Such a cleavage leads to formation of transient carbon-centered radicals and under proper conditions may result in alkylation[7,8] of the ligands of the iron species. We already demonstrated earlier that cysteine-iron chelates could effectively induce cleavage of QHS and the cystein ligand was alkylated at the sulfur atom. We reasoned[8] that intraparasitic Fe—S type species might also induce cleavage of QHS and lead to similar alkylation of the sulfur ligand. In that case, the Fe—S species would be covalently modified and hence irreversibly disabled, illustrating a potential mechanism through which QHS kills the intraerythocytic malaria parasite. Tetraoxanes are also found to be active antimalarials. However, no one seems to have studied their cleavage reactions. To examine if the tetraoxanes might also alkylate the sulfur ligands, we undertook the work disclosed below.

Results and discussion

We chose tetraoxane (**2**) as substrate because it appeared to be relatively easy to synthesize compared with other tetraoxanes, with a very common and inexpensive reagent (cyclohexanone) as the starting material. We first tried the one-step procedure (a, Scheme 1) of Xu *et al*.[9] To our surprise, no **2** could be isolated at all. As a compromise we next used the Sanderson's[10] two-step protocol (b and c, Scheme 1). Although this approach required step of reaction, it did work well in our hands, providing us with enough **2** for further study.

The cleavage reaction was carried out in aqueous THF at near physiological temperature. To avoid too high water solubility associated with cysteine itself encountered in the product isolation in previous studies, this time we chose to

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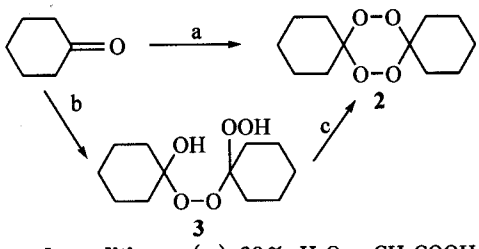
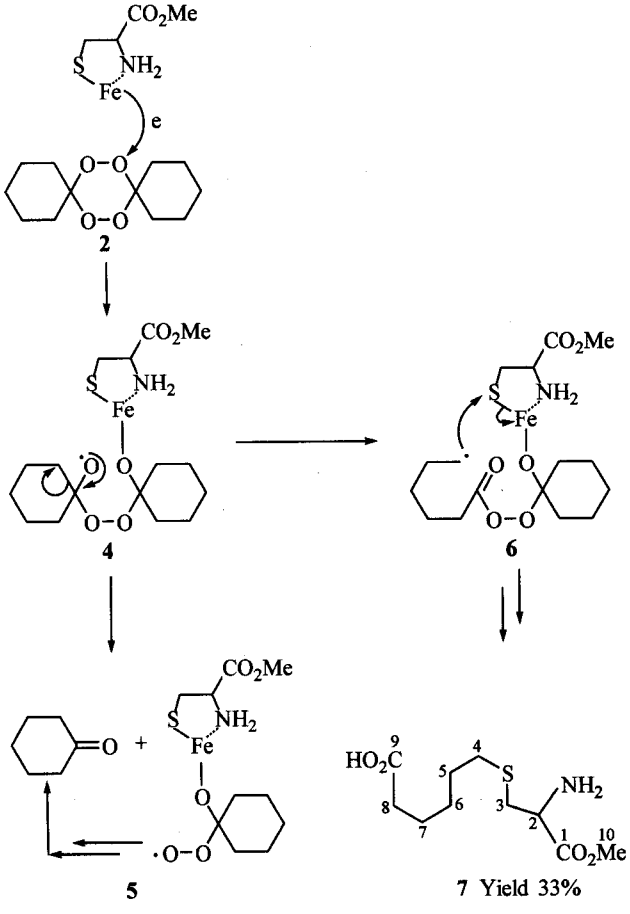
use methyl cysteinate to replace cysteine as we already noticed from many earlier experiments that the reducing property of the two species was almost the same. Thus, a mixture of **2** and methyl cysteinate hydrochloride (2 mol equiv.) in 1:1 (*V*/*V*) H_2O-THF containing NEt_3 (2 mol equiv.) and Fe^{2+} (*ca.* 1.9×10^{-2} mol equiv.) was stirred at 38—40 ℃ until TLC showed full consumption of the starting **2**. The products soluble in organic solvents appeared to be rather complex and difficult to identify due to their small molecular weights/high volatility. However, by reaction with 2,4-dinitrobenzylhydrazine, the presence of cyclohexanone was proven (Scheme 2).

Scheme 1



Reagents and conditions: (a) 30% H_2O_2, CH_3COOH, H_2SO_4; (b) 30% H_2O_2, 2 mol/L HCl, 2 h, r.t.; (c) 10% $HClO_4$/HOAc, 24 h.

Scheme 2 For clarity some of the ligands at the iron ion are not shown. Apart from cyclohexanone and **7**, there were also many other products that were highly volatile and thus difficult to identify.



From the aqueous phase in the work-up a compound that contained both methyl cysteine moiety and many saturated alkane protons was isolated in 33% yield. The ^1H NMR, ^{13}C NMR, IR, and MS spectra all pointed to a derivative of methyl cysteinate with the sulfur atom bonded to a six-carbon chain carrying a carboxylic functionality at the other terminal. Finally, with the assistance of 2D NMR and ESI-MS technologies, the precise structure of this novel compound was assigned as **7**.

Formation of **7** demonstrates that just like QHS-type compounds, the tetraoxanes may also alkylate the ligands in the reducing species and result in irreversible modification of the redox center. This kind of behavior may be responsible for their antimalarial activity as so far proposed for the QHS and arteflene,[11] an analog of anther natural antimalarial agent Yinzaosu.[12]

Experimental

To a 50 mL round-bottomed flask were added **2** (112 mg, 0.49 mmol) and methyl cysteinate hydrochloride (344 mg, 2.0 mmol). The flask was sealed and the air removed with an vacuum pump and the vacuum was released with argon (balloon) several times. Deaired (by bubbling N_2 gas through the liquid for 30 min followed by evacuation with stirring under aspirator vacuum) THF (10 mL), deaired water (10 mL) and NE_3 (0.139 mL) were introduced via syringes, followed by Fe^{2+}-cysteinate stock solution [5.0 mL, prepared from 0.194 mmol of $Fe_2(SO_4)_3$, 50 mL of deaired distilled H_2O, 0.50 mmol of methyl cysteinate hydrochloride and 0.25 mmol of Na_2CO_3]. The solution was then stirred at 38—40 ℃ (bath) overnight. The reaction mixture was concentrated on a rotary evaporator to remove most of the THF. The residue was extracted with EtOAc. The layers were separated. The aqueous phase was further concentrated on a rotary evaporator (under oil pump vacuum) and the residue was chromatographed on reverse phase silica gel (C-18, 50—70 μ), eluting in turn with H_2O, 5%, 10%, 15% and 20% of aqueous MeOH. The effluent fractions containing **7** were combined and evaporated to dryness and further purified on a normal phase silica gel column (eluting with 1 : 15 MeOH/CH_2Cl_2) to afford pure **7** as an oil (44 mg, yield 33%). ^1H NMR ($CDCl_3$, 300 MHz) δ: 5.13 (brs, 3H, NH), 3.76—3.73 (m, 4H, H-10 and H-2), 2.95 (dd, J = 13.5, 4.4 Hz, 1H, H-3), 2.83 (dd, J = 13.6, 7.1 Hz, 1H, H-3), 2.56 (t, J = 7.4 Hz, 2H, H-4), 2.31 (t, J = 7.2 Hz, 2H, H-8), 1.65—1.55 (m, 4H, H-7 and H-5), 1.47—1.40 (m, 2H, H-6); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 177.82 (C-9), 173.79 (C-1), 53.50 (C-10), 52.37 (C-2), 36.64 (C-3), 34.09 (C-4), 32.36 (C-8), 29.22 (C-7), 28.18 (C-6), 24.38 (C-5); (The carbon and proton assignments were made on the basis of 2D experiments COSY and HMQC); FT-IR (film) ν: 3418, 2925, 2854, 1750, 1537, 1444, 1405, 1231, 1005 cm^{-1}; ESI-MS: 250.2 ($[M + H]^+$); ESI-HRMS

calcd for $[C_{10}H_{19}NO_4S+H]^+$ 250.1103, found 250.1108.

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