Synthesis and Cleavage Studies of a 1,2-Dioxolane-type Peroxide[†]

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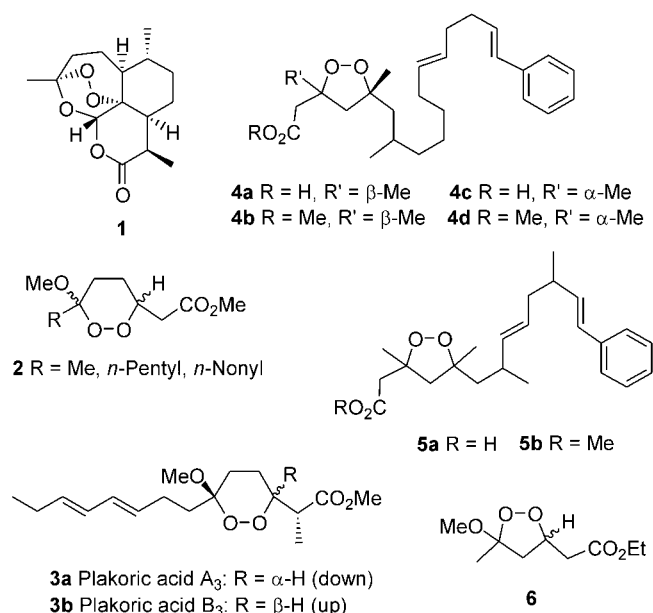
A [1,2]dioxolane-type peroxide was synthesized and tested for its cleavage behavior with Fe^{2+}-cysteinate as a simple model of biological redox species. No S-alkylation product was observed.

Keywords   cyclic peroxide, artemisinin, free radical, cleavage, mechanism

Introduction

Organic peroxides, with qinghaosu[1] (artemisinin, **1**) and its derivatives as the best known examples, are of great current interest[2] in malaria chemotherapy due to the wide-spreading and ever-increasing cases of multi-drug resistant malaria. Although some more effective derivatives[3] of QHS such as artemether and artesunate were later developed into marketed drugs, much effort[4] is still made in seeking structurally simple organic peroxides that remain at least part of the antimalarial potency of qinghaosu.[5] Recently, Kobayashi and co-workers[6] also reported that some simple 6-membered ring peroxides (**2**) related to peroxyplakoric acid (**3**)[7] possessed significant antimalarial activity. Apart from the 6-membered peroxides, naturally-occurring biologically active five-membered peroxides are also known, such as those (**4**, **5**) reported by Davidson[8] and Rinehart,[9] respectively.

It is broadly agreed[10] among the investigators in this field that the antimalarial activity of qinghaosu is related to the iron-induced cleavage of the peroxy bond. Therefore many cleavage studies[11] were carried out. However, to our knowledge, 1,2-dioxolane-type of peroxides has never been studied using single electron induced cleavage. Similar to the qinghaosu-related compounds,[12] some of such five-membered ring peroxides are also known to possess cytotoxicity to certain cancer cell lines, such as those (**4** and **5**) discovered by Davidson[8] and Rinehart,[9] respectively. It is hence interesting to study how the cleavage reactions of the 1,2-dioxolanes may proceed. Herein we wish to report on the synthesis and cleavage of **6**, the first cleavage investigation on the 1,2-dioxolane-type peroxides.

4a R = H, R' = β-Me   **4c** R = H, R' = α-Me
4b R = Me, R' = β-Me   **4d** R = Me, R' = α-Me

2 R = Me, *n*-Pentyl, *n*-Nonyl

5a R = H   **5b** R = Me

3a Plakoric acid A₃: R = α-H (down)
3b Plakoric acid B₃: R = β-H (up)

6

Results and discussion

Compound **6** was chosen as the first target of the cleavage study on the 1,2-dioxolane-type compounds mainly because its relative facile accessibility. Also, we wished to examine whether the Kobayashi's[13] methodology was applicable to the construction of five-membered ring. As shown in Scheme 1, starting from ethyl acetoacetate, protection of the keto carbonyl group according to Langer and Freifeld[14] gave the dioxolane **8** in 66% yield. The ester group was reduced with $LiAlH_4$ in THF and the resulting alcohol **9**[15] was oxidized with IBX[16] into the corresponding aldehyde **10**,[17] which was utilized directly (attempt to purify this compound led to extensive decomposition) in the next step of reaction
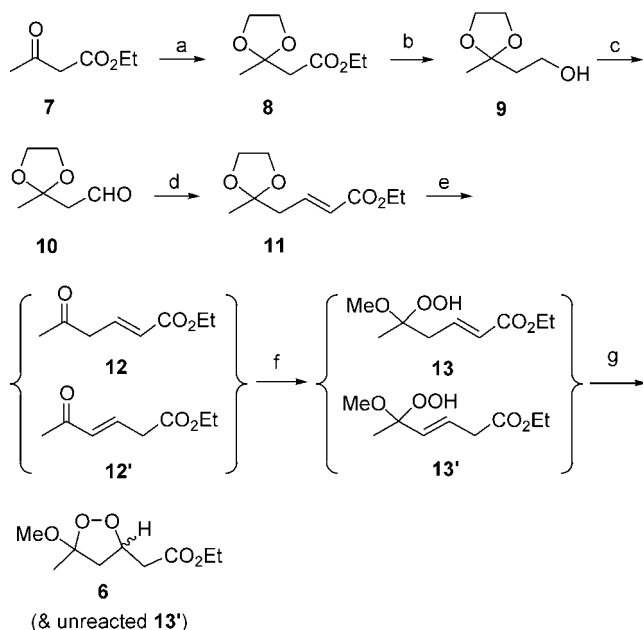
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with the Wittig reagent $Ph_3P{=}CHCO_2Et$ to yield the ethylene glycol protected α,β-unsaturated ester **11** in 42% yield (two-step yield, $E/Z{\approx}9:1$).

Scheme 1

(& unreacted **13'**)

Reagent and conditions: (a) *p*-TsOH/ethylene glycol/toluene, reflux, 66%; (b) LiAH$_4$/THF/0 ℃, 86%; (c) IBX/DMSO/r.t.; (d) Ph$_3$P$=$CHCO$_2$Et/ CH$_2$Cl$_2$, reflux, 42% (from **10**); (e) 80%HOAc-H$_2$O, 60 ℃, 65% (**12 & 12'**); (f) UHP/Sc(OTf)$_3$·XH$_2$O/MeOH/r.t., 38% (**13 & 13'**); (g) HNEt$_2$/ CF$_3$CH$_2$OH/r.t., 45%.

Hydrolytic removal of the ethylene glycol protecting group in **11** was rather difficult. Conventional methods such as pTsOH or PPTS in aqueous acetone did not work at all. H_5IO_6 in diethyl ether gave a complicated product mixture.[18] Aqueous HOAc (80%) appeared to be more effective. About 65% of **12**[19] could be obtained after reaction at 65 ℃ for *ca.* 4 h. However, partial rearrangement of the C—C double bond (leading to **12'**) also occurred as seen from the ^{1}H NMR of the mixture of the two inseparable isomers (3 : 2 mixture). Because in this work we were interested only in testing the cleavage behavior, no efforts were made to improve the synthesis of these compounds. The mixture of the deprotection of the ethylene glycol was used as such in the following reaction carried out under the conditions described by Kobayashi,[13] giving a mixture of **13** and **13'** (almost inseparable). This mixture (not stable) was then treated[13] with HNEt$_2$ in F$_3$CCH$_2$OH to afford the 1,2-dioxolane **6** in 45% yield, along with some "starting material" (presumably **13'**) that was resistant to react in spite of the prolonged time.

The cleavage reaction (Scheme 2) was carried out in aqueous THF at the ambient temperature (*ca.* 25 ℃). To avoid too high water-solubility associated with cysteine itself encountered in the product isolation in previous studies, this time we chose to 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.[20] Thus, a mixture of **6** and methyl cysteinate hydrochloride (2 mol equivalents with respect to the peroxide) in 1 : 1 (*V/V*) H$_2$O-THF containing NEt$_3$ (2 mol equivalents with respect to the peroxide) and a catalytic amount of Fe^{2+} (*ca.* $3.4{\times}10^{-3}$ mol equivalents with respect to the peroxide) was stirred until TLC showed full consumption of the starting **6** (*ca.* 9 h). The products recovered after aqueous workup accounted only for about 1/3 of the mass balance, suggesting formation of products of small molecular weights/high volatility.

Scheme 2
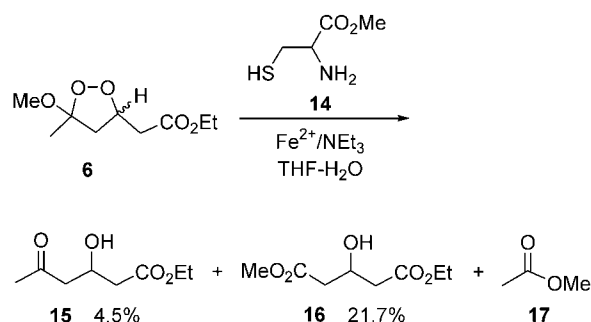
In the organic extracts, only two components were of large enough quantity to allow for isolation and identification. The structures of these two compounds were assigned to (previous known) **15**[21] and **16**,[22] respectively. By GC analysis of the reaction mixture, presence of methyl acetate (b.p. 55—58 ℃) in the reaction mixture was also unambiguously confirmed. The possible routes of formation of three cleavage products are shown in Scheme 3.

The diester **16** and methyl acetate **17** were all obviously derived from the radical anion **18b**. In the case of **16**, a methyl radical must be generated at the same time. However, unlike other peroxides we have examined before (such as the tetraoxane or qinghaosu) no alkylated cysteinate (with the sulfur atom methylated) was isolated. This might reflect the relative short life time of methyl radical (which is more reactive than any other alkyl radicals) in THF (which can easily lose one hydrogen from the carbon atoms α to the oxygen atom).

In principle, accompanying the formation of the methyl acetate **17**, there should be also radical **19** generated at the same time. However, the presence of the FeO— group at the carbon α to the unpaired electron in **19** might open up several further reaction paths, giving such products as **20** and/or **21**. Although so far we have not succeeded in identifying **20** and/or **21** in the product mixture, these possibilities can not be ruled out.

On the other hand, based on the GC analysis it was quite sure that in the cleavage reaction mixture there existed neither acetone **23** nor ethyl acetate **25**. This observation, along with the low yield of **15**, suggested that the single electron was mostly donated to the peroxy bond at the oxygen atom closer to the methoxy group (giving radical anion **18b**), although the steric

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Scheme 3[a]
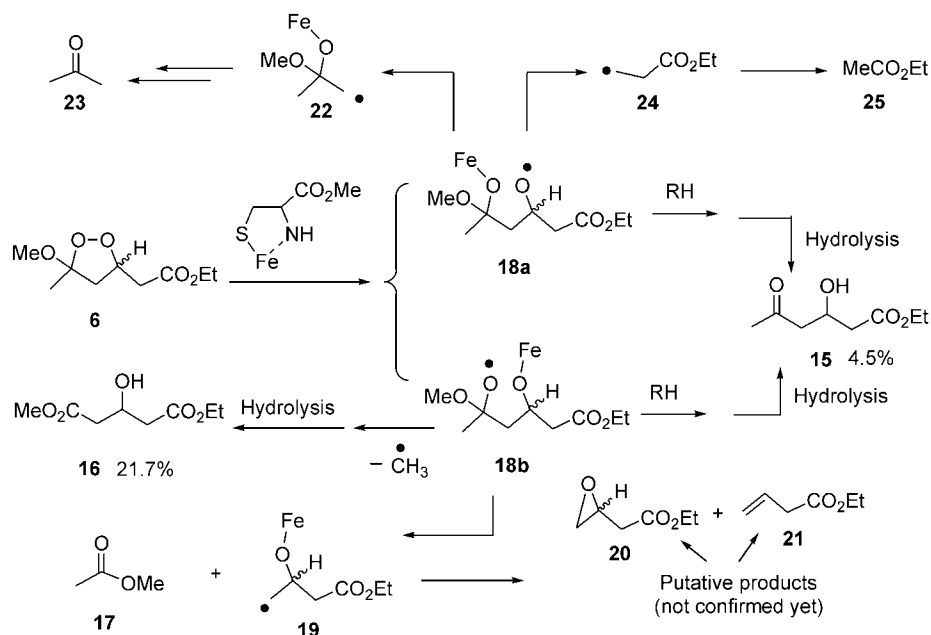
[a] For clarity some of the ligands at the iron ion in most species are not shown. Apart from the known compounds **15**, **16**, and **17**, there were also many other so far unidentified products. RH represents a hydrogen donor in the reaction mixture, which could be a methyl cysteinate or the solvent THF.

hindrance around this oxygen (bonded to a quaternary carbon) was expected to be more significant than around the other one (bonded to a tertiary carbon).

The above results imply that a methyl substituent at a [1,2]dioxolane as in our case may greatly reduce the probability to generate carbon-centered radicals of reasonably long life-time to cause irreversible damage to the redox center that induced the cleavage of the peroxy bond. As a consequence, such a compound might have drastically reduced antimalarial activity.

Experimental

General methods and materials

The ^{1}H NMR spectra were taken on either a Varian Mercury 300 (300 MHz) or a Bruker Avance 300 (300 MHz) NMR spectrometer with CDCl$_3$ as the solvent and Me$_4$Si as the internal standard. The FT-IR spectra were measured on a Nicolet Avatar 360 FT-IR. EI-MS were recorded on an HP 5989A mass spectrometer. ESI-MS spectra were taken on a PE Mariner API-TOF or an Agilent Technologies LC/MSD SL instrument. Elemental analyses were done using an Elementar Vario EL III autoanalyzer. Chromatography was performed on silica gel (300—400 mesh) unless otherwise stated. PE refers to petroleum ether (60—90 ℃). UHP stands for urea-hydrogen peroxide.

Synthesis of ethyl 4-(2-methyl-[1,3]dioxolan-2-yl)-but-2-enoate (11)

To a solution of **9** (2.64 g, 20 mmol) in DMSO (100 mL) stirred at the ambient temperature was added IBX (8.4 g, 30 mmol, 1.5 equiv.). When TLC showed full

consumption of the starting alcohol, the reaction mixture was diluted with EtOAc. The solids were filtered off with suction and the filtrate was washed in turn with aq. NaHCO$_3$, water, and brine, dried over anhydrous Na$_2$SO$_4$. After removal of the solvent on a rotary evaporator, the crude aldehyde was directly dissolved in CH$_2$Cl$_2$ (100 mL) and treated with Ph$_3$P=CHCO$_2$Et (10.3 g, 29.6 mmol, 1.5 equiv.) at reflux. When TLC showed completion of the reaction, the solvent was removed on a rotary evaporator and the residue was chromatographed (eluting with 10 : 1 PE/EtOAc) to give **11** (1.683 g, 42% overall yield) as a colorless oil. The data for the major component (*E*)-**11**: ^{1}H NMR δ: 6.96 (dt, *J*=15.9, 7.5 Hz, 1H), 5.90 (d, *J*=15.3 Hz, 1H), 4.19 (q, *J*=6.9 Hz, 2H), 4.00—3.94 (m, 4H), 2.55 (d, *J*=7.8 Hz, 2H), 1.36 (s, 3H), 1.29 (t, *J*=7.5 Hz, 3H); FT-IR (film) v: 1720, 1183 cm^{-1}; EI-MS *m/z* (%): 185 (M$^+$ − CH$_3$, 8.9), 113 (3.2), 87 (100), 43 (25.1); MALDI-HRMS calcd for C$_{10}$H$_{17}$O$_4$ ([M + H]$^+$) 201.1121, found 201.1134.

Synthesis of ethyl (5-methoxy-5-methyl-[1,2]dioxolan-3-yl)-acetate (6)

A mixture of **11** (242 mg, 1.21 mmol) in H$_2$O (1.0 mL) and glacial acetic acid (4.0 mL) was stirred at 65 ℃ until TLC showed full consumption of the starting ketal. The reaction mixture was then cooled down to the ambient temperature and poured into ice-aqueous NaHCO$_3$ and extracted with diethyl ether. The ethereal layer was separated and washed with brine, dried over anhydrous Na$_2$SO$_4$. After removal of the drying agent and the solvent, the residue was chromatographed (5 : 11, PE/EtOAc) to afford a mixture of **12** and the C=C

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bond shifted isomer as a colorless oil (133 mg, 65% yield). Part of this mixture (122 mg, 0.782 mmol) was dissolved in anhydrous MeOH (17 mL). To the solution were added Sc(OTf)$_3$•nH$_2$O (47 mg, *ca.* 0.1 equiv. with respect to the substrate) and UHP (555 mg, *ca.* 7.5 equiv.). The mixture was stirred at the ambient temperature (*ca.* 15 ℃) for 48 h before diluted with CH$_2$Cl$_2$ (50 mL). Solids were filtered off with suction and the filtrate was passed through a pad of neutral Al$_2$O$_3$. Rotary evaporation gave a residue, which was chromatographed (6 : 1 PE/EtOAc) to yield the unstable hydrogen peroxide **13** & **13'** (69 mg, 38% yield) as a yellowish oil. ^{1}H NMR (taken on a fraction containing mostly **13**) δ: 7.74 (s, OOH, 1H), 6.93 (dt, $J=15.6$, 7.7 Hz, 1H), 5.93 (d, $J=16$ Hz, 1H), 4.19 (q, $J=7.1$ Hz, 2H), 3.36 (s, 3H), 2.70—2.54 (m, 2H), 1.32 (br s, 3H), 1.30 (t, $J=7.8$ Hz, 3H); FT-IR (film) v: 3386, 1717, 1185 cm^{-1}.

The main portion of the above **13/13'** mixture (60 mg, 0.294 mmol) was dissolved in F$_3$CCH$_2$OH (3.0 mL) containing Et$_2$NH (5 μL, 53 μmol) and stirred at the ambient temperature for 8 h. The solvent was removed on a rotary evaporator and the residue was chromatographed (8 : 1, PE/EtOAc) to give **6** (27 mg, 45% yield) as a colorless oil. ^{1}H NMR δ: 4.87 (quint, $J=6.0$ Hz, 1H), 4.16 (q, $J=7.1$ Hz, 2H), 3.32 (s, 3H), 2.95 (dd, $J=12.6$, 7.7 Hz, 1H), 2.80 (dd, $J=15.8$, 7.4 Hz, 1H), 2.53 (dd, $J=15.5$, 6.2 Hz, 1H), 2.30 (dd, $J=12.5$, 4.4 Hz, 1H), 1.50 (s, 3H), 1.27 (t, $J=7.1$ Hz, 3H); FT-IR (film) v: 1736, 1449, 1378, 1188, 1100, 1030 cm^{-1}; ESI-MS m/z: 227.1 ([M＋Na]$^+$). Anal. calcd for C$_9$H$_{16}$O$_5$: C 52.93, H 7.90; found C 53.30, H 8.12 (ESI-HRMS calcd for C$_9$H$_{16}$O$_5$Na ([M＋Na]$^+$) 227.0890, found 227.0891).

Cleavage reaction of 6

To a 50 mL round-bottomed flask were added **6** (94 mg, 0.461 mmol) and methyl cysteinate hydrochloride (159 mg, 0.922 mmol). The flask was sealed and the air removed with a 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 (9 mL), deaired water (9 mL), and NEt$_3$ (0.14 mL) were introduced via syringes, followed by Fe^{2+}-cysteinate stock solution (4.0 mL, containing *ca.* 1.6×10^{-3} mmol of Fe^{2+} ion). The solution was then stirred at the ambient temperature (*ca.* 25 ℃) until TLC showed disappearance of the peroxide (*ca.* 9 h). The reaction mixture was concentrated on a rotary evaporator to remove most of the THF. The residue was extracted with EtOAc, washed with brine, and dried over anhydrous Na$_2$SO$_4$. After removal of the solvent, the residue was chromatographed (8 : 1 to 3 : 1 PE/EtOAc) on silica gel to afford the previously known compounds **15**[21] (17 mg, 0.100 mmol) and **16**[22] (4 mg, 0.021 mmol), both as almost colorless oil. The GC analysis of the reaction mixture before workup also showed many peaks, among them only methyl acetate was unambiguously identified by comparison with an authentic sample.

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