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A Carbonyl Oxide Route to Antimalarial Yingzhaosu A Analogues: Synthesis and Antimalarial Activity

Paul M. O' Neill,*[¥] Natalie L. Searle,[¥] Kaylene J. Raynes,[±] James L Maggs,[±] Stephen A. Ward,[±] Richard C. Storr,[¥] B. Kevin Park[±] and Gary H. Posner[§*]

[¥] Departments of Chemistry and of [±]Pharmacology and Therapeautics , University of Liverpool, P.O. Box 147, Liverpool L69 3GE, U.K.

[§] Department of Chemistry, The Johns Hopkins University, Baltimore MD 21218, USA

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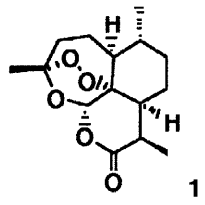
Abstract ; Ozonolysis of R-carvone and *in situ* trapping with primary alcohols ROH (R= Me, Et, Bu, Pent, Oct) produces hydroperoxy ketals (5a-e) as a 1:1 mixture of diastereomers. Cyclisation of these intermediates with catalytic sodium methoxide in methanol produces the corresponding endoperoxide derivatives (6a-6e). The pentyl and octyl endoperoxide derivatives demonstrate reasonable antimalarial potency *in vitro* against the HB3 strain of *Plasmodium falciparum*. A mechanism for antimalarial action involving the formation of a C-centred radical is proposed.

The discovery that artemisinin (**1**) and yingzhaosu A (**2**) possess potent antimalarial activity against chloroquine resistant *Plasmodium falciparum* malaria has led to the development of synthetically more accessible compounds of enhanced activity such as the bis(4-fluorophenyl)cyclopenta-1,2,4-trioxane **3**[1] and arteflene **4**.[2] Other simple endoperoxides possess antimalarial activity, at least *in vitro*, and it has been proposed that the peroxide class of drugs share a common mode of action.[3]



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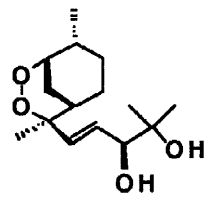

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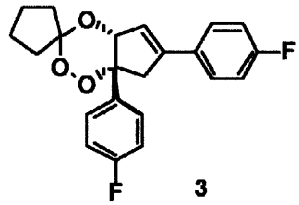

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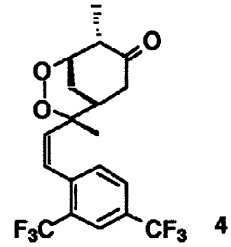

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As part of our interest in the synthesis of novel peroxide antimalarial analogues, we considered the possibility of a carbonyl oxide route (Scheme 1) as a flexible and attractive means of producing derivatives of **2**. Recent studies by the groups of Dussault,[4] Nojima and McCullough[5] have examined the utility of using carbonyl oxide derived hydroxyperoxy ketals as precursors to a range of cyclic peroxide products. In this study, we have examined the reaction of the carbonyl oxide derived from R-carvone with a range of different alcohols. The hydroperoxy ketals **5a-e** generated in this process were converted into the target antimalarial candidates **6a-e** by means of a base-catalysed intramolecular cyclisation reaction (catalytic sodium methoxide in methanol). Table 1 gives yields for the overall trapping and subsequent cyclisation reaction. Notably, for each of the peroxides obtained in the study, only one diastereomer was obtained following intramolecular cyclisation. This initially suggested the possibility of diasteroselective attack on the prochiral face of the carbonyl oxide with the diastereoselective formation of peroxyketal intermediates **5a-e.**

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Scheme 1

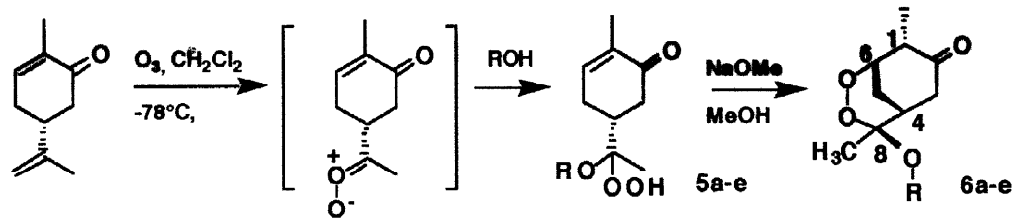
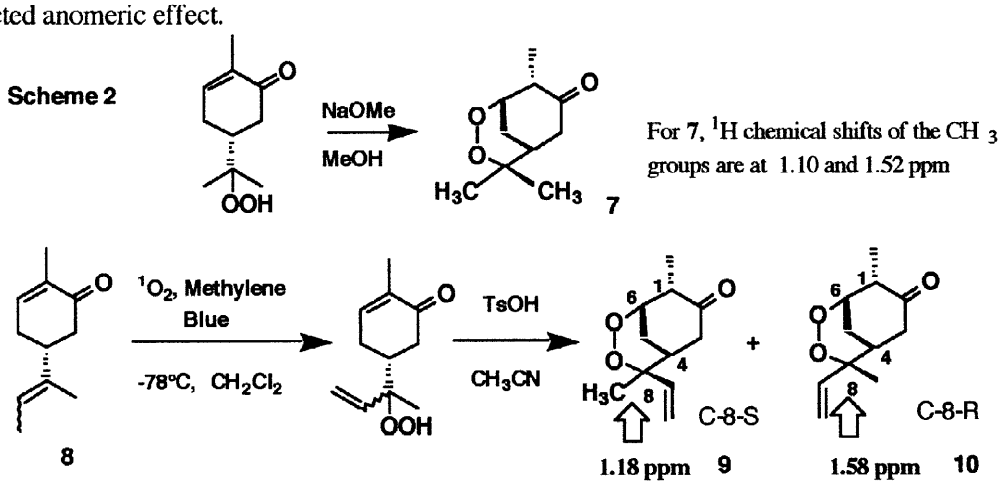


Table 1 Yields for the Synthesis of Endoperoxides 6a-6e

Endoperoxide 6n	6a (R=Me)	6b (R=Et)	6c (R=Bu)	6d (R=Pent.)	6e(R=Oct)[6]
Yield %*	38	32	35	28	24
*Unreacted R-carvone was recovered in all cases following Florisil chromatography					

However, closer examination of the reaction of the carbonyl oxide intermediate with methanol indicated that at the intermediate stage (**5a**, R=Me) there was a 1:1 mixture of peroxyketals as revealed by ^{1}H NMR of the reaction intermediates.[7] This observation is in line with that reported in an earlier study by Schreiber, whereby ozonolysis of (+)-trans-dihydrocarvone afforded a mixture of peroxyketals as a 1:1 mixture in high yield.[8] Thus, the fact that one diastereomeric endoperoxide is obtained in each case following cyclisation suggests thermodynamic equilibration to the more stable isomer. Assignment of the absolute configurations of the chiral centres in **6a-e** was made as follows. The X-ray crystal structure of the related peroxide **7** (Figure 1) shows the peroxide ring to be in the chair form with axial and equatorial methyl groups. The chirality of the C-1 and C-6 positions are as expected from intramolecular attack of the peroxide on the underface of the double bond of the α,β-unsaturated system. The two methyl groups of **7** have characteristic and significantly different ^{1}H chemical shifts (1.10 and 1.52 ppm). Significantly, the C-8 axial methyl group of arteflene **4** (R configuration at C-8) has chemical shift 1.54 ppm. The two related vinyl derivatives **9** and **10**, obtained from **8**, show methyl groups at 1.18 ppm and 1.58 and are assigned, therefore, as S and R respectively. All the new peroxides **6a-e** have methyl signals in the range 1.18-1.22 ppm and so are assigned the S configuration with the alkoxy groups axial, in line with an expected anomeric effect.

Scheme 2



The five new endoperoxide derivatives were subjected to testing against the HB3 strain of *Plasmodium Falciparum in vitro*. All of the derivatives tested had measurable antimalarial potency, with the pentyl and octyl derivatives having approximately a tenth of the antimalarial potency of Ro-42-611, arteflene **4**. It is

noteworthy that activity is apparently increased by lengthening the side-chain function. This suggests that activity may be increased even further by the incorporation of more lipophilic aromatic side-chains.

Figure 1. X-Ray Structure of 7;

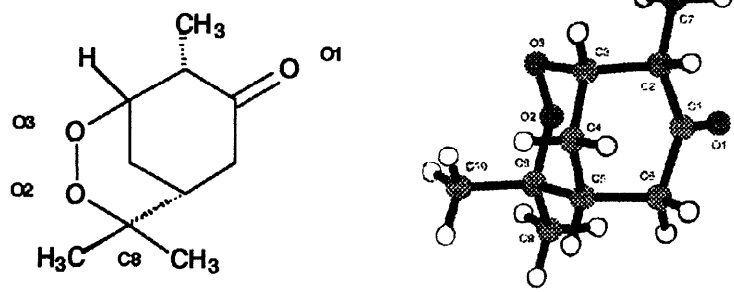
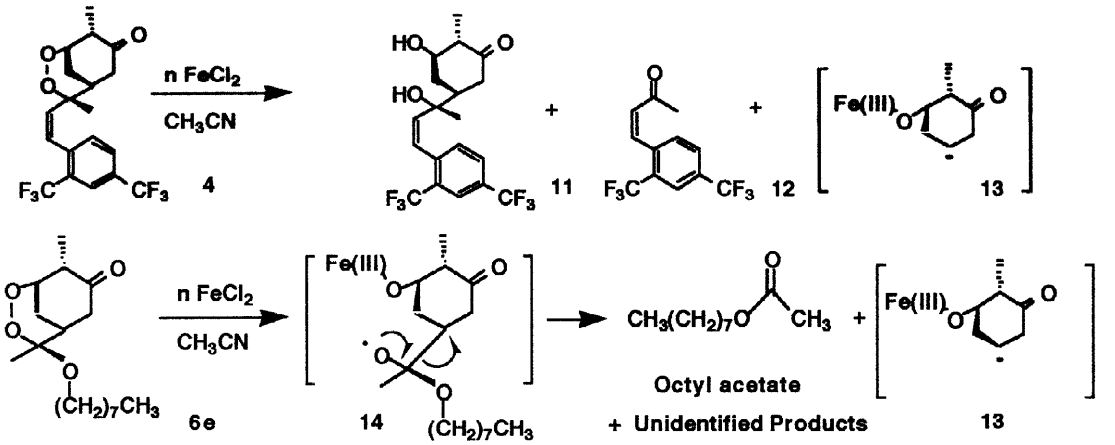


Table 2 *In Vitro* Antimalarial Potency of Peroxide Derivatives vs *Plasmodium falciparum* HB3

Compound	Antimalarial Activity (nM)
6a	666
6b	615
6c	503
6d	126
6e	123
Arteflene	15

Antimalarial activity was assessed by an adaptation of the 48 hour sensitivity assay of Desjardins et al.[9] which uses [^{3}H]-hypoxanthine incorporation as an assessment of parasite growth. IC_{50} values were calculated by interpolation of the probit transformation of the log-dose response curve. Each compound was tested in triplicate to ensure reproducibility of the results.

The malarial parasite *Plasmodium falciparum*, in the intra-erythrocytic stage of its life cycle, digests hemoglobin, which on proteolysis results in the generation of ferriprotoporphyrin IX (heme). Since "free heme" is toxic to the parasite, it is normally removed by oxidative polymerisation to hemozoin, an insoluble pigment. A number of model studies utilising iron (II) salts and heme iron (II) have been reported for the peroxide drug artemisinin with several intermediates being proposed as the ultimate cytotoxic species.[10] More recently, we have proposed a mechanism of action for arteflene based on similiar mechanistic studies using Fe(II) salts.[11] Arteflene, on reaction with $FeCl_2.4H_2O$ produced two products, the two electron reduction diol product **11** and the enone **12**, a surrogate marker for the formation of the cyclohexyl radical **13** (Scheme 3). In order to unravel whether a similiar mechanism of action might be available to our new derivatives, the octyl peroxide product **6e** was subjected to ferrous catalysed fragmentation. In addition to several unidentified, unstable products, a 20% yield of octyl acetate was obtained which was identical to a commercial sample. We therefore propose that these structurally related endoperoxides **6a-e** may have a similiar mechanism to arteflene, in that in the parasite food vacuole, Fe (II) mediated peroxide 1-electron reduction produces the oxyl radical **14** which then fragments to produce the C-centred radical **13** and octyl acetate. It is noteworthy that for artemisinin and bis(aryl)cyclopenta-1,2,4-trioxanes eg. **3**,[1] the formation of C-centred radicals is accompanied by a thermodynamically favourable formation of an ester moiety, as in this example.[10] In conclusion, we have synthesised a number of simple derivatives of yingzhaozu A, some of which possess reasonable antimalarial potency *in vitro*.



Scheme 3 Ferrous Mediated Fragmentation of Arteflene **4** and Peroxide **6e**

The mechanism of action of these derivatives is proposed to be similiar to that of other peroxide antimalarials such as the structurally related arteflene **4**, artemisinin **1** and the "fenozan " derivatives **3**.

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References and Notes

1. Jefford, C.W.; Kohmoto, S.; Jaggi, D.; Timari, J.; Rossier, J.C.; Rudaz, M.; Barbuzzi, O.; Gerard, D.; Burger, U.; Kamalaprija, P.; Mareda, J.; Bernardinelli, G.; Manzanares, I.; Canfield, C.J.; Fleck, S.L.; Robinson, B.L.; Peters, W. *Helv. Chim. Acta*, **1995**, *78*, 647.
2. Hofheinz, W.; Burgin, H.; Gocke, E.; Jaquet, R.; Masciadri, R.; Schmid, S.; Stohler, H.; Urwyler, H. *Trop. Med. Parasitol.*, **1994**, *45*, 261.
3. Meshnick, S.R.; Jefford, C.W.; Posner, G.H.; Avery, M.A.; Peters, W., *Parasitol. Today*, **1996**, *12*, 79.
4. Dussault, P.H.; Zope, U.R. *J. Org. Chem.*, **1995**, *60*, 8218.
5. Ushigoe, Y.; Masuyama, A.; Nojima, M.; Mc Cullough, K.J., *Tetrahedron Lett.* , **1997**, 38, 8753.
6. **6e**; R_f = 0.2 (20% EtOAc/ hexane, silica); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.34 (1H, s, CHOOR), 3.60-3.67 (1H, m, CHOR), 3.43-3.50 (1H, d, J = 4.40 Hz), 2.84-2.90 (1H, m,), 2.60-2.66 (1H, m), 2.40-2.47 (2H, m), 2.23 (1H, m), 1.72-1.77 (1H, m), 1.55-1.63 (3H, m), 1.25-1.40 (m, 12H), 1.18 (3H, s, CH_3), 0.866 (3H, t, CH_2CH_3); ^{13}C NMR 208.23, 104.12, 81.53, 61.09, 48.71, 43.59, 37.43, 31.82. 29.80, 29.40, 29.26, 28.19, 26.23, 22.62, 17.99, 14.03, 10.89; LCMS 614 ($2M^+ + NH_4$, 34), 316 ($M^+ + NH_4$, 100), 299 (36), 169 (25).
7. ^{1}H NMR of the intermediate hydroperoxy ketals revealed two methoxyl signals at 3.34 ppm and 3.32 ppm
8. Schreiber, S.L.; Liew, W.F., *Tetrahedron Lett.* , **1983**, *24*, 2363.
9. Desjardins, R.E.; Canfield, J ; Haynes, D.; Chulay, D.J. , *Antimicrob. Agents Chemother.*, **1979**, *16*, 710.
10. For the first report of a carbon-centered radical intermediate in iron (II) promoted reductive cleavage of an antimalarial trioxane see: Posner, G.H.; Cumming, J.N.; Ploypradith, P.; Oh. C.H. *J. Am. Chem. Soc*, **1995**, *117*, 5885.
11. O' Neill, P.M.; Bishop, L.P.; Searle, N.L.; Maggs, J.L.; Bray, P.G.; Ward, S.A.; Park, B.K.,*Tetrahedron Lett.* , **1997**, *38*, 4263.
Single Crystal X-RAY Analysis of (7) $C_{10}H_{16}O_3$. Data were collected with a Siemens R3m/V diffractometer. Wavelength 0.71073 A, Temperature, 153K. Crystal system, space group = monoclinic, P2 (1). Cystal size 0.35 x 0.10 x 0.08 mm, a = 6.429(3) b = 9.469(4) c = 8.526 (4). A total of 1373 reflections were collected in the 5°<2<45° range using 0.093 ω /2θ scans. 1247 reflections were unique. Lorentz and polarization but not absorbtion co-efficients were applied. The structure was solved by direct methods (SHEXS-86). The refinement was converged to R1 = 5.82% wR2 = 20.91 and a final difference map grater than 0.51 e/A. Complete details of the structure are available at request from the Cambridge Crystal Data Centre, 12 Union Road, Cambridge CB2 1EZ, England