Synthesis of some Stable Ozonides with Anti-malarial Activity

Luiz-Claudio de Almeida Barbosa,[a] David Cutler,[b] John Mann,[b] Geoffrey C. Kirby[c] and David C. Warhurst[c]

[a] *Departamento de Quimica, Universidade Federal de Vicosa, 36570, Vicosa-MG, Brazil*
[b] *Department of Chemistry, Reading University, Whiteknights, Reading RG6 2AD, UK*
[c] *Department of Medical Protozoology, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK*

We describe the synthesis of variously substituted 8,9,10,11-tetraoxatricyclo[5.2.1.$1^{2,6}$]undecan-4-ones. Several of these stable ozonides exhibited activity (IC_{50}'s of 10–20 μg cm^{-3}) against a chloroquine-resistant strain of the malaria parasite *Plasmodium falciparum*.

Several years ago we synthesised (completely by accident) the ozonide **1** by ozonolysis of 2,4-dimethyl-8-oxabicyclo[3.2.1]oct-6-en-3-one **3**.[1] This ozonide was unusually stable and survived complete structure elucidation including an X-ray structure determination. The recent intense research activity[2] centred upon the anti-malarial agent artemisinin **2** (quingaosu, ex. *Artemisia annua*), prompted us to carry out a simple computer modelling comparison of structures **1** and **2**† (see Fig. 1). The

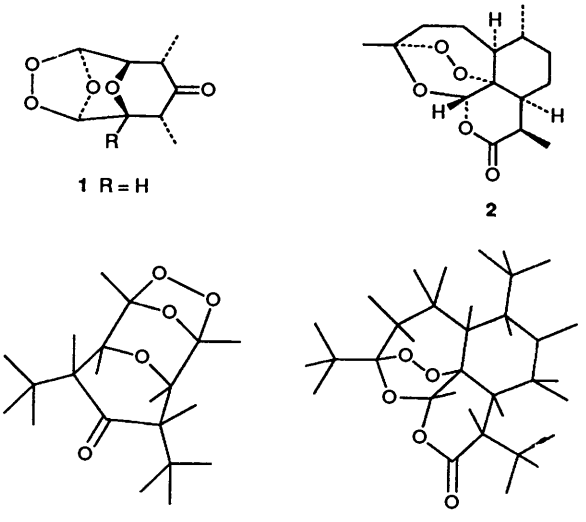
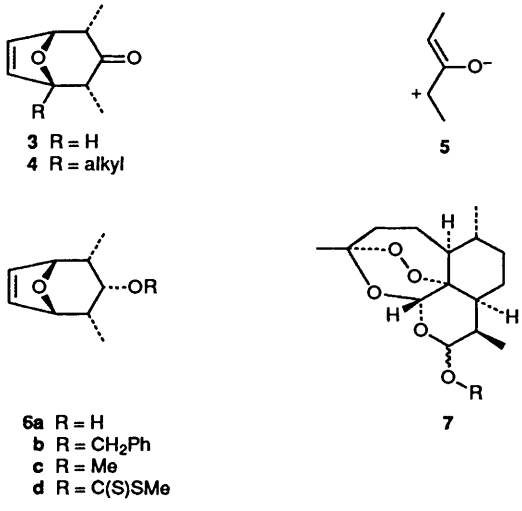
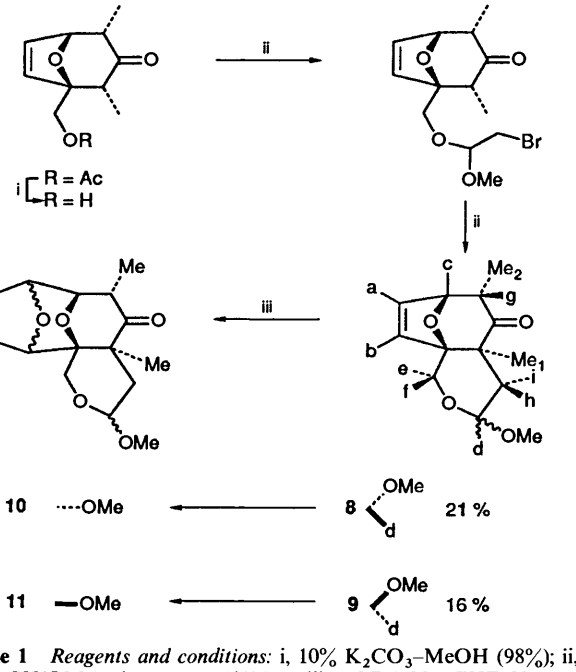


Fig. 1 Minimised structures of the ozonide **1** and artemisinin **2**.



root mean square difference calculations, based on all five oxygens of both structures, gave 'fits' of *ca.* 0.5 Å. This



Scheme 1 *Reagents and conditions:* i, 10% K_2CO_3–MeOH (98%); ii, $BrCH_2CH(OMe)_2$–benzene (64%); iii, LDA/NaI/THF–HMPA (1:1); iv, ozone–DCM at −5 °C for 30 min (*ca.* 70%)

encouraged us to have compound **1** evaluated for antimalarial activity which it was found to exhibit to high degree (*ca.* 10 μg cm^{-3}).‡ The synthesis and evaluation of other, more complex ozonides is the subject of this communication. Most of the substrates for ozonolysis were prepared from 1-substituted 2,4-dimethyl-8-oxabicyclo[3.2.1]oct-6-en-3-ones **4**, available from the cycloadditions of the oxyallyl cation **5**[4] with the requisite furans. Ozonolyses were usually carried out in dichloromethane (DCM) at −5 to 0 °C, and the ozonides either crystallised directly from the reaction mixture or were isolated, following evaporation of the DCM, and recrystallised. All the ozonides were apparently stable at room temperature, and gave clean m.p.s > 100 °C. In their ^1H NMR spectra they exhibited signals at δ 5.4–5.75 (2 s, 1-H and 7-H), together with the other expected proton signals. Interestingly, when the ketone **3** was reduced ($NaBH_4$ in ethanol) to the axial alcohol **6a**, and the benzyl and methyl ethers prepared **6b**, **c**, these gave intractable polymer

† We thank James Crabbe for the computer modelling carried out using Desktop Molecular Modeller, version 2, OUP, 1991.

‡ Compounds were tested *in vitro* against *Plasmodium falciparum* (K1 strain; chloroquine resistant; from Thailand) at concentrations up to 500 μg cm^{-3}, and the results were recorded as IC_{50}'s in μg cm^{-3}. See ref. 3 for details of the experimental protocol.

materials but no stable ozonides. Similarly, when the carbonyl group was removed completely, *via* the xanthate **6d** and reaction with tributyltin hydride, this too gave only polymeric material upon ozonolysis. We suggest that an interaction between an oxygen lone pair and the carbonyl π electron system helps to reduce the nucleophilicity of the bridgehead oxygen, thus stabilising the ozonides against destruction by this nucleophilic centre.

In addition, we have prepared a number of ozonides that more closely resemble the lactol ethers **7** derived from artemisinin, which have been shown to have greater anti-malarial activity.[5] Synthesis of the bicyclic alkenes **8** and **9** and their respective ozonides **10** and **11** (Scheme 1) are representative. These compounds had the expected structural and spectroscopic properties,* and their minimised structures more closely resembled **3** than did compound **1**. All the ozonides were evaluated *in vitro* for anti-malarial activity using a multi-resistant strain of *Plasmodium falciparum* from Thailand.[3] The results are shown in Table 1. Clearly the simpler compounds possess the best activity, and there is a suggestion that increasing the lipophilicity of the compounds could be beneficial. Further compounds are being prepared for evaluation.

Typical Experimental for Ozonization.—Ozone was passed through a solution of the alkene (2 mmol) in light petroleum (35 cm^3) held at 0 °C. After *ca.* 10 min the reaction was judged to be complete (by TLC analysis), and the solvent was removed under reduced pressure to leave the ozonide as a white crystalline solid, which could (usually) be recrystallised from dichloromethane–ether (1:1).

Table 1 Anti-Malarial activities of ozonides

Compd.[a]	IC_{50} (μg cm^{-3})[b]
1 (R = H)	18.2
1 (R = Et)	11.9
1 (R = C_7H_{15})	26.2
1 (R = CH_2OAc)	Inactive (> 500)
1 [R = CH(OMe)Ph]	Inactive (> 500)
10	250
11	Inactive (> 500)

[a] For comparison: artemisinin **2** IC_{50} 10^{-3} μg cm^{-3} and quinine IC_{50} 0.18 μg cm^{-3}. [b] Each result is the mean of two determinations; errors were ± 10%).

Acknowledgements

We thank the British Technology Group and the Wellcome Trust for generous financial support and Dr. Oliver Howarth for numerous NMR studies. David Warhurst is supported by the PHLS.

References

1 W. J. Cummins, M. G. B. Drew, J. Mann and E. B. Walsh, *J. Chem. Soc., Perkin Trans. 1*, 1983, 167.
2 D. L. Klayman, *Science*, 1985, **228**, 1049; W.-S. Zhou and X.-X. Xu, in *Studies in Natural Products Chemistry*, ed. Atta-ur-Rahman, 1989, vol. 3, Elsevier, Amsterdam; A. R. Butler and Y.-L. Wu, *Chem. Soc. Rev.*, 1992, **21**, 85.
3 R. M. Ekong, G. C. Kirby, G. Patel, J. D. Phillipson and D. C. Warhurst, *Biochem. Pharmacol.*, 1990, **40**, 297.
4 J. Mann, *Tetrahedron*, 1986, **42**, 4611.
5 A. J. Lin, L-q. Li, S. L. Anderson and D. L. Klayman, *J. Med. Chem.*, 1992, **35**, 1639 and refs therein.

Paper 2/05889H
Received 4*th November* 1992
Accepted 4*th November* 1992

* Compound **8** had the following ^1H NMR characteristics ($CDCl_3$, 400 MHz): δ 0.94 (d, 3 H, *J* 7 [*J* values in Hz], 2-Me), 1.24 (s, 3 H, 1-Me), 1.53 (d, 1 H, *J* 13.7, H^i), 2.29 (dd, 1 H, *J* 13.7 and 9.6, H^h), 2.84–2.95 (m, 1 H, H^g), 3.35 (s, 3 H, OMe), 3.57 (d, 1 H, *J* 12.2, H^f), 4.12 (d, 1 H, *J* 12.2, H^e), 4.78 (d, 1 H, *J* 4.1, H^d), 4.86 (dd, 1 H, *J* 5.2 and 1.8, H^c), 6.11 (d, 1 H, *J* 6.1, H^b) and 6.36 (dd, 1 H, *J* 6.1 and 1.9, H^a).

Compound **9** had the following ^1H NMR characteristics ($CDCl_3$, 400 MHz): 0.96 (d, 3 H, *J* 7, 2-Me), 1.16 (s, 3 H, 1-Me), 1.54 (dd, 1 H, *J* 12.8 and 2.2, H^i), 2.14–2.19 (m, 1 H, H^h), 2.94–2.97 (m, 1 H, H^g), 3.48 (2, 3 H, OMe), 3.91 and 4.00 (2 d, 2 H, *J* 12.7, H^e and H^f), 4.59 (dd, 1 H, *J* 9.6 and 2.3, H^d), 4.87 (dd, 1 H, *J* 5.1 and 1.8, H^c), 6.07 (d, 1 H, *J* 6.1, H^b) and 6.38 (dd, 1 H, *J* 6.1 and 1.8, H^a). The ozonides **10** and **11** possessed similar NMR spectra to the parent alkenes except for the disappearance of the alkene signals, and appearance of the expected singlets in the region δ 5.4–5.7. Mass spectral data (chemical ionisation) for the ozonides showed $M^+ + NH_4$ at 304.1396 for **10** and 304.1400 for **11** ($C_{13}H_{18}O_7 + NH_4$ requires 304.1390).