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# The structure and antimalarial activity of dispiro-1,2,4,5-tetraoxanes derived from (+)-dihydrocarvone

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ABSTRACT

An unsaturated dispiro 1,2,4,5-tetraoxane formed by peroxidation of (+)-dihydrocarvone was converted into four structurally diverse derivatives. X-ray crystallographic analysis shows that the structures possess central tetraoxane rings with spiro-2,5-disubstituted cyclohexylidene substituents and 6-membered rings in classical chair conformations. As polarity in the tetraoxane series increased, in vitro potency against *Plasmodium falciparum* decreased.

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The semisynthetic artemisinins derived from artemisinin (ART) rapidly reduce parasite burden and are particularly effective when used in 3-day artemisinin combination treatment (ACT) regimens.[1] At the core of the complex structure of artemisinin is its pharmacophoric 1,2,4-trioxane heterocycle. Recognition of this key structural element led to the quest to identify other more synthetically accessible peroxides[2–5] as potential antimalarials. As exemplified by WR 148999 (**1**),[6] one of the first of these were the dispiro-1,2,4,5-tetraoxanes (Fig. 1). Building on the discovery of **1**, significant efforts in the synthesis and evaluation of symmetrical[7–11] and more recently, unsymmetrical[12–16] tetraoxanes have resulted in the discovery of **2**[17] and **3**,[18] each of which has promising antimalarial potential.

Our previous data[6,9,19] demonstrate that, like WR 148999, tetraoxanes with flanking equatorial alkyl groups at the 1 and 10 positions provide substantial steric hindrance to the peroxide bonds and are required for good in vivo antimalarial activity. We also observed[9] that 1,4,10,13-tetralkyl substituted tetraoxanes had relatively high in vivo activities, although they were very lipophilic and offered little scope for chemistry to access more polar derivatives. Analogous to the diastereoselective formation of 1,2,4-trioxanes from reactions of endoperoxides with several chiral cyclohexanones,[20] we envisioned that (+)-dihydrocarvone, with its stereochemically defined α-methyl and isopropenyl substituents, could provide a solution as a starting material for the synthesis of an easily functionalized unsaturated tetraoxane. In this Letter, we describe the chemistry,[21] structure, and antimalarial activity of a set of dispiro-1,2,4,5-tetraoxanes derived from (+)-dihydrocarvone (**4**).

Using the method of McCullough et al.[22] diene tetraoxane **5** was prepared by peroxidation of (+)-dihydrocarvone (**4**) in $H_2SO_4$/$CH_3CN$ and was isolated as a single (vide infra) stereoisomer in low yield (Scheme 1). The crude yield of **5** was estimated to be 50% or more, but conversion of the initially formed viscous oil to a crystalline solid was inefficient. Ozonolysis of **5** in methanol followed by reduction with triphenylphosphine gave dione tetraoxane **6** in 77% yield. Reductive amination of **6** using $NH_4OAc$ and $NaBH_3CN$ in methanol followed by salt formation gave a low yield

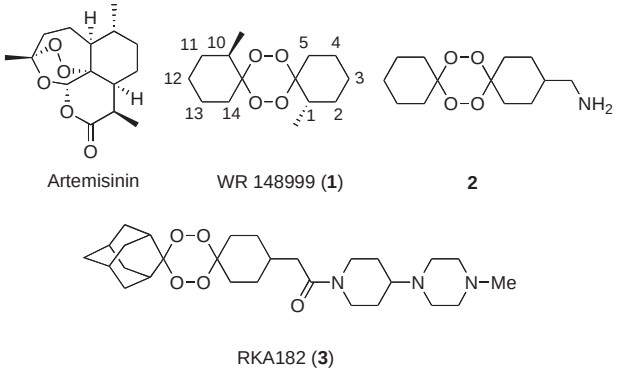


**Figure 1.**

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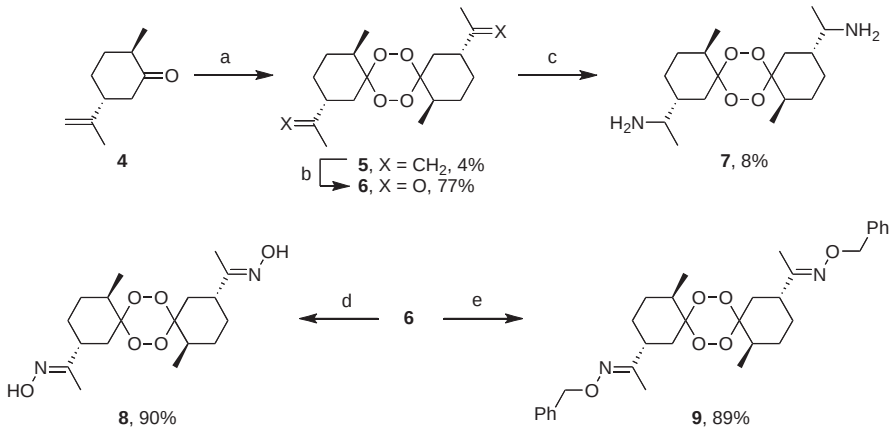


**Scheme 1.** Reagents and conditions: (a) 50% $H_2O_2$, $H_2SO_4$, $CH_3CN$, −20 °C; (b) $O_3$, $CH_3OH/CH_2Cl_2$ (1:1), −78 °C, then TPP, $CH_2Cl_2$, −78 °C to rt ; (c) $NH_4OAc$ (20 equiv), $NaBH_3CN$ (3 equiv), $CH_3OH$, rt, 48 h; (d) $NH_2OH{\cdot}HCl$, pyridine, $CH_3OH$, rt, 24 h; (e) $NH_2OCH_2Ph{\cdot}HCl$, pyridine, $CH_2Cl_2/CH_3OH$ (1:1), rt, 24 h.

of diamino tetraoxane dimesylate **7** as a 2:1 mixture of diastereomers. Treatment of **6** with $NH_2OH$ hydrochloride or $NH_2OCH_2Ph$ hydrochloride provided the corresponding *anti* bis-oxime **8** and bis-oxime ether **9** in high yields.

In order to unambiguously identify the stereochemistry of target tetraoxanes **5**–**8**, we prepared the higher molecular weight bis-oxime ether **9** for X-ray crystallographic structural analysis,[23] even though our previous NMR[8,24] and X-ray[19] data reveal that tetraoxanes synthesized from 2-substituted cyclohexanones, as exemplified by tetraoxane **1**, are predominately formed as a single centrosymmetric isomers.

Single crystals of tetraoxane **9** suitable for X-ray crystallographic analysis were grown from dichloromethane to petroleum ether (bp 60–80 °C) solution by slow evaporation. The X-ray diffraction data were collected on a Brucker X8 diffractometer equipped with a CCD area detector at 160 K using graphite-monochromated Mo Kα $\lambda = 0.71073$ Å. The structure was solved by direct methods and refined using least-squares techniques. All crystallographic calculations and preparation of structure plots and tables were carried out using the SHELXTL PC suite of programs. (Sheldrick, G. M. SHELXTL PC (vers. 5.1), Brucker AXS: Madison, WI, USA.)

The crystal structure[25] consists of discrete molecules of **9** which are comprised of a central tetraoxane ring with spiro-2,5-disubstituted cyclohexylidene substituents (Fig. 2). Each of the six-membered rings adopts a classical chair conformation. Although the absolute configurations of the chiral centres in **9** could not be determined unambiguously from the X-ray data, it is assumed that they are structurally correlated with those of the starting material, (+)-dihydrocarvone (**4**), and were assigned as the *R*-configuration. Thus, despite similarities in the locations of the methyl groups at C(3) and C(9), the molecular structure of **9** cannot be centrosymmetric like that of **1**. All derived geometrical parameters for **9** are within expected ranges.

In vitro and in vivo antimalarial activities[8] were measured using the chloroquine-resistant K1 and chloroquine-sensitive NF54 strains of *Plasmodium falciparum* and *Plasmodium berghei*-infected mice (Table 1). Groups of three *P. berghei*-infected mice were treated one day post-infection with 100 mg/kg oral (po) doses of compounds dissolved or suspended in a solubilizing 3% ethanol and 7% Tween 80 vehicle. Antimalarial activity was measured by percent reduction in parasitemia on day three post-infection compared to an untreated control group.

As manifested by log *P*/*D* values, we observed that as polarity increased in the tetraoxane series, in vitro potency decreased; for example, the hydrophilic diamino tetraoxane **7** was the least potent compound and the hydrophobic diene tetraoxane **5**, like **1**, had $IC_{50s} \leqslant 10$ ng/mL. Similarly, we observed that **5**, the most lipophilic tetraoxane of the series, had the highest in vivo antimalarial activity, a trend that is commonplace in diverse classes of antimalarial peroxides.[4] The order-of-magnitude lower potency of **7** and **8** compared to **6** suggests that H-bond acceptors, but not H-bond donors, are favored. Despite the low potency of **7** against *P. falciparum* in vitro, this tetraoxane had relatively high activity in vivo, better than that of artemisinin, suggesting that it must have attained relatively high plasma levels relative to its more potent and lipophilic analogs **5** and **6**. In summary, **5** had the best overall antimalarial

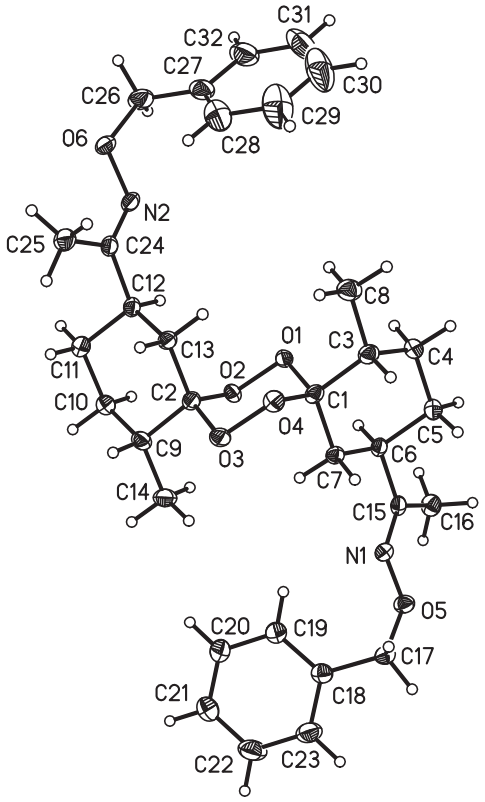


**Figure 2.** X-ray crystal structure of **9**. Crystal data for $C_{32}H_{42}N_2O_6$: $M = 550.68$, colorless blocks, crystal size $0.80 \times 0.65 \times 0.60$ mm$^3$, monoclinic, space group $P2_1$, $a$ 10.7247 (5), $b$ 11.3299 (6), $c$ 12.3872 (7) Å, $\beta$ 102.341 (3)°, $U$ 1470.39 (13) Å$^3$, $Z = 2$, $D_{calcd}$ 1.244 g cm$^{-3}$, $F(0\,0\,0)$ 592, $\mu$(Mo Kα) 0.085 mm$^{-1}$, 51,801 reflections measured, 6306 unique ($R_{int}$ 0.0710) which were used in all calculations. The final discrepancy factors were: $R_1 = 0.0454$ and $wR(F^2) = 0.0976$ (for data with $I > 2\sigma(1)$).

**Table 1**
Activity of **5**–**8** against *P. falciparum* in vitro and *P. berghei* in vivo

| Compd | $IC_{50}$ (ng/ml)[a] | | Activity (%)[b] po | Log *P*/*D*[c] |
|---|---|---|---|---|
| | K1 | NF54 | | |
| None | — | — | 0 | — |
| **1**[d] | 7.2 | 10 | 99.7 | 4.1 |
| **5** | 6.9 | 6.6 | 99.9 | 6.5 |
| **6** | 2.1 | 2.1 | 63 | 2.4 |
| **7** | 54 | 82 | 99.1 | −1.5 |
| **8** | 30 | 18 | <40 | 2.9 |
| ART[d] | 2.8 | 3.4 | 98 | 2.2[e] |

[a] Mean from $n = 2$–3 against chloroquine-resistant (K1) and chloroquine-sensitive (NF54) strains of *P. falciparum*.
[b] Groups of three *P. berghei*-infected NMRI mice were treated orally one day post-infection with trioxolanes (100 mg/kg) dissolved or suspended in 3% ethanol and 7% Tween 80. Antimalarial activity was measured by percent reduction in parasitemia on day 3 post-infection. Individual measurements generally differed by less than 10%.
[c] Calculated values for log *P*/*D* were obtained using ACD/Labs software V9.04.
[d] Data from Dong et al.[8]
[e] Experimentally determined value by Augustijins et al.[26]

profile, but it was no more effective than prototype **1**. In ongoing[18] and future researches, we anticipate substantial progress from a more flexible tetraoxane design strategy made possible by new methods[15] for the synthesis of unsymmetrical tetraoxanes.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.113.

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