Methyl-Substituted Dispiro-1,2,4,5-tetraoxanes: Correlations of Structural Studies with Antimalarial Activity

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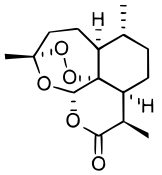
Two tetramethyl-substituted dispiro-1,2,4,5-tetraoxanes (7,8,15,16-tetraoxadispiro[5.2.5.2]-hexadecanes) **3** and **4** were designed as metabolically stable analogues of the dimethyl-substituted dispiro-1,2,4,5-tetraoxane prototype WR 148999 (**2**). For a positive control we selected the sterically unhindered tetraoxane **5** (7,8,15,16-tetraoxadispiro[5.2.5.2]hexadecane), devoid of any substituents. Tetraoxanes **3** and **4** were completely inactive in contrast to tetraoxanes **2** and **5**. We hypothesize that the two inactive tetraoxanes possess sufficient steric hindrance about the tetraoxane ring due to the two additional axial methyl groups to prevent their activation to presumed parasiticidal carbon radicals by inhibiting electron transfer from heme or other iron(II) species. For each of the tetraoxanes **2**−**4**, the tetraoxane and both spirocyclohexyl rings are in a chair conformation and the bond lengths and angles are all quite normal except for the C1−C2 bond which is slightly lengthened. Comparison of the modeled and X-ray structures for tetraoxanes **2**−**5** reveals that molecular mechanics (MMX and MM3) and 3-21G* calculations each gave accurate structural parameters such as bond lengths, bond angles, and dihedral angles. In contrast, semiempirical methods such as AM1 gave poor results.

Introduction

The discovery of artemisinin (qinghaosu, **1**), a naturally occurring endoperoxide sesquiterpene lactone,[1] initiated a substantial effort to elucidate its molecular mechanism of action[2] and to identify novel antimalarial peroxides.[3] Many synthetic 1,2,4-trioxanes have since been prepared, some with promising antimalarial potential.[2] There is now considerable evidence that heme or other iron(II) species are required for reductive activation of these antimalarial peroxides into carbon free radicals which are presumed to convey their parasiticidal effects.[2,4−8]



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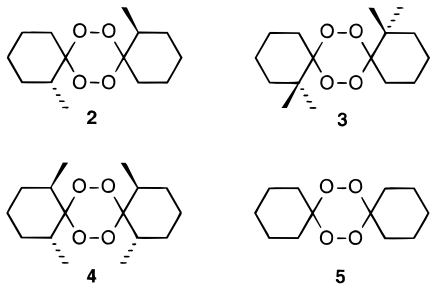
Among the many synthetic antimalarial peroxides, dispiro-1,2,4,5-tetraoxanes such as **2** (WR 148999) are notable in that they differ considerably in structure from artemisinin and are readily prepared in one step from substituted cyclohexanones.[9] Tetraoxane **2** possesses antimalarial activity comparable to **1**[10] but, like artemisinin,[11] has poor oral antimalarial activity.[12] We attribute the low oral activity of **2** to inactivating hepatic or gut metabolism.[12] Therefore, we set out to identify a metabolically stable analogue based on the hypothesis that the tetraoxane peroxide bonds of **2** were the point of metabolic attack. We envisioned that analogues of tetraoxane **2** with greater steric bulk flanking the peroxide oxygen atoms might serve this purpose.



2 **3** **4** **5**

Using tetraoxane **2** as a prototype, we designed target dispiro-1,2,4,5-tetraoxanes (7,8,15,16-tetraoxadispiro-[5.2.5.2]hexadecanes) bearing two additional methyl groups (**3**, **4**). For a positive control we selected the sterically unhindered tetraoxane **5**, devoid of any substituents. In this work, we also desired to unambiguously identify the structures of tetraoxanes **2**−**4** by X-ray crystallographic structural analysis, even though NMR analysis provides strong evidence that tetraoxanes formed from 2-substituted cyclohexanones, as exemplified by tetraoxane **2**, are formed exclusively as single centrosymmetric isomers.[13,14] Moreover, X-ray crystallographic analysis would also allow us to test the postulate[13] that the spirocyclohexyl rings in tetraoxane

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Table 1. Comparison of Modeled and X-ray Crystallographic Structures for Tetraoxane **2**

	MMX	MM3	3-21G*	crystal
	Bond Lengths (Å)			
O1−O2	1.477	1.457	1.466	1.478
C1−O1	1.421	1.429	1.448	1.436
C1−O2A	1.420	1.429	1.448	1.430
C1−C2	1.551	1.535	1.530	1.542
C1−C6	1.545	1.530	1.518	1.518
	Bond Angles (deg)			
O1−C1−O2A	108.7	106.8	107.0	108.0
C1−O1−O2	109.2	107.7	107.9	107.2
C2−C1−C6	109.4	109.9	112.7	113.2
C1−C2−C3	111.2	110.9	108.8	110.3
C1−C6−C5	112.0	112.2	109.9	111.6
	Dihedral Angles (deg)			
C1−O1−O2−C1A	−61.3	−65.2	−64.6	−64.2
O2−O1−C1−O2A	60.9	64.6	63.9	64.5
O1−C1−O2A−O1A	−60.9	−64.6	−64.1	−64.7
O1−C1−C2−C3	176.4	177.9	−179.5	173.9
C5A−C6A−C1A−O2	−63.3	−64.0	−61.0	−65.8
C6A−C1A−O2−O1	−62.3	−58.4	−59.4	−60.4
O2−O1−C1−C6	−63.1	−59.2	−59.5	−60.9
O1−O2−C1A−C2A	177.1	179.9	177.2	175.7
O1−C1−C6−C5	−176.4	−175.6	−178.5	−171.6
O2−C1A−C2A−C3A	66.7	67.2	65.8	72.6

3 exist in a spirocyclohexyl twist-boat conformation in order to minimize steric repulsion between the methyl groups and the peroxide oxygen lone pairs.

Results and Discussion

X-ray Crystallography. For each of the tetraoxanes **2**−**4**, their respective molecular structures in the solid state are ideally centrosymmetrical, with their point group and crystallographic inversion centers coincident. The bond lengths and angles observed for compounds **2**−**4** are all within normal ranges (see Table 1).

As predicted by NMR[13] and our molecular modeling experiments (vide infra), all rings in tetraoxane **2** are in the chair form with the tetraoxane equatorial carbon atom bearing equatorial methyl groups. In this structural arrangement, the two methyl groups are at the greatest possible distance from the peroxide oxygen lone pairs. For tetraoxane **3**, the X-ray crystallographic data reveal that both spirocyclohexylidene rings are in a chair, rather than the previously predicted twist-boat conformation,[13] with the tetraoxane equatorial carbon atoms at C1 and C1A bearing the four methyl groups (Figure 1). The geminal methyl groups adopt well-defined axial and equatorial positions with respect to the cyclohexylidene ring.

Compound **4** exhibits structural features which are generally similar to **2** (Figure 2). The methyl substituents at C2 and C6 are *trans* with respect to each other. To obviate potentially destabilizing steric interactions with the lone pairs on the distal ring oxygen atoms, the C6 methyl group, which is located in an axial positon, is directed away from the tetraoxane ring.

Least-squares fitting of the crystal structures of tetraoxanes **2**−**4** to that of the unsubstituted compound **5**[15] indicates that there is good agreement in the positions of the tetraoxane ring atoms in each case (weighted root-mean-squared (rms) deviations: 0.002, 0.017, and 0.005 Å, respectively). Conversely, since the C−C bond linking the spiro carbon C1 to the substituted carbon C2 shows a slight but significant lengthening

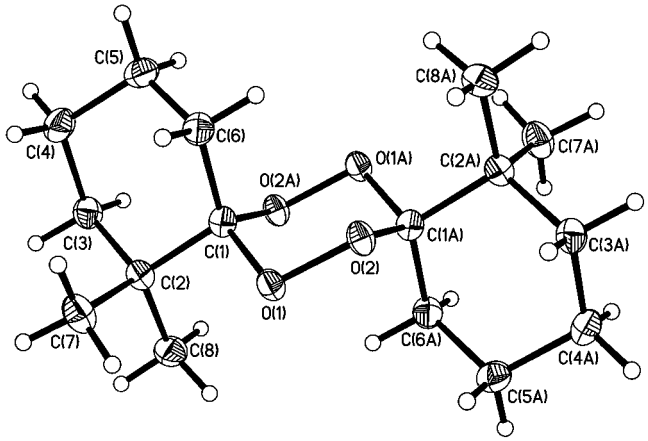

Figure 1. Solid-state structure of tetraoxane **3** (ORTEP[27]). The non-hydrogen atoms are represented by 50% probability ellipsoids and hydrogen atoms by spheres of arbitrary radius.

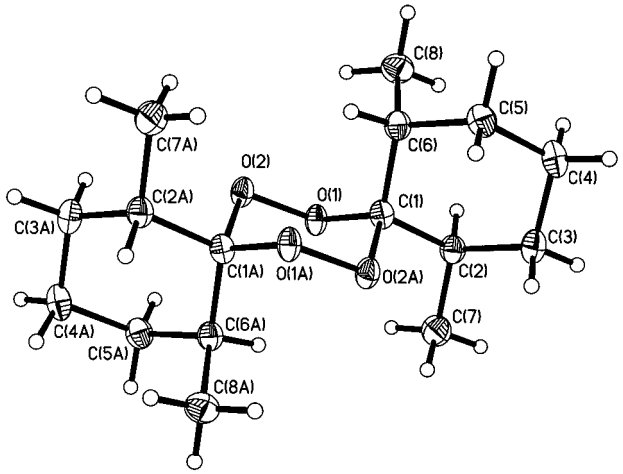

Figure 2. Solid-state structure of tetraoxane **4** (ORTEP[27]). The non-hydrogen atoms are represented by 50% probability ellipsoids and hydrogen atoms by spheres of arbitrary radius.

in each case when compared to **5** (Table 1), structural deviations are more pronounced for the positons of the cyclohexylidene ring atoms, particularly for compounds **3** and **4**.

Molecular Modeling. In the initial molecular modeling studies of these tetraoxanes, all of the isomers of 1,10-dimethyl-7,8,15,16-tetraoxadispiro[5.2.5.2]hexadecane (**2**) were built using PCModel 4.0 and the molecular mechanics method MMX. Considering planar representations of **2**, two stereoisomers are possible, a *cis*-10 isomer and a *trans*-10 isomer, each of which has three configurational isomers of center chirality (a pair of enantiomers and a *meso* structure). Each configurational isomer can give rise to four conformational isomers considering only chair conformations of the three six-membered rings of the dispiro system. The lowest strain energy of the 24 isomers was that of the *meso*-(*t*-10)-1,10-dimethyl-7,8,15,16-tetraoxadispiro-[5.2.5.2]hexadecane in the configuration eventually confirmed by the X-ray structure (vide supra).[16] The conformation of this modeled isomer also corresponded to that reported by Groth[15] for tetraoxane **5**. All other modeled tetraoxanes were subsequently built from this modeled structure.

As shown for tetraoxane **2** (Table 1), comparison of the modeled and X-ray structures for tetraoxanes **2**−**5**[17]

Table 2. Antimalarial Activity against *P. falciparum* in Vitro

	IC_{50} (nM)[a]	
compd	D6	W2
1	8.4	7.3
2	55	32
3	>1000	>1000
4	>1000	>1000
5	38	26

[a] Average of $n \geq 2$.

reveals that molecular mechanics (MMX and MM3) and 3-21G* calculations gave accurate structural parameters such as bond lengths, bond angles, and dihedral angles. MMFF94 calculations also produced reasonable parameters with bond lengths between those of MMX and MM3 and with dihedral angles very close to those obtained with MMX. The MMFF94 bond angles tended to match the higher values obtained with either MMX or MM3. Since the MMFF94 results were intermediate between MMX and MM3, they are not reported here. A potential advantage of MM3 over MMX is that MM3 is parametrized for peroxides,[18] whereas MMX is not.[19] Nonetheless, the MMX and MM3 tetraoxane structures were very similar, although MMX gave better O−O bond lengths and gave fewer atoms deviating more than 2.00 standard deviations from the matched atoms in the crystal structure than did MM3. However, MM3 structures always gave the smaller rms deviations.

The early MMX studies indicated a high degree of symmetry in these tetraoxanes that was substantiated by the other calculations and by the X-ray data. The brevity of Table 1 reflects this symmetry. Although molecular mechanics methods predict greater symmetry for each of the tetraoxanes than was apparent from the X-ray data, the 3-21G* results better reflect the X-ray data in the calculated values for the equatorial C1−O1 and axial C1−O2A bond lengths in tetraoxanes **3** and **4**. In contrast, semiempirical methods such as AM1 gave poor results (O−O bond length 1.298 Å). Shorter O−O bond lengths (1.396 Å) also resulted from the more time-intensive 6-31G* ab initio computations.

Antimalarial Activity. Notwithstanding any postulated increase in metabolic stability, tetraoxanes **3** and **4** were completely inactive in contrast to the tetraoxane prototype **2** (Table 2).[10] The completely unsubstituted tetraoxane **5** was as potent as **2**. With the possible exception of tetraoxane **5**, the very weak in vitro antimalarial activity of tetraoxanes **3** and **4** provides no justification for additional experiments to assess their metabolic stability or oral bioavailability.

We hypothesize that the two inactive tetraoxanes possess sufficient steric hindrance about the tetraoxane ring due to the two additional axial methyl groups to prevent their activation to presumed parasiticidal carbon radicals by inhibiting electron transfer from heme or other iron(II) species.[2] This steric hindrance probably precludes an energetically reasonable approach of these inactive tetraoxanes to heme − the putative antimalarial peroxide receptor.[20,21] A similar argument was recently put forth to account for the low antimalarial activity of the tetraoxane derived from phenylacetaldehyde[22] and for the complete loss of antimalarial activity for two C-5A methyl-substituted trioxane analogues of artemisinin.[23]

We note that steric hindrance affecting peroxide bond accessibility is only one of the many possibly significant factors influencing tetraoxane antimalarial activity. In the rationalization of antimalarial data other considerations such as stability of carbon radicals formed by β-scission after the initial electron transfer to the peroxide bond should also be considered. A clear delineation of the influence of steric and electronic effects and other physicochemical attributes of the tetraoxanes on their antimalarial activity is clearly not entirely possible. As a complementary exploration of SAR, iron(II) reactions of these and related tetraoxanes are underway.

Experimental Section

Melting points are uncorrected. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a Varian XL-300 spectrometer using $CDCl_3$ as a solvent. All chemical shifts are reported in parts per million (ppm) and are relative to internal $(CH_3)_4Si$ for ^{1}H and $CDCl_3$ (77.0 ppm) for ^{13}C NMR. Microanalyses were performed by M-H-W Laboratories, Phoenix, AZ. Vapor pressure osmometry MW analyses were performed by Galbraith Laboratories, Knoxville, TN. Cyclohexanone and 2,2-dimethyl- and 2,6-dimethylcyclohexanone were available from Aldrich Chemical Co.

Tetraoxane Synthesis. The tetraoxanes were prepared by acid-catalyzed peroxidation of the corresponding cyclohexanone derivative and were isolated as single stereoisomers as indicated by ^{13}C NMR spectra which displayed only one peak for the two identical spiro carbon atoms at 105−111 ppm. Tetraoxane **2** was prepared as previously described.[10] Tetraoxanes **3** and **4** were prepared by a modified method developed by McCullough et al. using 50% rather than 86% H_2O_2.[9] Tetraoxane **5** was synthesized according to a modified method developed by Bertrand et al. using 50% rather than 30% H_2O_2.[24] Although we encountered no difficulties in working with these rather stable and friction-insensitive peroxide compounds,[25] routine precautions such as the use of shields and fume hoods and the avoidance of metal salts should be observed whenever possible.

1,1,10,10-Tetramethyl-7,8,15,16-tetraoxadispiro[5.2.5.2]-hexadecane (3): yield, 26%; mp 161−162 °C (lit.[9] mp 161−163 °C); ^{1}H NMR 1.04 (s, 12H), 1.37−1.73 (m, 12H), 2.32 (t, J = 6.2 Hz, 4H); ^{13}C NMR 21.30, 22.37, 22.85, 25.58, 37.45, 39.01, 110.72.

1,5,10,14-Tetramethyl-7,8,15,16-tetraoxadispiro[5.2.5.2]-hexadecane (4): yield, 18%; mp 164−165 °C dec (CH_3CN); ^{1}H NMR 1.04 (br s, 12H), 1.25−2.15 (m, 14H), 3.34 (br s, 2H); ^{13}C NMR 13.76, 19.68, 28.73 (br s), 29.69 (br s), 31.26 (br s), 33.78 (br s), 110.78; VPO MW 274; calcd MW 284. Anal. ($C_{16}H_{28}O_4$) C, H.

7,8,15,16-Tetraoxadispiro[5.2.5.2]hexadecane (5): yield, 90%; mp 130−131 °C (lit.[26] mp 131−132 °C); ^{1}H NMR 1.35−1.51 (m, 4H), 1.52−1.72 (m, 12 H), 2.29 (br s, 4H); ^{13}C NMR 21.94 (br s), 22.10 (br s), 25.38, 29.55 (br s), 31.77 (br s), 108.12.

X-ray Crystallographic Determinations. Single crystals of tetraoxane derivatives **2** (methanol), **3** (ethanol), and **4** (methanol−hexane) were grown by slow evaporation from the solvents indicated in parentheses. The X-ray diffraction data (Mo Kα λ = 0.71073 Å) were collected on a Siemens P4 diffractometer at 160 K. The structures were solved by direct methods and refined by full least-squares techniques using anisotropic temperature factors for the non-hydrogen atoms. All crystallographic calculations were carried out using the SHELXTL suite of progams.[27]

Molecular Modeling Experiments. Molecular modeling was done using PCModel by Serena Software, SYBYL by Tripos Inc., Spartan by Wavefunction Inc., and Gaussian94 (revision A.1) by Gaussian Inc. MMX is a molecular mechanics component of PCModel, and MM3 is a separate molecular mechanics program from Tripos Inc. and is interfaced with both Spartan and SYBYL. Using a systematic search technique

as implemented in Spartan, the lowest energy and most abundant tetraoxane conformers were selected for subsequent ab initio 3-21G* calculations on Gaussian94. Molecular electrostatic potential profiles were sampled over the entire accessible surface of the tetraoxanes (van derWaals contact to a distance of approximately 1.5 Å).

Antimalarial Screens. In vitro activity against *P. falciparum* was determined using a modification of the semiautomated microdilution technique of Desjardins et al.[28] and Milhous et al.[29] Two *P. falciparum* malaria parasite clones, designated as Sierra Leone (D6) and Indochina (W2), were used in susceptibility testing.[30] The former is resistant to mefloquine and the latter to CQ, pyrimethamine, sulfadoxine, and quinine. Test compounds were dissolved in dimethyl sulfoxide and solutions serially diluted with culture media. Erythrocytes with 0.25−0.5% parasitemia were added to each well of a 96-well microdilution plate to give a final hematocrit of 1.5%. Inhibition of uptake of tritiated hypoxanthine was used as an index of antimalarial activity. Results were initially recorded as IC_{50} (ng/mL) values and then converted to nM values.

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Supporting Information Available: Crystal data along with tables of atomic coordinates, anisotropic thermal displacement parameters, and derived geometrical parameters for tetraoxanes **2**−**4.** This material is available free of charge via the Internet at http://pubs.acs.org.

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