THE STRUCTURE AND ANTIMALARIAL ACTIVITY OF SOME 1,2,4-TRIOXANES, 1,2,4,5-TETROXANES, AND BICYCLIC ENDOPEROXIDES. IMPLICATIONS FOR THE MODE OF ACTION

Charles W. Jefford*, Jean-Claude Rossier, and Wilbur K. Milhous[#]

Department of Organic Chemistry, University of Geneva
1211 Geneva 4, Switzerland

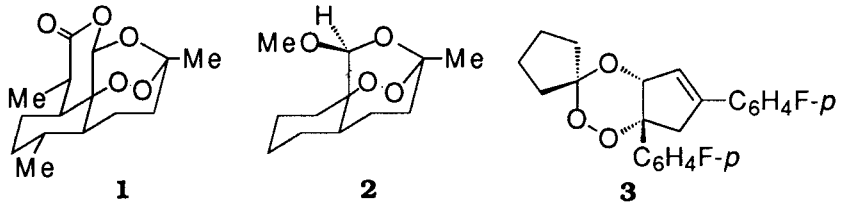
[#]Walter Reed Army Institute of Research, Division of Experimental Therapeutics, Washington, DC 20307, USA

Abstract- The mode of antimalarial action of *cis*-fused cyclopenteno-1,2,4-trioxanes (**7-13**), dispiro-1,2,4,5-tetroxanes (**14-17**), and bridged bicyclic endoperoxides (**18-22**) was examined by systematically changing substituents at positions remote from the O-O bond. It is concluded that peroxides of high activity (**7-11**, **14-15**, **18**, and **19**) are able to intimately dock on heme and rearrange efficiently to an ethyl radical which kills the parasite by alkylation. Dimethyl substituents on the spirocyclohexane ring in the trioxanes (**12** and **13**) and tetroxane (**16**) diminish activity by hampering either docking or the reactivity of a C-centered radical. Endoperoxides derived from methylnaphthalenes (**20-22**) are inactive owing to their inability to generate an ethyl radical.

INTRODUCTION

Many of the features of the tetracyclic skeleton of artemisinin (**1**), a potent antimalarial agent, are not necessary for ensuring high parasiticidal activity.[1] Far simpler structures such as the tricyclic non-lactonic analogue (**2**) and the *cis*-fused bicyclic 1,2,4-trioxane (**3**) display substantial artemisinin-like activity in different *in vitro* and *in vivo* models.[2-5] We recently showed that minor modifications to the cyclopentene moiety of related trioxanes, exemplified by the epoxide (**4**) and the epimine (**5**), leave the essential activity intact, whereas the dichlorocarbene adduct (**6**) suffers a diminution of activity.[6] We now describe preliminary results on three sets of 1,2,4-trioxanes, 1,2,4,5-tetroxanes, and endoperoxides in which systematic variations in ring substitution affect activity. We will discuss how these changes provide insights into the mode of action of this new class of antimalarial peroxides.

Dedicated to Professor Teruaki Mukaiyama on the occasion of his 73rd birthday



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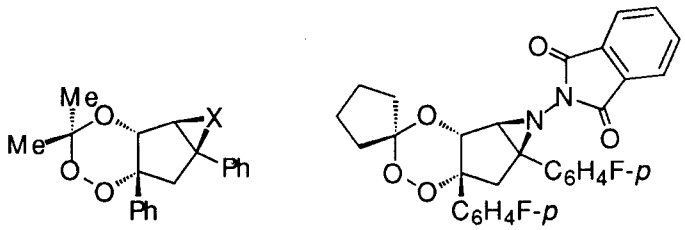
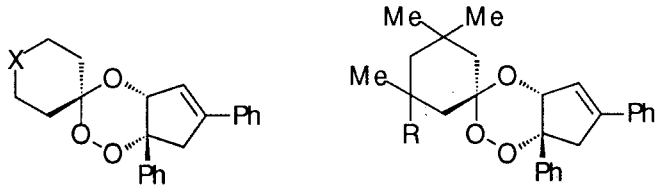


4 X = O
6 X = CCl_2

5

RESULTS AND DISCUSSION

The 1,2,4-trioxanes chosen (**7-13**) are all *cis*-fused to the same diphenylcylopentene unit.[7] At the C3 position is located a spirocyclic cyclohexane ring which is progressively modified. Tests of the antimalarial activity of **7-13** together with appropriate reference compounds were performed *in vitro* against *Plasmodium falciparum* clones by using a standard method.[8] In each case the concentrations of sample that inhibited parasite growth by 50% (IC_{50}) and by 90% (IC_{90}) of the Indochina W-2 and Sierra Leone D-6 clones were determined. The clones respond differently to the usual nitrogen-containing antimalarial agents. The D-6 clone is relatively resistant to mefloquine, but sensitive to chloroquine, quinine, pyrimethamine, and sulfadoxine. Conversely, the W-2 clone is considerably resistant to chloroquine, quinine, pyrimethamine, and sulfadoxine, but susceptible to mefloquine.



7 X = CH_2
8 X = S
9 X = O
10 X = CO
11 X = C(-OCH_2CH_2O-)

12 R = H
13 R = Me

Inspection of the activities (Table 1) reveals that introducing a distal hetero atom into the cyclohexane ring of **7** only brings about a small change, the oxa derivative (**9**) being somewhat more active than the thia analogue (**8**) which resembles the parent (**7**) (entries 1-3). Introduction of a carbonyl function (**10**) in the same position behaves like an inserted oxygen atom by improving activity (entry 4). Conversion of the carbonyl group to its acetal derivative (**11**), despite its bulk, is without effect; activity being similar to that of the parent (**7**) (entry 5). It is noted that all fluctuations are quite small and show the same trend in both the sensitive and resistant clones. In contrast, a dramatic change is observed when geminal methyl substituents are placed on the cyclohexane ring

The trimethyl derivative (**12**) experiences a sharp drop in activity in both clones compared with **7** (entry 6). An equally large loss of activity is seen for the tetramethyl derivative (**13**) (entry 7). Clearly, a steric effect is responsible, because the basic trioxane pharmacophore remains the same throughout. In other words, while the trioxanes (**7-11**) are not so active as artemisinin (**1**) (entry 18), their antiparasitic effect is nonetheless high, unlike the methylated derivatives (**12**) and (**13**) which would hardly be considered as drug candidates. It is worth noting that the trioxanes with the exception of the sulfur and trimethyl derivatives (**8**) and (**12**) are more effective against the resistant than the sensitive clone.

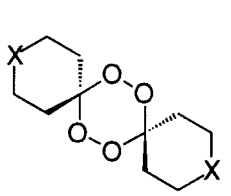
Table 1. *In vitro* antimalarial activity of some 1,2,4-trioxanes, 1,2,4,5-tetroxanes, and endoperoxides against *P. falciparum* clones (IC_{50} and IC_{90} values in ng/ml)

entry	Compound[a]	Sierra Leone D-6		Indochina W-2	
		IC_{50}	IC_{90}	IC_{50}	IC_{90}
1	**7**	5.7	12.2	3.9	5.0
2	**8**	7.2	16.2	6.0	15.6
3	**9**	2.2	12.5	0.1	0.4
4	**10**	1.7	6.5	0.8	2.3
5	**11**	4.3	7.5	2.4	3.7
6	**12**	147.0	245.0	208.0	302.0
7	**13**	140.0	345.0	94.0	211.0
8	**14**	25.9	50.4	19.75	32.4
9	**15**	102.0	300.5	68.0	150.0
10	**16**	252.0	794.0	255.0	440.0
11	**17**	inactive		inactive	
12	**18**	124.0	175.0	96.0	288.0
13	**19**	322.0	654.0	191.0	1207.0
14	**20**	inactive		inactive	
15	**21**	inactive		inactive	
16	**22**	inactive		inactive	
17	Chloroquine	1.9	-	55.2	-
18	Artemisinin	2.0	-	1.2	-

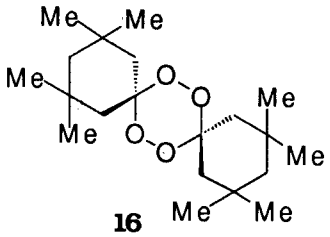
[a] All synthetic compounds are racemic mixtures if chiral.

Dispiro-1,2,4,5-tetroxanes have long been known to possess antimalarial activity.[1,9] They have the advantage of being easy to prepare, but are limited in their structural variety. For reasons which will become clearer later, the 3,3,6,6-tetrasubstituted derivatives (**14-17**) were chosen for testing.[10] It is immediately evident that the parent, the cyclohexane derivative (**14**), is 4-5 times less active than its trioxane counterpart (**7**), but still reflects the same distinction between the sensitive and resistant clones (Table 1, entry 8). The insertion of an oxygen atom

in the cyclohexane ring, unlike the same process in the trioxanes, diminishes activity; **15** being less active than **14** (entry 9). However, the tetroxane (**16**) bearing two pairs of geminal methyl groups on the cyclohexane rings is striking in that its activity is strongly reduced compared to that of **14** (entry 10). The simple 3,3,6,6-tetramethyl-1,2,4,5-tetroxane (**17**) is inactive (entry 11). Once again it can be concluded that steric encumbrance at the C3 position relative to the spirocyclic carbon atom is a critical feature that interferes with the mode of parasiticidal action.



14 X = CH_2
15 X = O



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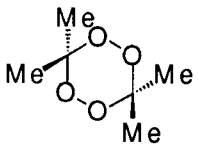
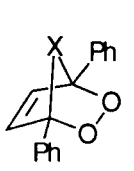
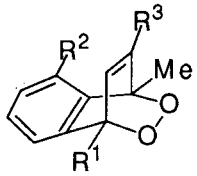


17

Many endoperoxides, exemplified by the natural product ascaridole, are endowed with antimalarial properties.[1,11-14] The bridged bicyclic endoperoxides (**18-22**) were selected because of their ready availability.[15] Their antimalarial activities are revealing. The most active of the set is 1,4-diphenylbicyclo[2.2.2]oct-2-ene (**18**) (entry 12). Its activity falls below that of artemisinin by about 50-100 fold. There is little differentiation between the two clones. The lower homologue (**19**) is much less active (entry 13). Significantly, the three naphthalene endoperoxides (**20-22**) are all ineffective (entries 14-16).



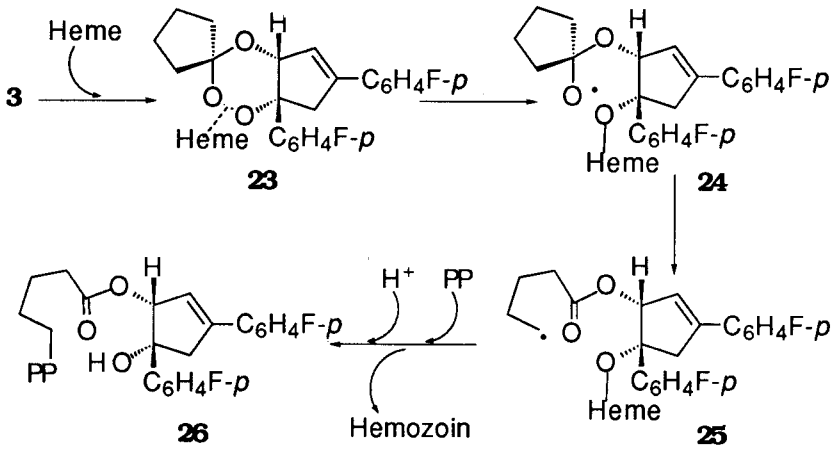
18 X = CH_2CH_2
19 X = CH_2



20 R^1 = Me, R^2 = R^3 = H
21 R^1 = H, R^2 = Me, R^3 = H
22 R^1 = Me, R^2 = H, R^3 = Me

The foregoing results provide further evidence in support of current ideas regarding the mode of action of peroxide antimalarials. It is now fairly certain that artemisinin (**1**) and the synthetic trioxane (**3**) kill the parasite inside the red blood cell by a process of chemical induction.[5,18-20] During the trophozoïte stage of the intraerythrocytic cycle within the host, the parasites invade the red blood cells and digest the hemoglobin therein, liberating amino acids as a nutritional source. The heme so discarded, because of its toxicity to the parasite, is immediately oxidized and polymerized to the insoluble malarial pigment, hemozoin. However, when a peroxidic antimalarial is administered it interacts with heme and diverts the normal detoxification process. The behavior of **3** provides a pertinent illustration. The *cis*-fused entity of **3** presents its convex face to the iron atom of heme so that the O-O bond coordinates closely with it forming a complex (**23**) (Scheme 1).[21] Next, an electron jumps from iron to the antibonding orbital of the peroxide bond causing it to break. The resulting oxygen radical (**24**) spontaneously rearranges to the crucial reactive carbon-centered radical (**25**) which then alkylates the protein (PP) of a nearby parasite causing its death. Lastly, protonation releases the alkylated protein (**26**) together with hemozoin. What happens overall is that a toxic radical replaces toxic heme.

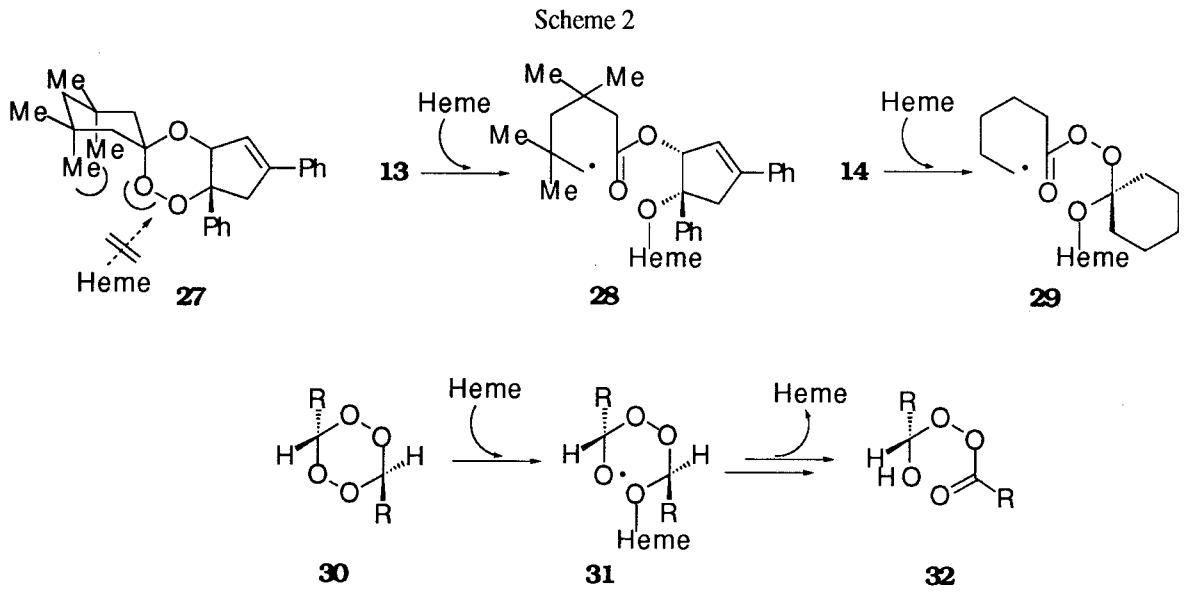
Scheme 1



Evidently, the effectiveness of a particular peroxidic drug candidate depends crucially on the efficient operation of the aforementioned chemical events. Of course, the drug must first penetrate the parasitized red blood cell. Next, it must closely dock with heme. Electron transfer must be easy. Once the O-centered radical is formed, enough thermodynamic driving force must be available to break the adjacent C-C bond and give a C-centered radical of sufficient reactivity to alkylate parasite protein.

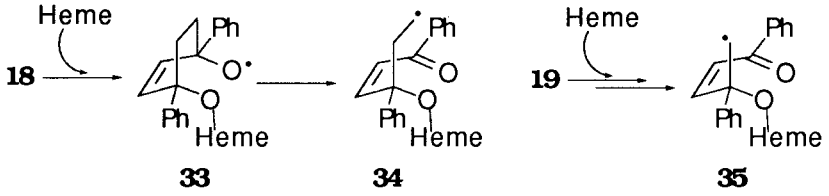
The trioxanes (**8-11**) behave just like **3**. They all complex effectively with heme and generate the lethal primary ethyl-type radical corresponding to **25**. Even **11** manages to closely coordinate with iron, presumably because the bulky ethanedioxy substituent lies far away from the O-O bond. However, the poor antimalarial performance of the trimethyl and tetramethyl derivatives (**12** and **13**) could be due to two factors. The diaxial interaction between two methyl groups with a ring oxygen atom (cf. **27**) could hamper a tight fit with heme and therefore stop radical formation (Scheme 2). Alternatively, complexation could occur as before, but the radical so generated, e.g. **28** from **13**, would be unable to alkylate parasite protein (PP) because of its unreactive neopentyl nature.

Scheme 2



Similar arguments can be advanced for the tetroxanes. The activity of **14** and **15** undoubtedly springs from the heme-induced scission of the O-O bond to give the primary C-radical. Thus, **14** affords **29** (Scheme 2). Alkylation of PP by **29** takes place without impediment as attested by the parasitic toxicity. When the tetramethyl grouping is present as in **16** either complexation or the relevant radical, if produced, is sterically hindered thereby accounting for the observed lack of activity. In the case of the simple tetroxane (**17**), it is possible that methyl radicals are not formed and that another reaction course is followed. Alternative radical pathways are also likely for 3,6-dialkyl-1,2,4,5-tetroxanes (**30**), which were reported to be inactive.[22] A possible avenue circumventing primary C-radicals is the heme-catalyzed rearrangement of **30** to the perester **32** *via* 1,5 hydrogen atom shift of the initial oxy radical (**31**). Choosing the dispiro-3,3,6,6-tetrasubstituted tetroxanes as we did avoids such mechanistic detours.

Scheme 3



The endoperoxides provide a further commentary on the mechanism. The rigid bicyclo[2.2.2]octane skeleton is perfectly set up for complexation and scission to the required key ethyl radical. Heme opens the peroxide bond of **18** successively to the oxy and carbon radicals (**33** and **34**); the driving force being the formation of the stable α,ß-unsaturated phenone entity tethered to an unencumbered ethyl radical (Scheme 3). The lower homologue (**19**) is broken apart just as easily as **18**, but does not do as well in killing the parasite, presumably because the resulting lethal agent is the more hindered methylene radical (**35**). Although the naphthalene endoperoxides (**20-22**) like artemisinin have an exposed O-O bond making them well-disposed for complexation with heme, they are structurally incapable of providing the requisite ethyl radicals, whence their inactivity. Apparently, like the tetramethyltetroxane (**17**), excision of methyl radicals from **20-22** is feasible, but seems not to occur. We predict however that by reducing the double bond in **20-22** high antimalarial activity would be conferred.

CONCLUSIONS

The activity profiles of the three sets of trioxanes, tetroxanes and peroxides are all consistent with the effect of substituents on the proposed sequence of chemically induced mechanistic events responsible for ultimate parasiticidal action. It is worth emphasizing that the motor for antimalarial activity is the thermodynamic stability accruing from the rearrangement of an O-radical to a C-centered one. Ideally, the C-centered radical so formed should be unhindered and unstabilized, in other words, like a pendent ethyl radical. The ability of the peroxide bond to closely bind with heme is important for activity. Docking studies, particularly on the tetramethyl derivatives (**13**) and tetroxane (**16**), should cast light on the origin of poor antimalarial performance. Concurrently, the deployment of $FeCl_2.4H_2O$, recently developed as a diagnostic reagent for activity and tool for investigating mechanism,[19,20] should reveal how the O-heterocyclic rings of the least active peroxides actually unravel.[23]

ACKNOWLEDGMENTS

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