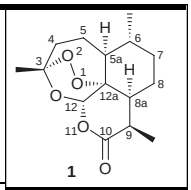
Is alkylation the main mechanism of action of the antimalarial drug artemisinin?



CHEMICAL SOCIETY REVIEWS

Anne Robert and Bernard Meunier*

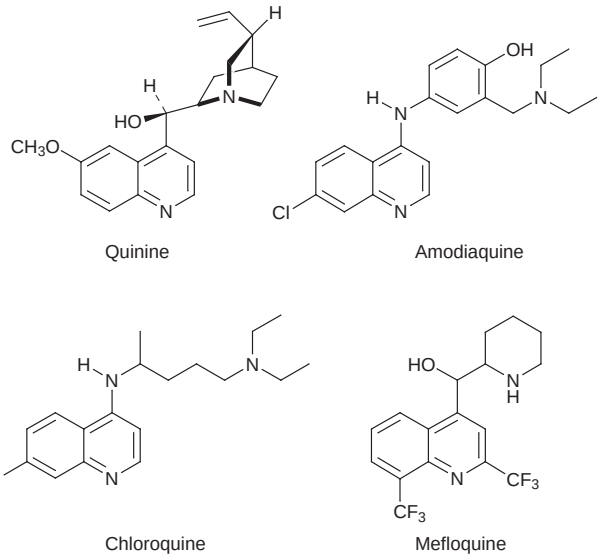
Laboratoire de Chimie de Coordination du CNRS, 205 route de Narbonne, 31077 Toulouse cedex 4, France

Artemisinin is a sesquiterpene lactone with an endoperoxide function essential for its antimalarial activity against chloroquine-resistant strains of *Plasmodium falciparum*. The mechanism of action of this paradigm molecule for endoperoxide-containing antimalarial drugs is still open to debate. Are the artemisinin derivatives only responsible for oxidative stress or are they able to alkylate heme and parasite proteins? The characterization of a covalent artemisinin-heme model adduct supports the role of C-centered radicals generated by the reductive activation of the peroxidic bond of this class of drugs. Artemether (an artemisinin analogue) and BO7 (a synthetic antimalarial trioxane) are also able to alkylate a porphyrin cycle.

1 Introduction

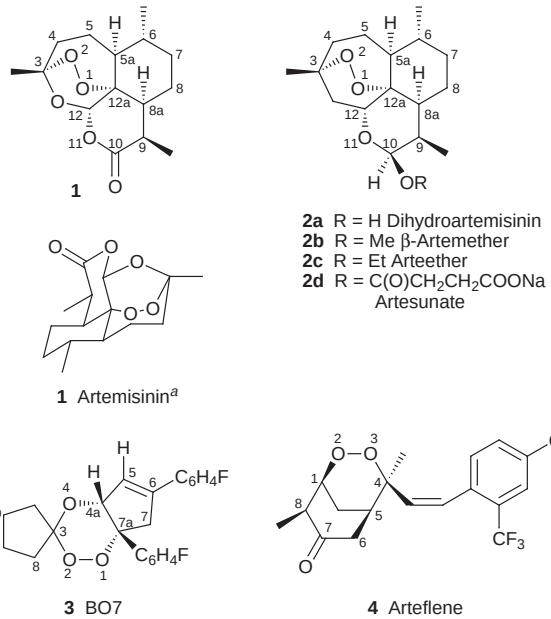
Nearly two billion people are at risk of malaria and the incidence of this disease is dramatically increasing since many *Plasmodium falciparum* strains, the parasite responsible for the majority of fatal malaria infection, have now become resistant to chloroquine. Some strains have also developed resistance to mefloquine, and even to the naturally occurring and highly efficient antimalarial quinine. A major event in the history of malaria was the discovery, in the early 17th century, of the antimalarial activity of the bark of *Cinchona*, the 'Peruvian fever tree'. In 1820, Pelletier and Caventou, two French pharmacists, isolated the two main alkaloids responsible for this activity: quinine and cinchonine. Selective breeding of a new variety of *Cinchona* trees was then developed, and the new trees were producing up to 13% quinine, ten times more than the older varieties in cultivation. Quinine was the main treatment for malaria until the 1930s, up to the development of synthetic antimalarials based on a quinoline moiety, of which chloroquine, mefloquine and amodiaquine are the most commonly used. Quinine is now considered as being too toxic for prophylaxis or routine treatment of malaria, but cases of resistance are rare, and this molecule is still used *via* intravenous infusion to treat severe malaria. Resistance to chloroquine first appeared in Thailand and South America in the early 1960s,[1] and many strains of *P. falciparum* resistant to nearly all quinoline drugs are now present in large parts of the world. Resistance is now so widespread that chloroquine is virtually useless in some parts of the world.[2] The alarming spread of drug resistance has led the WHO to predict that, in the absence of new antimalarial strategies, the number of people suffering from malaria will double by the year 2010. Thus, to circumvent this phenomenon of drug resistance, it is imperative that novel drugs should be developed to treat the desease.

In 1967, the government of China launched a programme to discover new antimalarial drugs and indigeneous plants used in traditional medicine have been systematically examined.[3,4] The antipyretic activity of a decoction of leaves of *Artemisia annua* was described as long ago as 340 and its antimalarial activity in 1596 (the year of publication of the Chinese *Compendium of Materia Medica*). In 1972, Chinese researchers recovered, by extraction at low temperature from this plant, a crystalline compound that they named *qinghaosu* (the name artemisinin is preferred by *Chemical Abstracts*, RN 63968-64-9). Artemisinin

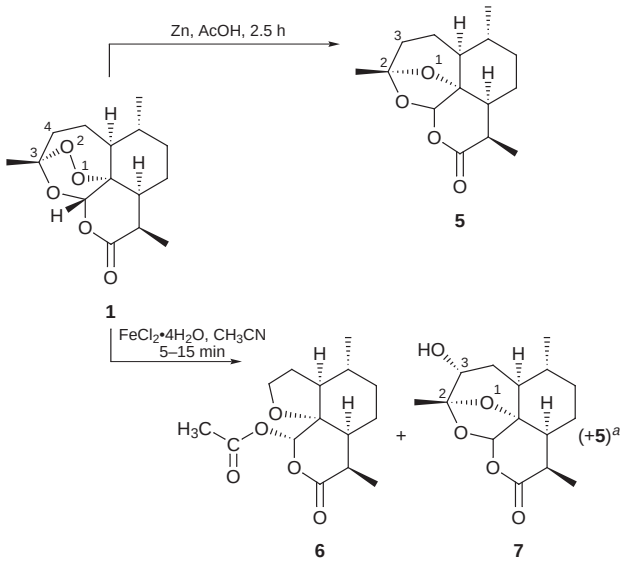


Structure of some antimalarial drugs based on a quinoline moiety

1 is an enantiomerically pure sesquiterpene lactone bearing an endoperoxide function which has been proved to be essential for antimalarial activity, the reduced peroxide deoxyartemisinin **5** (Scheme 1) being completely inactive. It is highly potent and is currently used in China, South-East Asia and in some parts of Africa, to treat more than a million people. However, although they are highly active against polyresistant *P. falciparum*, artemisinin and its effective analogues, such as β-artemether **2b** and sodium artesunate **2d**, have to be obtained by semi-



Structure of antimalarial drugs artemisinin, β-artemether, BO7, and arteflene. [a] Two different possible drawings are given for artemisinin.



Scheme 1 Reactivity of artemisinin according to the reaction conditions: zinc powder in acetic acid, or iron(II) chloride in acetonitrile (after reference 23). [a] Compound **5** was also obtained when the reaction was carried in the presence of $Fe^{II}Br_2$ in tetrahydrofuran (reference 25).

synthesis from extracts of the original plant *Artemisia annua*, with all the drawbacks and cost that this entails [the growing of *A. annua* is possible only in limited geographical areas, namely in the South Chinese and Vietnamese uplands, and the yield of extraction is low (0.4%).[5] Possible biosynthetic pathways have recently been described[6]]. Under these circumstances, it will be difficult to extend the use of these artemisinin derivatives to the scale of billions of people.

Fully synthetic compounds that share the benefits of artemisinin without its disadvantages (neurotoxicity has been reported at high doses of arteether **2c** in monkeys[7]) and which can be made at low cost are clearly highly desirable. This has drawn attention to a new class of products having an endoperoxide function and are therefore capable of having a similar activity.[8,9] Some of them have been prepared, in particular RO 42-1611 (arteflene **4**) and BO7 ('Fenozan-50F' **3**), the latter being based on a *cis*-fused cyclopenteno-1,2,4-trioxane, exhibiting good activity on chloroquine-resistant *Plasmodium* strains and with a remarkably high safety margin.[10,11] The development of this strategy obviously requires a precise knowledge about the mechanism of action of artemisinin at a molecular level.

Like chloroquine and quinine, these peroxidic antimalarial drugs act as blood schizonticides. However, from the chemical structure of artemisinin and synthetic trioxanes, it is apparent that these molecules share a common mode of action which will be different from that of the traditional quinoline-based antimalarials. In this review article, we will focus on the molecular aspects of the mechanism of action of artemisinin (**1**) and the related semi-synthetic derivative β-artemether (**2b**) or a synthetic related peroxide having a significant biological activity, BO7 (**3**).

2 What is known about the reactivity of artemisinin derivatives in infected erythrocytes?

After infection of a person by the bite of an infected female *Anopheles*, *Plasmodium* parasites first accumulate in hepatocytes, then invade the erythrocytes for the next stage of their maturation. After a few days, the infected red cells burst open and the merozoites are released causing the periodic fevers of malaria. These merozoites infect new erythrocytes and the intraerythrocyte cycle starts again. Within erythrocytes, the parasite degrades the hemoglobin of the host and digests 30% or more of the protein moiety, using it as a source of amino-acids for the synthesis of its own proteins. The resulting free and potentially toxic heme residues are polymerized as a microcrystalline, redox inactive iron(III)–heme pigment called hemozoin, which is insoluble in biological conditions and accumulates in the food vacuole.[12,13] Only a small amount of heme is degraded by the parasite to be used as an iron source for its own metalloenzymes.

Artemisinin and related peroxide-containing drugs are active on intraerythrocytic parasites at nanomolar concentrations, the toxic concentration toward normal erythrocytes being in the range of micromolar. It has been proposed that free intraparasitic heme liberated during hemoglobin digestion might play an important role in the selective toxicity of artemisinin toward the parasite.[14] Hemin was found to catalyze the reductive decomposition of artemisinin and dihydroartemisinin *in vitro*.[15] Since the activity of artemisinin is inhibited by antioxidants (including catalase), it was initially proposed that the mechanism of action of this drug involved an oxidative stress leading to the destruction of the parasite.[16] When incubated with normal erythrocytes, artemisinin was shown to increase the methemoglobin concentration and to reduce slightly the intracellular glutathione and membrane fatty acid concentrations, resulting in a dose-dependent increase of cell lysis.[17] A combination of hemin and artemisinin oxidize erythrocyte membrane thiols *in vitro*, whereas artemisinin alone or hemin alone has little effect. This oxidation is reduced in the presence of a free radical scavenger (α-tocopherol) or in the presence of deferoxamine which binds to iron ions resulting from the parasitic degradation of heme. Thus, the artemisinin–hemin mediated oxidation reactions, dependent on iron and mediated by free radicals, were considered as due to an iron-catalyzed cleavage of the endoperoxide function of the drug.[18] However it should be noted that all these experiments were made at concentrations ranging from 50 to 1000 μM, *i.e.* at concentrations 10^3 to 10^5 times higher than effective *in vitro* antimalarial concentrations. It is therefore reasonable to consider that the parasite death in the presence of artemisinin is not due to non-specific or random cell damage caused by free radicals, but might involve specific radicals and targets which have yet to be identified at the molecular level.[19]

For this purpose, radioactive [^{14}C]-artemisinin **1**,[20] [^{3}H]-dihydroartemisinin **2a**, [^{3}H]-arteether **2c** or [^{14}C]-arteflene **4** were incubated with *P. falciparum* infected erythrocytes.[21] After treatment, a proportion of the total parasite-associated radioactivity was bound to hemozoin as a low molecular mass artemisinin–heme adduct.[20] Furthermore, with radioactive dihydroartemisinin[21] used at a pharmacologically relevant concentration (34 nM), this drug was able to alkylate some specific and not particularly abundant parasite proteins, one of which has a similar size to that of 'HRP' (histidine-rich protein, 42 kDa), a protein involved in the polymerization of heme in infected erythrocytes.[22] The two other endoperoxides, arteether **2c** and arteflene **4**, reacted with the same proteins.[21] In contrast, no proteins were alkylated under the same conditions in normal erythrocytes. None of the parasitic proteins were alkylated by the inactive reduced analogue deoxyartemisinin **5**, supporting a specific mode of action of these peroxide-containing drugs. But the precise identities of the artemisinin–heme adduct(s) and target proteins are not yet known.

Recent *in vitro* studies reported two possible modes of reactivity of artemisinin: (i) is artemisinin an oxygen atom donor with respect to heme and (ii) is artemisinin an alkylating agent?

3 Is artemisinin an oxygen atom donor with respect to heme?

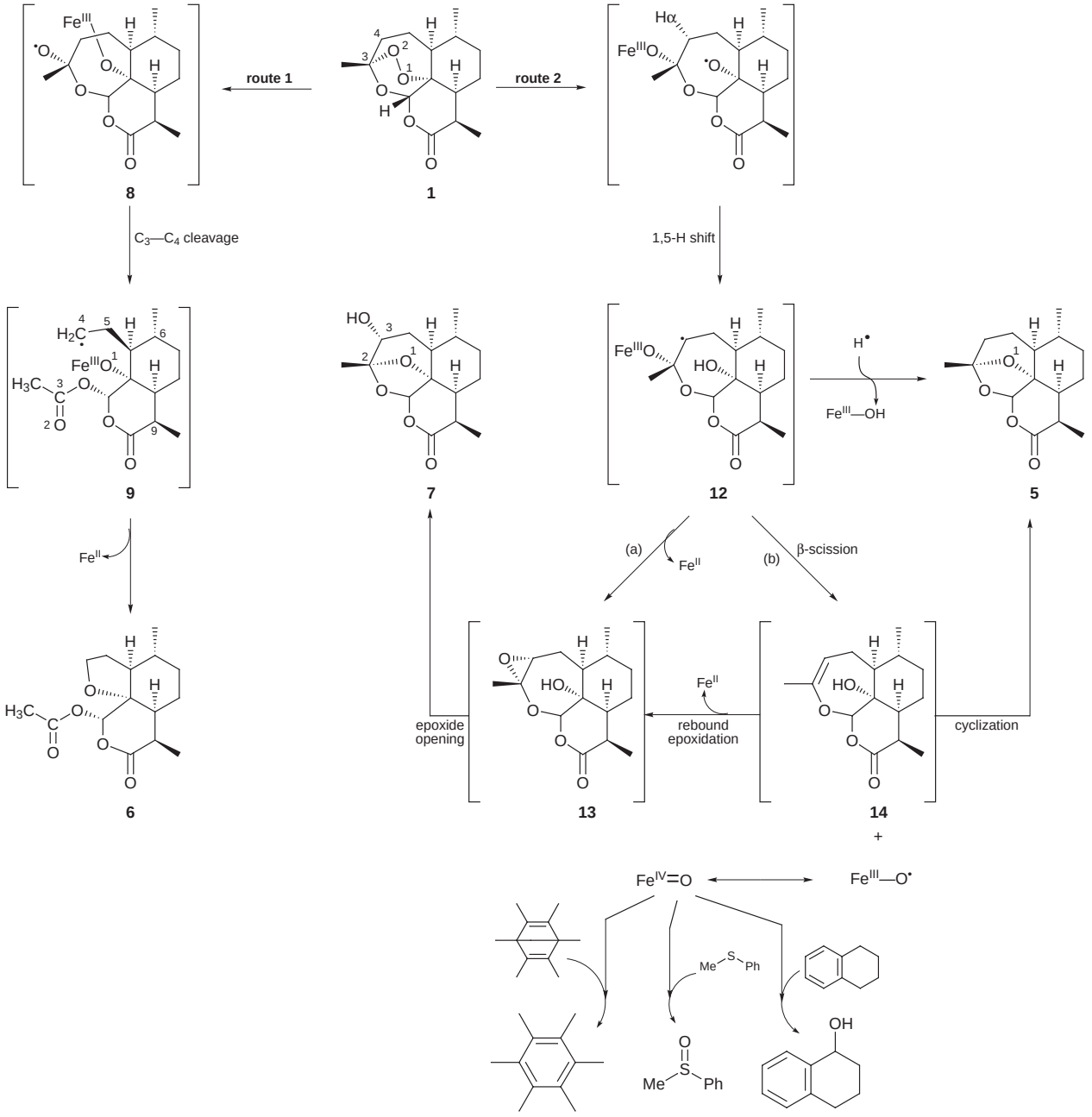
We will consider two different cases: the reactivity of artemisinin with simple transition metal ions and then with synthetic metalloporphyrins as heme models.

3.1 In the presence of transition metal salts

The formation of deoxyartemisinin **5**, an artemisinin metabolite, has been considered as an argument in favor of artemisinin acting as an oxygen atom donor to a metal complex (see reference 3 for the identification of artemisinin metabolites). Reaction of artemisinin in the presence of a $Pd/CaCO_3$ catalyst caused the loss of a peroxidic oxygen atom to give the bis-ether derivative deoxyartemisinin **5**.[3] More recently, it has been reported that the addition of zinc powder to a solution of artemisinin in acetic acid resulted after a few hours in a nearly quantitative conversion to deoxyartemisinin (Scheme 1). The same experiment carried out with **2b** produced deoxy-β-artemether.[23] The same result was previously obtained with the synthetic trioxane **3**.[24] This deoxygenation is obviously a two-electron process, which might be enzymatically induced *in vivo*, leading to the inactive deoxy metabolite. However, there is absolutely no indication that such a reaction is implicated in the parasiticidal event.

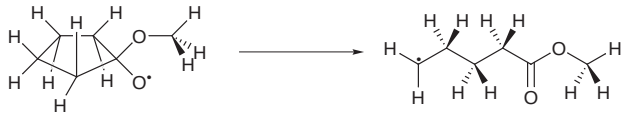
In the presence of iron(II) chloride tetrahydrate in acetonitrile for no more than fifteen minutes, artemisinin was completely converted to a ring-contracted furano acetate derivative **6** and 3α-hydroxydeoxyartemisinin **7** (resulting from a hydroxylation at position $C_{4\alpha}$ of artemisinin) with yields of 78 and 17%, respectively.[24] In the presence of iron(II) bromide in tetrahydrofuran,[25] the degradation of artemisinin produced the same products **6**, **7**, and also **5** in 29, 10 and 59% yields, respectively.

Posner *et al*. proposed that compounds **6** and **7** arose from an iron(II)-induced homolytic cleavage of the peroxidic bond, a $RO^{\bullet}$ radical being formed either on O_2 or on O_1 of artemisinin (routes 1 and 2 respectively, Scheme 2).[8] The $RO_2^{\bullet}$ radical **8** (Scheme 2, route 1) rearranged by homolytic cleavage of the C_3–C_4 bond to produce the non-sterically hindered C_4-centered primary radical **9**. In the absence of an easy substrate to alkylate, this radical might react with the Fe–O_1 bond to give rise to the ring-contracted derivative **6** with release of a Fe^{II} salt. This reactivity, which supposes the rapid isomerization of the O-centered radical to a C-centered radical by cleavage of the adjacent C–C bond has also been evidenced by Jefford, using BO7 as substrate.[24]



Scheme 2 Mechanism of iron(II) mediated degradation of artemisinin (the right part of the scheme—route 2—is reproduced from reference 8)

That the ester formation is a driving force of the rearrangement of the intermediate radical **9** was confirmed by calculations on models using 1-methoxycyclopentan-1-yloxyl radical **10**: the heats of formation of **10** and **11** indicated that the conversion of the O-radical **10** to the C-radical **11** was a strongly exothermic process (Scheme 3).[11] The analogue of compound **6** (but with an ethyl ether at position 12 instead of C=O) was one of the microbial metabolites of arteether **2c**, but was not found in mammalian metabolism.[26] These data indicate that the reaction pathway with a C_4-centered radical might be involved *in vivo*.



10
1-methoxycyclopentan-1-yloxyl
Heats of formation; –59 kcal mol^{-1}

11
δ-radical of methyl pentanoate
–77 kcal mol^{-1}

Scheme 3 Isomerization of an O-centered radical to a C-centered radical (after reference 11)

In route 2 of Scheme 2, the $RO_1^{\bullet}$ radical initially formed rearranged *via* a stereospecific 1,5-H shift from $H_{4\alpha}$, leading to the C_4-centered secondary radical **12**. It should be noted that a reaction pathway proceeding *via* a carbon-centered radical at position $C_{4\alpha}$ has already been suspected to be important for the antimalarial activity of artemisinin.[27] From radical **12**, it has been proposed that deoxyartemisinin **5** was formed *via* a capture of $H^{\bullet}$ and release of Fe^{III}-OH, the presence of cyclohexa-1,4-diene as hydrogen atom donor increasing the yield of **5**. In fact, the initial alkoxyl radical $RO_1^{\bullet}$ is a better H-atom abstracting agent from an external source than the alkyl radical **12** (this has been evidenced in autoxidation reactions). The formation of **5** might therefore occur directly from RO_1H *via* the release of Fe^{III}-OH. From the C_4-centered radical **12**, the $C_{3\alpha}$-hydroxy deoxyartemisinin **7** might be formed by two different pathways: a direct intramolecular epoxidation with release of iron(II) followed by a OH^- mediated intramolecular hydrolysis of the intermediate epoxide **13** [Scheme 2, route 2(a)], or by an indirect route involving the elimination of Fe^{III}-$O^{\bullet}$ from the $C_{4\alpha}$-radical **12**, followed by epoxidation of the C_3–C_4 double bond of the intermediate **14**, and then intramolecular opening of the α-oriented epoxide [Scheme 2, route 2(b)]. It should be noted that this latter proposed alternative pathway (and only that one) requires a high-valent epoxidizing iron-oxo species such as Fe^{III}-$O^{\bullet}$ (or Fe^{IV}=O). In fact, the formation of all the characterized products from route 2 can be explained by route 2(a) alone without the second hypothetical route, 2(b). Furthermore, it should be noted that the aromatization of hexamethyl Dewar benzene, sulfoxidation of thioanisole and hydroxylation of tetrahydronaphthalene, all of which have been invoked as proof for an iron-oxo intermediate, are reactions which can be achieved by efficient one-electron oxidants, but which do not absolutely require an oxygen atom transfer. Hexamethyl Dewar benzene can be isomerized to hexamethylbenzene *via* a radical cation chain rearrangement, even in the absence of high valent metal-oxo species [up to 48% isomerization was obtained in the presence of tris(*p*-bromophenyl)aluminium hexachloroantimonate alone].[28] Methyl phenyl sulfoxide and hydroxytetrahydronaphthalene can be obtained by a one-electron oxidation followed by nucleophilic attack of a water molecule.

One of the most efficient and selective reactions of a putative iron(IV or V)-oxo species should be the epoxidation of an electron-rich olefin such as cyclohexene. Exposure of artemisinin to one equivalent of $FeCl_2{\cdot}4H_2O$ in acetonitrile for 15 min at room temperature provided cleanly the same two products as above: the furano acetate as the major product and 3-hydroxydeoxyartemisinin (**6** and **7**, respectively, Scheme 1). In the presence of cyclohexene, the same products were obtained in 84% and 8% respectively, and no traces of cyclohexene oxide were detected.[23] The same lack of epoxidation was observed with β-artemether, and with a synthetic trioxane close to BO7 (bearing two phenyl groups instead of *p*-fluorophenyl at positions 6 and 7a) in the presence of $Fe^{II}Cl_2$ in acetonitrile, acetic acid or tetrahydrofuran.[24]

However, it must be noted that, even with good oxygen atom donors such as PhIO, NaOCl or $KHSO_5$, no oxygen atom transfer reaction, hydroxylation or epoxidation, is efficiently catalyzed by simple iron salts. Only porphyrin or Schiff base ligands provide a suitable coordination sphere around manganese or iron to catalyze oxygenation reactions.[29,30]

3.2 In the presence of a metalloporphyrin

We therefore decided to investigate the possibility of generating manganese(IV)-oxo or manganese(V)-oxo species from artemisinin and a synthetic manganese tetraarylporphyrin.[31] Several attempts to epoxidize cyclohexene with artemisinin (2 equiv. compared to the substrate) in the presence of catalytic amounts of Mn^{III}(TMP)Cl or $Mn^{II/III}(Cl_{12}TMP)Cl$[32] (5 mol% with respect to the olefin) were performed. These experiments were unsuccessful: there was no olefin conversion and no traces of epoxide after 30 min at room temperature, suggesting that no metal-oxo species was generated by artemisinin in the presence of a metalloporphyrin.

In fact, hydroperoxides are known to be poor oxygen atom donors with respect to metalloporphyrins or non-heme metal complexes,[29,33] and no evidence has been reported up to now that dialkylperoxide compounds are better oxygen atom donors than hydroperoxides. The main reaction pathway when a peroxide is activated by a transition metal complex corresponds to the homolytic cleavage of the weak peroxidic O–O bond.

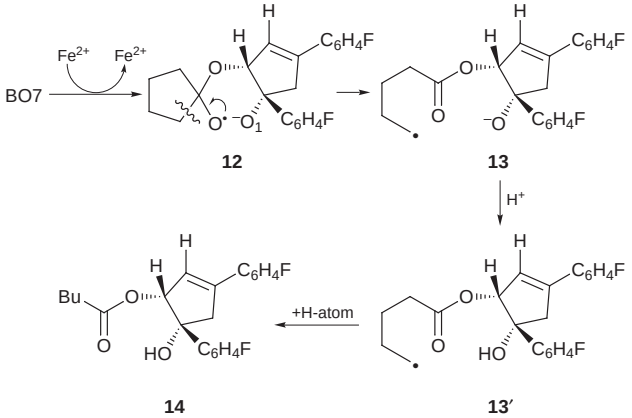
4 Is artemisinin an alkylating agent *via* C-centered radicals?

The best way to answer this question will be to isolate and to characterize fully a covalent adduct of artemisinin with a low weight molecule like heme (or a heme model) or with a parasitic protein. In fact, early studies on the activation of artemisinin derivatives have been described using simple metal salts, and then developed with metalloporphyrins.

4.1 In the presence of metal salts

Artemisinin was reported to form adduct(s) with heme after generating an unidentified oxy radical which, after rearrangement, can also alkylate malarial proteins.[4,21]

The reaction products of BO7 with iron(II) chloride tetrahydrate have been carefully identified, thus giving information about the possible intermediates.[24] Under different reaction conditions, **14** was a constant product of the reaction (Scheme 4). The origin of this product is rationalized by the rupture of the



Scheme 4 Mechanism of iron(II) mediated degradation of BO7 (from reference 24)

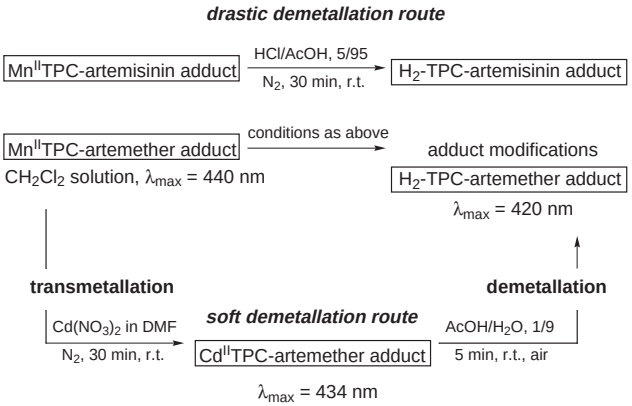
peroxidic bond of BO7 after a single electron transfer from an iron(II) ion, giving the radical anion **12**, which quickly isomerizes to **13** or **13′** after protonation. The alkyl radical **13′** is trapped in the presence of a thiol to afford the pentanoate **14**. The intermediate radical **13** is completely analogous to radical **9** (Scheme 2) produced by activation of artemisinin under similar conditions. This common pathway for artemisinin and BO7 is an indication that these compounds probably share the same reaction mode.

The radicals R–$CH_2^{\bullet}$ **9** and **13** are potentially alkylating species. We therefore decided to trap these intermediates by characterization of covalent adducts between these radicals and a synthetic heme model.

4.2 In the presence of a metalloporphyrin

As a heme model, we used a hydrophobic complex $Mn^{II}TPP$ generated *in situ* by borohydride reduction of $Mn^{III}(TPP)Cl$ (TPP stands for the dianion of tetraphenylporphyrin). This synthetic analogue of heme was expected to provide a limited number of products because of its four-order symmetry.

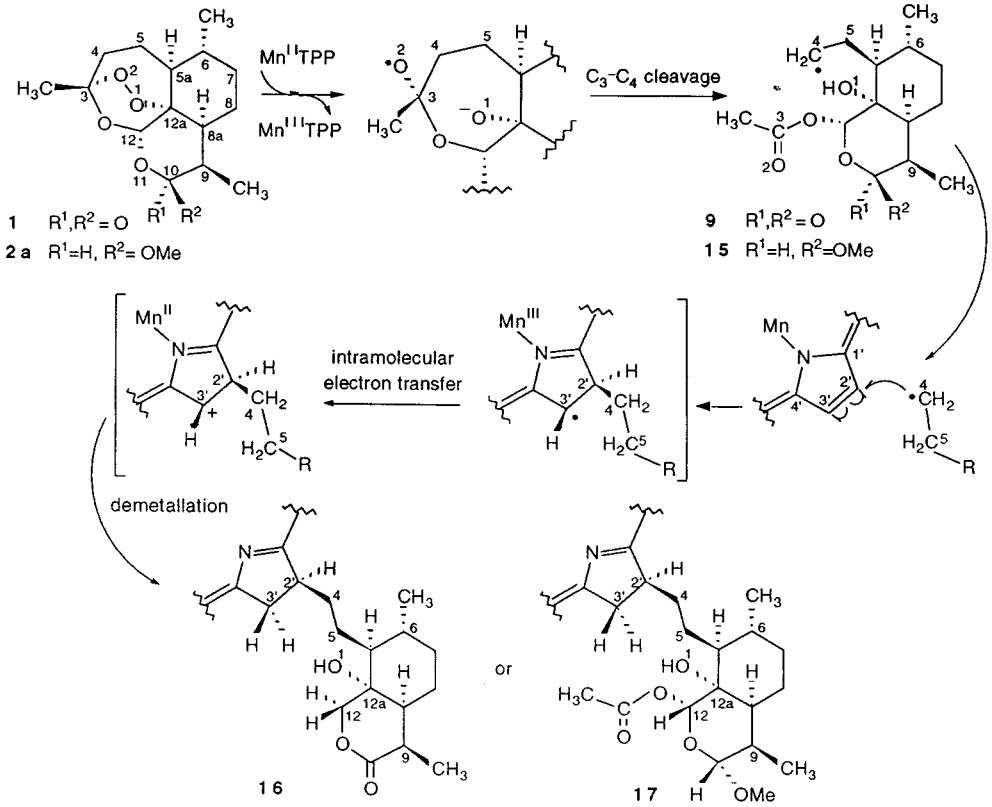
By reacting $Mn^{III}(TPP)Cl$ with three equivalents of artemisinin, β-artemether or BO7, in the presence of borohydride in dichloromethane, manganese chlorin-type adducts were formed between the macrocycle and one of these antimalarial drugs. The demetallation of these manganese(II) adducts was achieved by adding a solution of acetic and hydrochloric acids (95 : 5, v/v) directly to the reaction mixture. In the cases of artemisinin or BO7, this treatment allowed the removal of manganese without any other modification of the corresponding adducts. However, in the case of β-artemether, this drastic demetallation procedure could not be applied, owing to the lability of the B cycle of β-artemether itself under such strongly acidic conditions. A gentler demetallation was then carried out, by transmetallation of the Mn(II)-chlorin-β-artemether adduct to its analogous cadmium(II) derivative, followed by demetallation of the cadmium(II) adduct under very mild conditions (Scheme 5). The resulting demetallated adducts between the



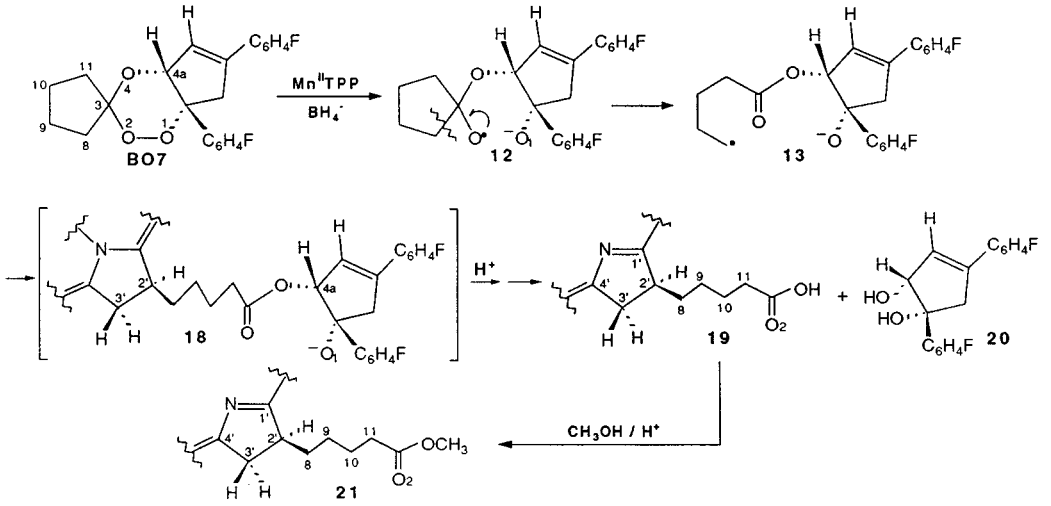
Scheme 5 Demetallation of the manganese(II)–tetraphenylchlorin–artemisinin and tetraphenylchlorin-β-artemether adducts

tetraphenylchlorin and the trioxane-derived moiety were completely characterized by the usual analytical methods, including 2D-NMR (adducts **16** and **17** with artemisinin and artemether, respectively, Scheme 6; adduct **21** with BO7, Scheme 7).

In all three cases, the covalent chlorin-drug adducts were not minor compounds: 20–30% of pure adducts were obtained. These adducts resulted from addition at the β-pyrrolic position $C_{2'}$ of the macrocycle of a non-sterically hindered alkyl radical derived from the antimalarial drug, namely radical **9** in the case of artemisinin (or its analogue in the case of artemether) and **13** in the case of BO7. A secondary alkyl radical was therefore formed at the adjacent position $C_{3'}$ of the macrocycle, leading after an intramolecular electron abstraction by Mn(III) to a carbocation at $C_{3'}$. The addition of borohydride at this position produced the dihydropyrrole ring. Borohydride also mediated the reductive elimination of the ester at positions C_{12} of artemisinin and C_{4a} of BO7. In the case of artemisinin, the introduction of two hydrogen atoms from borohydride at positions $C_{3'}$ of the macrocycle and C_{12} of the artemisinin



Scheme 6 Mechanism of alkylation of the heme model $Mn^{II}TPP$ by artemisinin

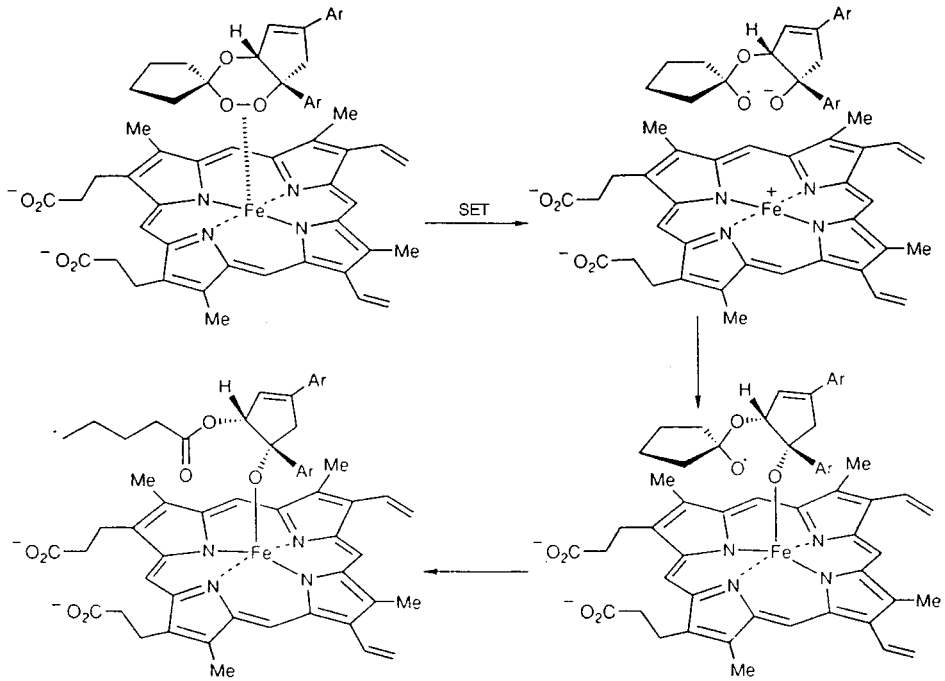


Scheme 7 Mechanism of alkylation of the heme model Mn^{II}TPP by BO7

fragment was confirmed by using borodeuteride instead of borohydride as reducing agent. The reduction at position 12 did not occur in the case of β-artemether. The 'entire' drug was conserved in the addition product of tetraphenylchlorin and the β-artemether-derived radical. Adduct **17** was therefore isolated without loss of any fragment from the drug (for a preliminary communication about alkylating properties of artemisinin, see ref. 34; complete characterization of these products, including artemether and BO7 derived adducts and analysis of the mechanism of their formation have been reported in reference 35).

The antimalarial activity of BO7 was not influenced by the absolute configuration of the molecule, the pure enantiomers being no more active than the racemate.[11] However, its mode of action on the intraerythrocytic parasite was rationalized in terms of close docking by a twist-boat conformer of the 1,2,4-trioxane cycle with a heme molecule (Scheme 8). the close interaction between the iron(II) of heme [or manganese(II)] and the peroxide bond results in a one-electron transfer to the O–O antibonding orbital, causing the fast scission of the O–O bond. The generated acetal radical then irreversibly isomerizes to a non bulky C-centered radical, the ultimate alkylating, and probably lethal, agent.

In addition, it should be noted that BO7 (with a cyclopentane and two *p*-fluorophenyl substituents) is a highly hydrophobic molecule like artemisinin, indicating that this non-aqueous solubility is commonly shared by promising antimalarial drugs.[4] Among the semi-synthetic derivatives of artemisinin, many hydrosoluble molecules have been synthesized, bearing for instance an amine, a glycosyl, an ether or, more recently, a butyric acid side-chain.[36] Only few of them are pharmacologically active, and usually on a narrow spectrum of parasite strains, considerably reducing interest in them. Furthermore, conversion of esters derived from artemisinin to the corresponding acids drastically reduces their antimalarial activity, suggesting again that the lipophilicity, probably related to a fast diffusion through membranes, plays an important role in determining the antimalarial activity of this series.[4] Since heme itself is a rather hydrophobic molecule, the lipophilicity and the boat conformation of 1,2,4-trioxanes are two necessary factors



Scheme 8 Nestling of the twisted-boat conformer of BO7 on the surface of heme, and activation of the peroxide bond. (Reproduced with permission from reference 11, SET stands for single electron transfer).

for their docking with heme, and then for the initiation of the alkylation reaction.

5 Concluding remarks

All these results based on a heme model clearly indicate that one of the major modes of reactivity of artemisinin and related synthetic trioxanes is their reductive activation leading to the homolytic cleavage of their peroxide bond. The resulting alkoxyl radicals are rearranged in non-sterically hindered C-centered radicals acting as powerful alkylating agents. These data on the alkylating properties of artemisinin derivatives also indicate that a heme molecule in the presence of artemisinin is not going to perform cytochrome P-450-type chemistry involving high-valent metal-oxo species. These alkylation reactions involve the generation of drug radicals in the vicinity of heme in a solvent-cage controlled reaction, instead of as freely diffusible radicals.

The characterization of these artemisinin-type adducts confirms that the alkylating properties of artemisinin is not limited to this natural compound, but is a common feature probably required for the antimalarial activity of endoperoxide-containing drugs. These compounds should be able to alkylate either heme itself or parasitic proteins. This alkylation process, which it is proposed occurs at a pharmacologically relevant concentration of the drug,[21] would inhibit the proteases responsible for the hemoglobin digestion within infected erythrocytes, thus starving the parasite of essential amino acids. The alkylation and inactivation of proteins involved in the heme polymerisation, namely the 'histidine-rich protein', would poison the parasite with redox active heme molecules. High heme concentrations are supposed to be responsible for oxidative stress within the cell inducing a disruption of membranes and they have also been shown to inhibit a parasitic hemoglobinase.[2] Alkylation of heme by a drug-derived radical may also be directly involved in the parasite death through the accumulation of non-polymerizable redox-active heme adducts which could also behave as inhibitors for heme-polymerisation enzymes.

Finally, the characterisation of these covalent adducts resulting from C-alkylation of a heme model by radicals derived either from artemisinin, artemether or a synthetic peroxide will be useful for the interpretation of the mass spectra of parasitic proteins alkylated by artemisinin and related derivatives. It will also contribute to giving a better molecular basis for the rational design of new synthetic antimalarial drugs having an endoperoxide function.

6 Acknowledgements

This work was supported by CNRS and by a grant from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (Director's Initiative Fund). Professor Charles W. Jefford (University of Geneva, Switzerland) is gratefully acknowledged for a gift of synthetic trioxane BO7 and Rhône-Poulenc-Rorer Doma (Antony, France) for a gift of β-artemether (Paluther®).

7 References

1 S. J. Foote and A. F. Cowman, *Acta Tropica*, 1994, **56**, 157.
2 M. Foley and L. Tilley, *Int. J. Parasitol.*, 1997, **27**, 213.
3 D. L. Klayman, *Science*, 1985, **228**, 1049.
4 S. R. Meshnick, T. E. Taylor and S. Kamchonwongpaisan, *Microbiol. Rev.*, 1996, **60**, 301.
5 K. L. Chan, C. K. H. Teo, S. Jinadasa and K. H. Yuen, *Planta Med.*, 1995, **61**, 285.
6 R. K. Haynes and S. C. Vonwiller, *Acc. Chem. Res.*, 1997, **30**, 73.
7 J. M. Petras, D. E. Kyle, M. Gettayacamin, G. D. Young, R. A. Bauman, H. K. Webster, K. D. Corcoran, J. O. Peggins, M. A. Vane and T. G. Brewer, *Am. J. Trop. Med. Hyg.*, 1997, **56**, 390.
8 J. N. Cumming, P. Ploypradith and G. H. Posner, *Adv. Pharmacol.*, 1997, **37**, 253.
9 C. W. Jefford, *Adv. Drug Res.*, 1997, **29**, 271.
10 W. Peters, B. L. Robinson, G. Tovey, J.-C. Rossier and C. W. Jefford, *Ann. Trop. Med. Parasitol*, 1993, **87**, 111.
11 C. W. Jefford, S. Kohmoto, D. Jaggi, G. Timari, J.-C. Rossier, M. Rudaz, O. Barbuzzi, D. Gérard, U. Burger, P. Kamalaprija, J. Mareda, G. Bernardinelli, I. Manzanares, C. J. Canfield, S. L. Fleck, B. L. Robinson and W. Peters, *Helv. Chim. Acta*, 1995, **78**, 647.
12 A. F. G. Slater, W. J. Swiggard, B. R. Orton, W. D. Flitter, D. E. Goldberg, A. Cerami and G. B. Henderson, *Proc. Natl. Acad. Sci. USA*, 1991, **88**, 325.
13 D. S. Bohle, R. E. Dinnebier, S. K. Madsen and P. W. Stephens, *J. Biol. Chem.*, 1997, **272**, 713.
14 S. R. Meshnick, A. Thomas, A. Ranz, C.-M. Xu and H.-Z. Pan, *Mol. Biochem. Parasitol.*, 1991, **49**, 181.
15 F. Zhang, D. K. Gosser, Jr. and S. R. Meshnick, *Biochem. Pharmacol.*, 1992, **43**, 1805.
16 S. R. Krungkrai and Y. Yuthavong, *Trans. R. Soc. Trop. Med. Hyg.*, 1987, **81**, 710.
17 M. D. Scott, S. R. Meshnick, R. A. Williams and D. T.-Y. Chiu, *J. Lab. Clin. Med.*, 1989, **114**, 401.
18 S. R. Meshnick, Y.-Z. Yang, V. Lima, F. Kuypers, S. Kamchonwongpaisan and Y. Yuthavong, *Antimicrob. Agents Chemother.*, 1993, **37**, 1108.
19 S. R. Meshnick, *Lancet*, 1994, **344**, 1441.
20 Y.-L. Hong, Y.-Z. Yang and S. R. Meshnick, *Mol. Biochem. Parasitol.*, 1994, **63**, 121.
21 W. Asawamahasakda, I. Ittarat, Y.-M. Pu, H. Ziffer and S. R. Meshnick, *Antimicrob. Agents Chemother.*, 1994, **38**, 1854.
22 D. J. Sullivan Jr., I. Y. Gluzman and D. E. Goldberg, *Science*, 1996, **271**, 219.
23 C. W. Jefford, M. G. H. Vicente, Y. Jacquier, F. Favarger, J. Mareda, P. Millasson-Schmidt, G. Brunner and U. Burger, *Helv. Chim. Acta*, 1996, **79**, 1475.
24 C. W. Jefford, F. Favarger, M. G. H. Vicente and Y. Jacquier, *Helv. Chim. Acta*, 1995, **78**, 452.
25 G. H. Posner, J. N. Cumming, P. Ploypradith and C. H. Oh, *J. Am. Chem. Soc.*, 1995, **117**, 5885.
26 I.-S. Lee and C. D. Hufford, *Pharmac. Ther.*, 1990, **48**, 345.
27 G. H. Posner, C. H. Oh, D. Wang, L. Gerena, W. K. Milhous, S. R. Meshnick and W. Asawamahasakda, *J. Med. Chem.*, 1994, **37**, 1256.
28 T. G. Traylor and A. R. Miksztal, *J. Am. Chem. Soc.*, 1987, **109**, 2770.
29 B. Meunier, *Chem. Rev.*, 1992, **92**, 1411.
30 W. Nam and J. S. Valentine, *J. Am. Chem. Soc.*, 1993, **115**, 1772.
31 A. Robert, M. Boularan and B. Meunier, *C. R. Acad. Sci. Paris*, 1997, **324II**, 59.
32 P. Hoffmann, A. Robert and B. Meunier, *Bull. Soc. Chim. Fr.*, 1992, **129**, 85.
33 P. A. MacFaul, I. W. C. E. Arends, K. U. Ingold and D. D. M. Wayner, *J. Chem. Soc., Perkin Trans. 2*, 1997, 135.
34 A. Robert and B. Meunier, *J. Am. Chem. Soc.*, 1997, **119**, 5968.
35 A. Robert and B. Meunier, *Chem. Eur. J.*, 1998, in the press.
36 A. J. Lin, A. B. Zikry and D. E. Kyle, *J. Med. Chem.*, 1997, **40**, 1396.

Received 9th March 1998
Accepted 9th April 1998