Characterization of the Main Radical and Products Resulting from a Reductive Activation of the Antimalarial Arteflene (Ro 42−1611)

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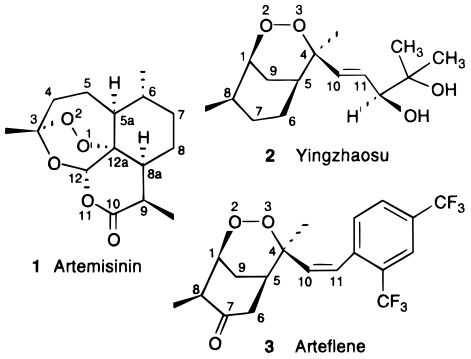
The peroxide-containing antimalarial drug arteflene (Ro 42-1611) generates an alkyl radical after the reductive homolytic cleavage of the peroxide bond in the presence of a heme model Mn^{II}(TPP). This alkyl radical has been trapped by TEMPO, and the different products of the reduction activation of arteflene have been characterized. These data suggest that, in these experimental conditions, arteflene is not a significant alkylating agent compared to artemisinin, a trioxane-containing antimalarial drug.

Introduction

Artemisia a. is a plant with a long history of medical use against fever and malaria in China.[1] The active molecule, artemisinin (**1**, Scheme 1), isolated in 1972, is a sesquiterpene lactone containing an endoperoxide function critical for its activity. This drug and its hemisynthetic derivatives artesunate and artemether are currently being developed to treat severe and multidrug-resistant malaria, including cerebral malaria.

A reasonable hypothesis on the mechanism of action of artemisinin is the reductive activation of its endoperoxide bridge by Fe^{II}-heme resulting from the digestion of hemoglobin by *Plasmodium*,[2,3] which leads to dioxygen-derived radicals responsible for an oxidative stress[4] within infected erythrocytes or to C-centered radicals.[5−10] We recently illustrated the alkylating properties of one of these C-centered radicals by reporting the characterization of a covalent adduct between artemisinin and a heme model based on *meso*-tetraphenylporphyrin.[11,12] The isolated chlorin-type adduct resulted from a C-alkylation of a β-pyrrolic position of the porphyrin macrocycle by a carbon radical at the C4 position of artemisinin produced after reductive cleavage of the peroxide bridge. In similar conditions, we observed the same alkylating behavior of

Scheme 1. Structure of Artemisinin 1, Yingzhaosu 2, and Arteflene 3



β-artemether, a modified analogue of artemisinin. We also found that the synthetic antimalarial trioxane BO7 gave a chlorin adduct after reductive activation.[10,12] These results suggested that the alkylating ability of artemisinin, which is responsible for the death of the parasite, is not limited to this naturally occurring product but is a general feature probably required for the antimalarial activity of endoperoxide-containing drugs (for a recent review on the alkylating ability of artemisinin and related trioxanes, see ref 13).

Unfortunately, the extraction and, therefore, the cost of artemisinin derivatives is a limiting factor to the wide use of these efficient drugs in tropical and subtropical regions, where malaria is endemic and chloroquine-resistant strains are spreading. There is a need for new low-cost synthetic drugs with pharmacological properties similar to those of artemisinin. Many artemisinin models[5,14−19] and other naturally occurring peroxide compounds,[20] different than artemisinin, with good antimalarial activity have been reported. Yingzhaosu A (**2**, Scheme 1) is one of these natural peroxide compounds, but little information is available about the biological activity of this compound, probably because its isolation from the plant source, a rare ornemental vine, *Artabotrys uncinatus (L.) Merr.*, was difficult and erratic and did not provide sufficient amounts of material for a proper

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evaluation of its antimalarial activity.[21,22] However, a series of analogues have been prepared and evaluated as antimalarial agents. In particular, molecules having a keto group within the ring system and a lipophilic substituent possessed activities comparable to those of artemisinin. One of these models, arteflene (**3**, Scheme 1), has been taken through clinical trials.[22−24] However, this drug is associated with a certain degree of recrudescence.[3]

The reactivity of arteflene in the presence of a heme model, under experimental conditions described for artemisinin and BO7, has been investigated. We found that a manganese(II) porphyrin can activate the peroxide function of **2** by electron transfer and induces the homolytic cleavage of the C4−C5 bond. The resulting alkyl radical centered at C5 was not in a suitable position to alkylate the porphyrin ligand located in the vicinity via an intramolecular process. The porphyrin ligand was recovered unchanged, but trapping of the C5-centered radical by TEMPO allowed us to isolate and characterize the two fragments of arteflene. These data provide a deeper insight into the mechanism of action of this antimalarial agent.

Experimental Section

Materials. (1*S*,4*R*,5*R*,8*S*)-4-[(*Z*)-2,4-Bis(trifluoromethyl)-styryl-4,8-dimethyl-2,3-dioxabicyclo[3.3.1]nonan-7-one, namely, arteflene (Ro 42−1611), was a gift from F. Hoffmann-La Roche. Dichloromethane (stabilized with amylene) and hexane supplied by Fluka were of low evaporation residue content (≤0.0005%). All other commercially available reagents and solvents were obtained from Aldrich or Fluka. The tetra-*n*-butylammonium borodeuteride was prepared by reaction of tetra-*n*-butylammonium chloride with sodium borodeuteride 98%D: $NaBD_4$ (100 mg, 2.4 mmol, 1.5 equiv) and NaOH (4 mg) were dissolved in deuterated water (1 mL). This solution was mixed with a solution containing *n*-$Bu_4N^+Cl^-$ (443 mg, 1.6 mmol, 1 equiv) in D_2O (1 mL) and stirred for 1 min. The resulting tetra-*n*-butylammonium borodeuteride was extracted with CH_2Cl_2 (5 mL). The organic layer was dried over sodium sulfate, and the product was recovered by evaporation of the solvent to dryness (yield: 80%; 95+ atom % D measured by ^{1}H NMR). [IR (KBr) ν = 1756, 1721, 1681 cm^{-1} (BD_4^-)]. Mn^{III}(TPP)Cl was prepared by metalation of chlorin-free H_2TPP with $Mn^{II}(OAc)_2 \cdot 4H_2O$ in DMF in the presence of 2,4,6-collidine.[25] The *cis*-benzylideneacetone was obtained by irradiation of the commercially available *trans*-isomer with a 200 W tungsten lamp in acetonitrile under argon. After 48 h, the *cis*/*trans* ratio was 30/70. The *cis* olefin was purified by column chromatography (SiO_2, pentane/diethyl ether 95/5 to 90/10, v/v).[26] [^{1}H NMR, 250 MHz in CD_2Cl_2: δ, ppm 7.30−7.60 (m, 5H), 6.88 and 6.21 (2 × d, 2 × 1H, J = 12.6), 2.18 (s, 3H)]. Aluminum oxide 90, 70−230 mesh, activity II−III (Merck) and silica 60, 70−200 μm (SDS, France) were used for column chromatography.

Reaction of Arteflene with Mn^{III}(TPP)Cl in the Presence of Tetra-*n*-butylammonium Borohydride. Isolation of Compounds 6−11. Mn^{III}(TPP)Cl (30 mg, 43 μmol, 1 equiv) and arteflene (53 mg, 129 μmol, 3 equiv) were dissolved in CH_2Cl_2 (5 mL). This solution was carefully degassed and kept under a nitrogen atmosphere. Tetra-*n*-butylammonium borohydride (110 mg, 430 μmol, 10 equiv) was then added as a solid. The mixture was allowed to stand for 3.5 h at room temperature with magnetic stirring under nitrogen. Demetalation was performed as previously decribed[12] by addition of a degassed solution of cadmium(II) nitrate [$(Cd(NO_3)_2 \cdot 4H_2O$, 263 mg, 860 mmol, 20 equiv) in DMF (2 mL)] to the reaction mixture, and stirring was continued for 20 min. An aqueous solution of acetic acid (10 vol %, 10 mL) was then added under air. The organic layer was extracted with CH_2Cl_2, washed with water, dried over sodium sulfate, and evaporated to dryness. Purification of the crude product was performed by column chromatography on silica gel using a hexane/dichloromethane mixture (gradient from 60/40 to 10/90, v/v). The tetraphenylporphyrin ligand was eluted at the solvent front followed by a mixture of compounds **6**, **7**, and **8**, which was recovered by evaporation of the solvent (**6**/**7**/**8** ratio was in the range of 45/36/19 to 30/30/40, overall yield 28% with respect to arteflene). NMR characterization was performed on the mixture of compounds **6**−**8**. For clarity, the spectra of the three compounds are described separately. Compound **6**: ^{1}H NMR (δ, CD_2Cl_2) 8.00−7.70 (m, 3H), 6.69 (br d, J = 11.7, 1H), 5.91 (dd, J = 11.7 and 9.5, 1H), 4.36 (m, J = 9.5, 6.2 and 1.0, 1H), 1.27 (d, J = 6.2, 3H). Compound **7**: ^{1}H NMR (δ, CD_2Cl_2) 8.00−7.70 (m, 3H), 6.96 (br d, J = 15.8, 1H), 6.39 (dd, J = 15.8 and 5.8, 1H), 4.54 (m, J = 6.5, 5.8 and 1.7, 1H), 1.37 (d, J = 6.5, 3H). Compound **8**: ^{1}H NMR (δ, CD_2Cl_2) 8.00−7.70 (m, 2H), 7.50 (d, J = 7.5, 1H), 3.87 (m, J = 6.2 and 6.2, 1H), 2.95 (m, 2H), 1.73 (m, 2H), 1.23 (d, J = 6.2, 3H).

The mixture of compounds **6**−**8** (2.9 mg) was stirred at room temperature with 4-(dimethylamino)pyridine (DMAP, 1.5 mg, 12 μmol, 1.2 equiv) and acetic anhydride (9.4 μL, 100 μmol, 10 equiv) in CH_2Cl_2 (500 μL). After 1 h of reaction, the resulting products were passed through an alumina column with CH_2Cl_2 as eluent. The mixture of acetylated derivatives **9**−**11** was recovered by evaporation of solvent. MS (DCI/NH_3^+) *m*/*z* 343(15), 344(100)[M^+ + 18 for **9** and **10**], 345(22), 346(64)[M^+ + 18 for **11**], 347(10). NMR characterization was performed on the mixture of compounds **9**−**11**, but for clarity, the spectra of the three compounds are described separately. Compound **9**: ^{1}H NMR (δ, $CDCl_3$) 8.00−7.60 (m, 3H), 6.69 (br d, J = 11.8, 1H), 5.84 (dd, J = 11.8 and 9.0, 1H), 5.42 (m, J = 9.0, 6.2 and 1.0, 1H), 1.99 (s, 3H), 1.25 (d, J = 6.2, 3H). Compound **10**: ^{1}H NMR (δ, $CDCl_3$) 8.00−7.60 (m, 3H), 6.95 (br d, J = 15.8, 1H), 6.26 (dd, J = 15.8 and 5.8, 1H), 5.54 (m, J = 6.5, 5.8 and 1.7, 1H), 2.09 (s, 3H), 1.42 (d, J = 6.5, 3H). Compound **11**: ^{1}H NMR (δ, $CDCl_3$) 8.00−7.60 (m, 2H), 7.45 (d, J = 7.5, 1H), 4.97 (m, J = 6.2 and 6.2, 1H), 2.86 (m, 2H), 2.04 (s, 3H), 1.88 (m, 2H), 1.27 (d, J = 6.2, 3H).

Isolation of Compound 12. Mn^{III}(TPP)Cl (12 mg, 16 μmol, 1 equiv), arteflene (20 mg, 49 μmol, 3 equiv), and 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO, 15 mg, 98 μmol, 6 equiv) were dissolved in CH_2Cl_2 (2 mL). This solution was degassed and kept under argon. Tetra-*n*-butylammonium borohydride (42 mg, 163 μmol, 10 equiv) was then added as a solid. The mixture was stirred at room temperature for 3.5 h. Upon exposure to air, the dark green solution was washed with water (pH 5), dried over sodium sulfate, and evaporated to dryness. The crude mixture was then acetylated by addi-

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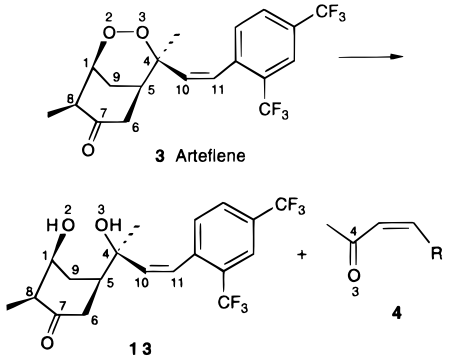
tion of a solution of DMAP (16 mg, 130 μmol, 8 equiv) and $(CH_3CO)_2O$ (92 μL, 978 μmol, 60 equiv) in CH_2Cl_2 (2 mL). After 1 h of stirring at room temperature, the reaction mixture was washed with water (successively at pH 5, pH 8, and pH 5) and dried over sodium sulfate. The purification step was performed using an alumina column with a mixture hexane/dichloromethane (gradient from 100/0 to 0/100, v/v). A mixture of the acetylated derivatives **9** and **11** was first eluted (ratio **9**/**11** = 100/0 to 85/15, v/v); the adduct **12** was then recovered in 6% yield with respect to arteflene. R_f = 0.5 (SiO_2, hexane/dichloromethane, 60/40 v/v); ^{1}H NMR (δ, CD_2Cl_2) 5.14 (m, J = 3.5, 3.5 and 3.5, 1H), 4.74 (m, J = 11.0, 11.0 and 4.0, 1H), 3.98 (m, J = 12.0, 12.0, 4.0 and 4.0, 1H), 2.55 (m, J = 11.4, 4.0, 4.0 and 2.0, 1H), 2.34 (m, J = 13.8, 4.0, 3.5 and 2.0, 1H), 2.01 and 2.03 (2 × s, 6H), 1.72 (m, J = 11.0, 7.0, and 3.5, 1H), 1.40−1.25 (m, 2H), 1.44 (m, 6H), 1.07 (m, 12H), 0.87 (d, J = 7.0, 3H); MS (DCI/NH_3^+) m/z 370(100)[M^+ + 1], 371(24), 372(3). $[\alpha]_{365}$ = +180 (c = 290 × 10^{-6}, CH_2Cl_2).

Reaction of Arteflene with Mn^{III}(TPP)Cl in the Presence of Tetra-*n*-butylammonium Borodeuteride. Isolation of Compounds 9-*d* to 11-*d*$_2$. Mn^{III}(TPP)Cl (17 mg, 24 μmol, 1 equiv) and arteflene (30 mg, 73 μmol, 3 equiv) were dissolved in CH_2Cl_2 (1.5 mL). This solution was degassed and kept under an argon atmosphere. Tetra-*n*-butylammonium borodeuteride (63 mg, 244 μmol, 10 equiv) was then added as a solid. After 1.5 h of stirring at room temperature, the reaction mixture was washed with water (pH 5), dried over sodium sulfate, and evaporated to dryness. The crude product was purified using a silica column and hexane/dichloromethane (gradient from 50/50 to 0/100, v/v). Acetylation was carried out by addition of a solution of DMAP (8 mg, 655 μmol) and $(CH_3CO)_2O$ (24 μL, 254 μmol). After 1 h of stirring, the product was washed with water (successively at pH 5, pH 8, and pH 5), dried over sodium sulfate, and purified on silica gel with dichloromethane as eluent. After evaporation of the solvent, the mixture of acetylated derivatives **9-*d*** to **11-*d*$_2$** was recovered. For clarity, NMR spectra are described separately. Compound **9-*d***: ^{1}H NMR (δ, CD_2Cl_2) 8.00−7.50 (m, 3H), 6.72 (br d, J = 11.7, 1H), 5.87 (d, J = 11.7, 1H), 1.97 (s, 3H), 1.27 (s, 3H). The signal at 5.42 (H4) disappeared. Compound **10-*d***: ^{1}H NMR (δ, CD_2Cl_2) 8.00−7.50 (m, 3H), 6.96 (br d, J = 15.8, 1H), 6.32 (d, J = 15.8, 1H), 2.08 (s, 3H), 1.40 (s, 3H). The signal at 5.54 (H4) disappeared. Compound **11-*d*$_2$**: ^{1}H NMR (δ, CD_2Cl_2) 8.00−7.60 (m, 2H), 7.50 (d, J = 7.5, 1H), 2.87 (m, 1.5H), 2.03 (s, 3H), 1.85 (m, 1.5H), 1.23 (s, 3H). The signal at 4.97 (H4) disappeared.

Isolation of Compound 12-*d*. The synthesis and workup were the same as for **12**, but with tetra-*n*-butylammonium borodeuteride as reducing agent [Mn^{III}(TPP)Cl (12 mg, 16 μmol, 1 equiv), arteflene (20 mg, 49 μmol, 3 equiv), 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO, 15 mg, 98 μmol, 6 equiv), and tetra-*n*-butylammonium borodeuteride (43 mg, 163 μmol, 10 equiv) were dissolved in CH_2Cl_2 (2 mL)]. ^{1}H NMR (δ, CD_2Cl_2) signals were the same as for **12** except that the signal at 4.74 (H7) disappeared. MS (DCI/NH_3^+) m/z 370(18), 371(100)[M^+ + 1], 372(22), 373(4).

Reaction of *cis*-Benzylideneacetone with Mn^{III}(TPP)Cl in the Presence of Tetra-*n*-butylammonium Borohydride. A solution of Mn^{III}(TPP)Cl (24 mg, 34 μmol, 1 equiv) and *cis*-benzylideneacetone (15 mg, 103 μmol, 3 equiv) in CH_2Cl_2 (3 mL) was degassed and kept under a nitrogen atmosphere. Tetra-*n*-butylammonium borohydride (61 mg, 342 μmol, 10 equiv) was then added as a solid. After 4 h of stirring at room temperature, the reaction mixture was washed with water (pH 5), dried over sodium sulfate, and evaporated to dryness. The crude product was purified using a silica column with a hexane/dichloromethane gradient (from 60/40 to 0/100, v/v) initially, followed by a dichloromethane/methanol mixture (gradient from 100/0 to 97/3, v/v). After evaporation of the solvent, a mixture of *cis*-4-phenyl-3-buten-2-ol and 4-phenylbutan-2-ol (ratio 54/46) was recovered. For clarity, NMR spectra are described separately. ***cis*-4-Phenyl-3-buten-2-ol**: ^{1}H NMR (δ, CD_2Cl_2) 7.65−7.10 (m, 3H), 6.49 (d, J = 11.9, 1H), 5.68 (dd, J = 11.9 and 9.0, 1H), 4.75 (m, 1H), 1.33 (d, J = 6.0, 3H). **4-Phenylbutan-2-ol**: ^{1}H NMR (δ, CD_2Cl_2) 7.65−7.10 (m, 3H), 3.79 (m, 1H), 2.70 (m, 2H), 1.71 (m, 2H), 1.20 (d, J = 6.0, 3H).

Scheme 2. Isolated Products from the Reductive Activation of Arteflene by Zn/CH_3COOH, $FeCl_2$ or Hemin Chloride/*N*-Acetylcysteine[28]



Results and Discussion

The activation of the peroxide function of antimalarial 1,2,4-trioxanes and cyclic dialkylperoxides such as arteflene by heme resulting from the digestion of host hemoglobin by *Plasmodium* is probably able to generate in vivo alkylating species responsible for the parasite death. Moreover, when [^{14}C]-arteflene or [^{3}H]-dihydroartemisinin were incubated with *P. falciparum* infected erythrocytes, beside a small amount of low-molecular heme−drug adducts (13−15% of the total parasite-associated radioactivity), these drugs were able to specifically alkylate the same and not particularly abundant parasite proteins, one of which has a similar size to that of the histidine-rich protein (HRP, 42 kDa)[27] involved in the polymerization of heme in infected erythrocytes (the radioactivity associated with parasite proteins was ca. 65%).[8] The reductive activation of arteflene by zinc in acetic acid, by iron(II) chloride, or by hemin chloride associated with *N*-acetylcysteine as reducing agent has been recently investigated,[28] in conditions similar to those previously reported for artemisinin or synthetic trioxane derivatives.[29−31] In these conditions, the two identified products (Scheme 2) of reaction of arteflene were the diol **13**, resulting from the reductive cleavage of the O−O bond followed by the reduction of the generated RO$^\bullet$, and the α,β-unsaturated ketone **4** (this latter one is the major one in the presence of hemin chloride/*N*-acetylcysteine). The formation of the ketone **4** suggests the parallel formation of a C-centered radical **5** (Scheme 3), but this radical was not trapped in these reaction conditions.

Identification of Compounds 6−8. In the present study, we have been able to characterize different products resulting from the reductive activation of arteflene **3** by manganese(II) tetraphenylporphyrin. In the experimental conditions leading to the alkylation of the por-

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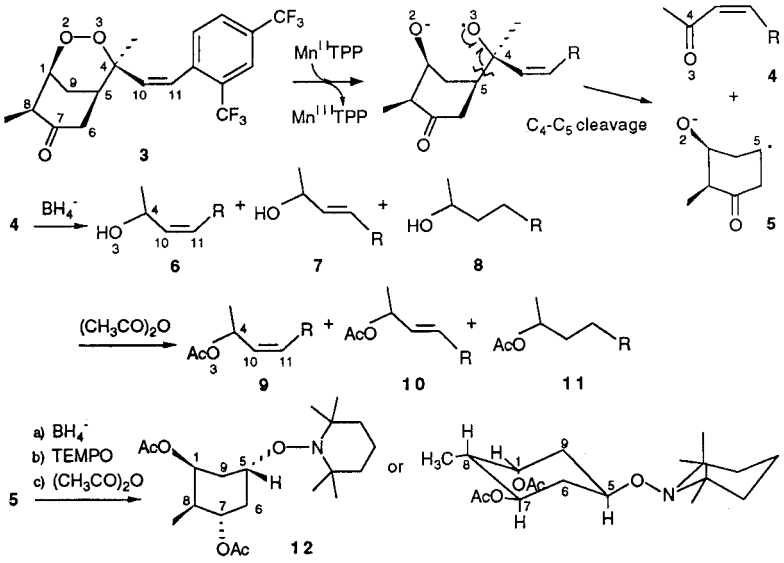
Scheme 3. Mechanism of Reductive Activation of Arteflene by Mn^{II}(TPP) in the Presence of Borohydride; R = 2,4-Bis(trifluoromethyl)phenyl



phyrin ligand by artemisinin, β-artemether, and BO7 at a β-pyrrolic position,[11,12] arteflene was not able to alkylate the porphyrin ligand (Mn^{II}TPP being generated in situ from Mn^{III}(TPP)Cl and BH_4^- in CH_2Cl_2). After the reaction of Mn^{II}TPP with arteflene, the crude reaction mixture was treated to demetalate the metalloporphyrin via a transmetalation from manganese(II) to cadmium (II).[12,13] This mild demetalation procedure avoids possible modification of chlorin or porphyrin adducts due to strongly acidic conditions that are usually required for the demetalation of manganese porphyrins. The unmodified free-base tetraphenylporphyrin was recovered after chromatography, but no macrocycle modified by arteflene alkylation was detected in the reaction mixture.

However, the products **6**−**8**, resulting from arteflene fragmentation, were isolated in a 28% yield with respect to the initial amount of arteflene and characterized by NMR as a mixture (see Scheme 3 for structures of **6**−**8**). The H4 protons were detected at 4.36 and 4.54 ppm for the allylic alcohols **6** and **7**, respectively, and the values of the coupling constants between H10 and H11 (11.8 and 15.8 Hz in compounds **6** and **7**, respectively, and 13.3 Hz in arteflene) indicated the isomerization of the C10−C11 double bond of **4** to produce the thermodynamically more stable *trans*-isomer during the reduction.

It should be mentioned that, by activation of arteflene, a significant amount of product **8** resulting from the hydrogenation of the C10−C11 double bond was also recovered. The H4 proton was detected at 3.87 ppm, and a multiplet at 2.95 was attributed to the benzylic CH_2 group.

The acetylation of the mixture of **6**−**8** with acetic anhydride in the presence of DMAP afforded quantitatively the derivatized alcohols **9**−**11**. In ^{1}H NMR spectra, the acetyl groups were detected at 1.99, 2.09, and 2.04 ppm for compounds **9**, **10**, and **11**, respectively, as proofs for the presence of hydroxyl functions in compounds **6**−**8**. The signals of the protons H4 for **9**, **10**, and **11** were deshielded by 1.0−1.1 ppm compared to corresponding protons in alcohols **6**, **7**, and **8**. The other signals were not significantly modified by acetylation ($\Delta\delta < 0.15$ ppm). By mass spectrometry (DCI/NH_3^+), the molecular peaks were identified at $m/z = 344$ ($M^+ + 18$ for **9** and **10**) and 346 ($M^+ + 18$ for **11**), whereas no molecular peaks could be detected under similar conditions for the alcohols **6**−**8**.

Isolation of Compound 12. The stable free radical 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO) is a specific trap for alkyl radicals (rate constant for trapping R$^\bullet$ radicals = 10^9 M^{-1} s^{-1}; it reacts 10^6 times slower with alkoxyl radicals such as *t*-BuO$^\bullet$ and not at all with alkylperoxyl radicals). Furthermore, the reaction between TEMPO and R$^\bullet$ gives only the coupled product TEMPO−R, and TEMPO does not abstract hydrogen atoms that are bound to the carbon adjacent to the radical center in R$^\bullet$.[32,33] Arteflene was reacted with Mn^{III}(TPP)Cl and BH_4^- in the presence of TEMPO (2 equiv versus arteflene). The manganese porphyrin was not demetalated (it was easier to remove Mn(TPP)Cl than the free-base porphyrin ligand by chromatography). The crude reaction mixture was directly acetylated, and compound **12**, resulting from the addition of the cyclohexyl radical **5** to TEMPO, was recovered by chromatography (yield 6%). The TEMPO adduct **12** was completely characterized by its ^{1}H NMR spectrum. The presence of two acetyl resonances at 2.01 and 2.03 ppm indicated the derivatization of two hydroxyl functions at positions C1 and C7, the second one due to the reduction of the ketone function of arteflene by borohydride ($\delta = 4.74$ ppm for H7). At C7, the introduction of H^- was stereospecifically achieved in an axial position,[34] as confirmed by the large coupling constants of H7 with the axial protons H8 and H6a (both being equal to 11.0 Hz). By using borodeuteride as reducing agent (compound **12-*d***), the complete introduction of D^- at position C7 resulted in the extinction of the resonance at 4.74 ppm. The analysis of the ^{1}H−^{1}H coupling constants also indicates that the configurations of C1 and C8 were unchanged with respect to arteflene and that the TEMPO moiety was introduced at C5 in an equatorial position, as expected for a bulky substituent.

Mechanism of the Activation of arteflene by Reduced Metalloporphyrins. The formation of the

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alcohols **6**−**8** can be explained by the reductive activation of the peroxide function of **3** by the manganese(II) porphyrin, resulting in the homolytic cleavage of the O−O bond (Scheme 3). The alkoxyl radical formed on O3 quickly induces the β-scission of the C4−C5 bond, producing the α,β-unsaturated ketone **4** and the cyclohexyl radical **5**. (a Priori, the O−O bond cleavage might also produce an alkoxyl radical centered on O2, able to isomerize by β-scission to other alkyl radicals; although this pathway was not identified until now, it cannot be completely ruled out owing to the moderate yield of the identified products. However, the interaction between the manganese(II) porphyrin and arteflene may also favor the formation of the radical on O3 if the docking of arteflene with $Mn^{II}TPP$ favors the approach of O2 on the metal center). Compound **4** is not stable under the reaction conditions. In the presence of an excess of borohydride, the reduction of the ketone to give the alcohol **6** was expected. The α,β-unsaturated ketone **4** can also be reduced by addition of borohydride to the conjugated double bond to give the saturated ketone which, after reduction of the carbonyl, produces the saturated alcohol **8**. It has been well-documented that the reduction of conjugated ketones by $NaBH_4$ can follow either 1,2-addition to the carbonyl bond or 1,4-addition to the conjugated double bond, leading in general to substantial amounts of fully saturated alcohols (for example, the reduction of substituted *cis*- and *trans*-cinnamates to give dihydrocinnamates was reported in ref 34). When the model compound *cis*-benzylideneacetone was submitted to the same reaction conditions [$Mn^{III}(TPP)Cl + BH_4^-$], we observed, beside the reduction of the carbonyl, the reduction of the conjugated double bond with a 25% yield, the products of the reaction being *cis*-4-phenyl-3-buten-2-ol and 4-phenylbutan-2-ol (ratio 54/46). When *trans*-benzylideneacetone was reacted in the same reaction conditions, the ratio of *trans*-allylic alcohol to the fully saturated alcohol was 70/30, close to that obtained in the case of *cis*-benzylideneacetone.

The production of the *trans*-allylic alcohol **7**, obtained after reductive activation of arteflene, which contains a *cis* double bond, is difficult to explain. It has to be mentioned that this product is not a minor one, the ratio **6**/**7** being close to 1/1. In addition, when *cis*-benzylideneacetone was used as substrate, no significant amount of the corresponding *trans*-allylic alcohol could be obtained. It is noteworthy that no traces of the *trans*-allylic alcohol **10** were obtained after acetylation of the reaction mixture when the activation was performed in the presence of the alkyl radical trap TEMPO. This result suggests that the isomerization of the C10−C11 double bond of the olefin **4** may occur via a radical species (probably an allyl radical) involving a reduced-metal species.

When borohydride was replaced in the reaction mixture by borodeuteride, the reduction of ketone at position C4 was achieved by introduction of D^-, leading to complete disappearance of the signals due to H4 at 5.42, 5.54, and 4.97 ppm, in compounds **9-*d***, **10-*d***, and **11-d_2**. In compound **11-d_2**, a second deuterium atom was introduced in the molecule; the resonances of CH_2 groups at C10 and C11 were detected as two multiplets at 1.85 and 2.87 ppm, respectively, accounting for 1.5 proton each, indicating the introduction of one deuterium atom without selectivity between the positions C10 and C11. The statistic incorporation of deuterium during the BD_4^--mediated 1,4-addition to conjugated double bonds was reported in the case of α-phenylcinnamates.[35]

The reductive activation of arteflene reported in this article is an illustration that the mechanism recently proposed for the reductive activation of artemisinin and related trioxanes by a Mn(II) porphyrin,[11,12] involving the formation of an O-acetal radical that is thermodynamically driven to an ester and a C-centered radical, is operating for many different peroxidic antimalarial drugs. In the case of arteflene, an alkoxyl radical is formed instead of an acetal radical, and it is driven to a ketone, consequently releasing an alkyl radical in the medium. Arteflene activated by a manganese(II) porphyrin provided the cyclohexyl radical **5** but did not produce any species able to alkylate the porphyrin ligand. The formation of a manganese−O2 bond concomitant with the formation of the alkoxyl radical on O3, followed by the β-fragmentation between C4 and C5, generates the alkylating carbon-centered radical (**5**) that does not alkylate the porphyrin cycle, contrary to what has been observed with the primary alkyl radical produced by activation of artemisinin.[11] In principle, the rate of nucleophilic attack of an alkyl radical to alkene is controlled by steric and polar effects: on a monosubstituted olefin, the rate of addition of the cyclohexyl radical was found to be at least five times that of ethyl radical, consistent with the frontier orbital theory.[36] The absence of alkylation of the porphyrin by **5** is therefore probably due to steric hindrance factors and not to electronic ones. The pyrrolic double bond of the metalloporphyrin, the target of the alkyl radical, is not a simple olefin but a conjugated one with two bulky phenyl groups on both sides of these alkylable positions. Consequently, the cyclohexyl radical **5** derived from arteflene is probably too hindered to attack a β-pyrrolic position. Furthermore, the close interaction of the peroxide function of arteflene with the central ion of the metalloporphyrin suggests that **5** is probably formed away from any alkylable position of the macrocycle, precluding its alkylation. It should be noted that when [^{14}C]-radiolabeled arteflene (at C11 position) was incubated with *P. falciparum* infected erythrocytes, the radioactive center was recovered in the covalent adducts between the drug and parasitic proteins. Because C11 is not present in **5**, this radical cannot be involved in the arteflene-derived adducts observed in vivo with the radiolabeled drug and is probably not responsible for the parasite death. In fact, **5** can react with molecular oxygen to generate the corresponding cyclohexylperoxyl radical, which can dimerize and produce, via a Russel reaction, the corresponding ketone and the secondary alcohol at position C5. This cyclohexylperoxyl radical might also be responsible for lethal radical chain autoxidation processes. At high concentration, artemisinin derivatives induce nonspecific oxidative damage leading to the destruction of the parasite, but it should be noted that this is observed at concentrations 10^3−10^5 times higher than in vitro effective antimalarial concentrations.[37,38]

In contrast, the C11 atom, incorporated in the radioactive drug−protein adducts, is present in the α,β-unsatur-

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ated ketone **4**, which is a primary fragment generated by reductive activation of arteflene (the isolated alcohols **6**−**8** are derived from **4** because of the presence of borohydride in the presently used reaction conditions). In vivo, the enone **4** may undergo a 1,4-addition from nucleophilic side chains of amino acid residues, namely, amine or thiol functions, giving rise to covalent addition to the protein chain through an amine or thioether link, respectively. Another possibility during the reductive activation of arteflene by the reduced heme is the formation of an alkoxyl radical on O2, instead of O3 as observed when arteflene is activated by $Mn^{II}TPP$. In that case, the β-scission of the $RO^{\bullet}$ radical will induce the cleavage of the C1−C9 bond, giving rise to a primary alkyl radical centered at C9 still containing the whole structure of arteflene, and therefore the radiolabeled C11 position will be retained. Subsequent alkylation of parasitic proteins by this nonsterically hindered alkyl radical may be responsible for the parasite death. However, such selectivity in the docking of arteflene onto the metal center during the reductive peroxide opening by metalloporphyrins is rather unexpected.

Conclusion

The reductive activation of the peroxide bridge of arteflene by a metalloporphyrin generates the α,β-unsaturated ketone **4**, leading to alcohol derivatives in the presently used reaction conditions and to a cyclohexyl radical **5**, which has been trapped by TEMPO. No arteflene−macrocycle adducts have been obtained, contrary to what has been observed with artemisinin derivatives. These data suggest that the C-centered radical generated in the reductive activation of arteflene is probably not responsible for the alkylation of parasite proteins, in contrast to the behavior of trioxane-containing antimalarial drugs such as artemisinin.

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Supporting Information Available: IR spectrum of tetra-*n*-butylammonium borodeuteride. ^{1}H NMR spectra, with peak assignment, of *cis*-benzylideneacetone, mixture of compounds **6**/**7**/**8**, mixture of compounds **9**/**10**/**11**, compound **12**, mixture of compounds **9-*d***/**10-*d***/**11-*d*$_2$**, compound **12-*d***, and mixture of *cis*-4-phenyl-3-buten-2-ol and 4-phenylbutan-2-ol. MS spectra (DCI/NH_3^+) of mixture of compounds **9**/**10**/**11**, compound **12**, and compound **12-*d***. This material is available free of charge via the Internet at http://pubs.acs.org.

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