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The Antimalarial Artemisone Is an Efficient Heme Alkylating Agent

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The reductive activation by iron(II)-heme of artemisone, a C-10 substituted derivative of the antimalarial artemisinin, generates covalent heme-drug adducts in high yields. This result confirms that the substitution at C-10 of artemisinin does not alter the alkylating ability of artemisinin derivatives when activated by a redox-active metal center.

Togetherwith AIDS and tuberculosis, malaria is one of the three main causes of mortality by infectious diseases in the world. The naturally occurring artemisinin, and its semisynthetic derivatives artemether and artesunate are nowadays the most efficient and rapidly acting antimalarial drugs. Despite a wide use for more than 30 years, no clinically relevant parasite resistance to these drugs has been reported up to now.[1–3]

The peroxide function of artemisinin (**1**; Figure 1) and synthetic trioxanes is known to play a key role in their antimalarial activity. The possible formation of alkylating species after reductive activation of the O–O bond by heme- or non-heme-iron species was early suspected.[4,5] The full characterization of a covalent porphyrin-artemisinin adduct after reductive activation with a heme model confirmed the strong alkylating ability of artemisinin.[6,7] Covalent heme-artemisinin adducts were also isolated and characterized.[8] Furthermore, these heme-drug adducts have been detected in the spleen and the urine of malaria-infected mice, whereas they are absent from healthy mouse organs treated under the same conditions. This experimental fact indicates that alkylation of heme by artemisinin is triggered by the presence of the parasite within erythrocytes.[9] In fact, the reductive activation of peroxide-containing antimalarials to produce heme-drug adducts has been found to correlate well with their biological efficacy.[10,11] The active semisynthetic derivatives of artemisinin substituted at C-10 are also efficient heme-alkylating agents,[12,13] despite a claim by R. K. Haynes et al. to the contrary.[14]

Artemisone (**3**; Figure 1) is a promising artemisinin derivative substituted at the C-10α position by a thiomorpholine 1,1-dioxide residue.[15] This compound was recently reported to extensively react with iron(II) salts. This reaction produced a C-4-centered radical which has been trapped by 4-oxo-TEMPO in a rather low yield [10% when activation occurred in the presence of $Fe(OAc)_2$].[16] For this reason, the authors considered that "the structural flexibility of the C radicals from artemisinins allows facile extrusion of Fe^{2+} and collapse to benign isomerization products". Then, they rule out the possible importance of these radicals for the antimalarial activity, and state that "as an explanation for the antimalarial activity of artemisinins (...) the C-radical hypothesis is not feasible". In fact, under the conditions used by Haynes et al. (activation by an iron salt), the experimental mixture does not contain any component that could be considered as a putative biological target of the C-centered radical.

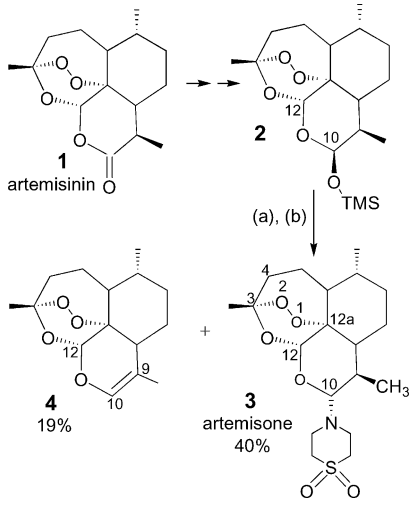

Figure 1. Synthesis of artemisone **3**; (a) TMSBr, 25 min, 0 °C; (b) thiomorpholine 1,1-dioxide, 1,1,1,3,3,3-hexafluoropropan-2-ol (HFIP), 75 min, 0 °C. TMS = trimethysilyl.

Therefore, we decided to evaluate the alkylating capacity of artemisone in the presence of a potential target such as heme. We found that the alkylation of iron(II)-heme by artemisone is indeed achieved in high yield as reported below.

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A patent reports the large-scale synthesis of artemisone (**3**) by using gazeous HCl.[17] In ref.[15b] is also reported the laboratory-scale synthesis and the characterization of this compound. However, we prepared artemisone from dihydroartemisinin using the route that we chose for the preparation of the 10α-benzylpiperazinyl derivative of artemisinin.[12] The 10β-trimethylsilyl ether derivative **2** was first prepared by reaction of chlorotrimethylsilane with dihydroartemisinin in pyridine as solvent. Reaction of bromotrimethylsilane (1.2 mol-equiv.) with compound **2** in dichloromethane for 25 min, followed by addition of thiomorpholine 1,1-dioxide (10 mol-equiv.) in the presence of 1,1,1,3,3,3-hexafluoropropan-2-ol (HFIP) at 0 °C provided artemisone **3** (Figure 1). After purification by chromatography and recrystallization (from ethyl acetate and hexane), the yield of **3** was 40% with respect to dihydroartemisinin, close to the reported value (44%).[15b] Elimination product **4** was also isolated in 19% yield. The (*R*) configuration at C-10 of artemisone was assessed by the ^{1}H NMR coupling constant 9-H,10-H (J = 10.3 Hz).[18]

Iron(II)-heme, generated in situ by reduction of its iron(III) analogue with sodium dithionite, reacted quickly with artemisone (**3**) to provide the covalent heme-drug adduct **5** (heme/**3**/dithionite = 1:1:5 molar ratio, room temp., DMSO). As previously described for artemisinin, reductive activation of the peroxide bond produces an alkoxy radical centered at O-2. Rapid and subsequent C-3–C-4 β-scission leads to the alkyl radical centered at C-4. This radical quickly alkylates the *meso* positions of the porphyrin macrocycle by an intramolecular reaction. The reaction was monitored by high-pressure liquid chromatography (C18 RP column). After 1 h, the conversion of heme monitored by HPLC was 93% (R_t = 18.2 min, λ_{max} = 398 nm). Seven resolved peaks were detected with retention times between 23 and 26 min, and λ_{max} = 405–407 nm. These peaks can be assigned to the four regioisomers of the two different heme-drug adducts, resulting from the alkylation of the four *meso* positions of heme without regioselectivity (eight peaks expected, but two peaks overlapped; Figure S2, Supporting Information). The crude reaction product was analyzed by ESI$^+$-MS after dilution with acetonitrile. The complete adduct **5** (Figure 2) was not detected; however, its formation was assessed by the detection of adducts **6** and **7**. Compound **6** (Figure 2; at m/z = 957.5) is generated by rearrangement of the artemisone-derived moiety of **5**. The drug-derived fragment of adduct **6** has been previously trapped using 4-oxo-TEMPO (Figure 2, inset), and a pathway has been proposed for its formation.[16] Adduct **7**, detected at m/z = 840.3, arose from the hydrolysis of the C-12–O bond of **5**, followed by the intramolecular attack of the hydroxy function at C-12 onto the C-10 and the release of the thiomorpholine dioxide residue (Figure 3). As an additional identification of compound **7**, dilution with methanol caused hydration of the aldehyde at C-12 and methylation of the alcohol function at C-10 (m/z = 872.5, compound **8**; Figure 2). These analytical results are fully consistent with those reported for the adducts between heme and other artemisinin derivatives (higher retention times than heme itself, and a bathochromic shift of absorbance due to the substitution at the *meso* position of the porphyrin macrocycle).[8,12] In particular, the alkylation of heme with another C-10-substituted artemisinin derivative also led to adduct **7** after release of the C-10 substituent.[12] To confirm these results, a similar reaction was carried out by using heme dimethyl ester instead of heme. Under these conditions, covalent heme-drug adducts were detected at m/z =

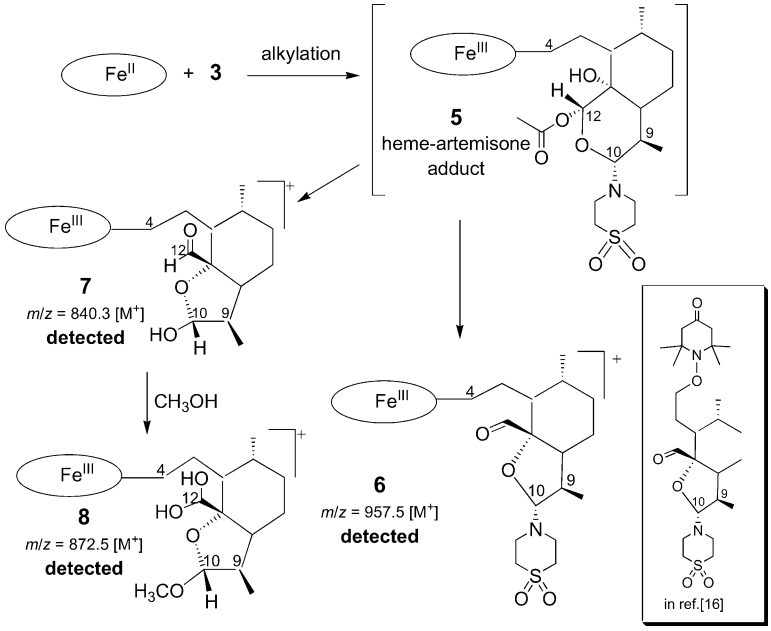

Figure 2. Alkylation of iron(II)-heme by artemisone. The oval stands for the protoporphyrin-IX macrocycle. Inset: adduct between artemisone and 4-oxo-TEMPO, reported in ref.[16]

985.5, 868.4, and 900.5, corresponding to the dimethyl ester analogues of adducts **6**, **7**, and **8**, respectively. From HPLC and mass spectra, the yield of heme-drug adducts was ranging from 80 to 90% with respect to starting heme.

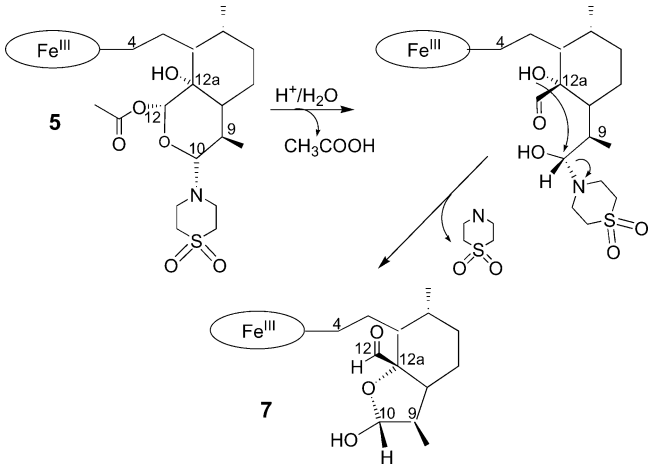

Figure 3. Mechanism of rearrangement of the covalent heme-artemisone adduct **5**.

There is no doubt that the substitution of dihydroartemisinin by the thiomorpholine dioxide moiety at C-10α does not prevent the reductive activation of the peroxide bond of artemisone by iron(II)-heme, leading to the alkylation of the four *meso* positions of heme. Artemisone, as all the other active artemisinin derivatives (artemether, artesunate, ...) is a very efficient alkylating agent toward heme. This property of artemisone is an experimental fact that should be taken into consideration in discussions about possible mechanisms of action of this antimalarial drug.

Supporting Information (see footnote on the first page of this article): Experimental conditions of the alkylation reaction and characterization of the heme-drug adducts **6**, **7**, and **8** by mass spectrometry; ^{13}C NMR spectrum of **3** (Figure S1), and HPLC monitoring of the alkylation reaction (Figure S2).

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[18] Analytical data for **3**: MS (DCI/NH_3^+): *m*/*z* = 402.5 [M + H^+], 419.5 [M + NH_4^+]. $C_{19}H_{31}NO_6S$ (401.5): calcd. C 56.84, H 7.78, N 3.49; found C 56.67, H 7.61, N 3.41. ^1H NMR (500 MHz, $CDCl_3$): δ = 5.31 (s, 1 H, 12-H), 4.24 (d, $^3J_{10\text{-H},9\text{-H}}$ = 10.3 Hz, 1 H, 10-H), 3.54–3.49 (m, 2 H, CH_2), 3.43–3.38 (m, 2 H, CH_2), 3.23 (m, 4 H, 2 CH_2), 2.61 (m, 1 H, 9-H), 2.38 (m,1 H, 4-H), 2.03 (m,1 H, 4-H), 1.90 (m, 1 H, 5-H), 1.78–1.73 (m, 2 H, 7-H, 8-H), 1.60 (m,1 H, 8a-H), 1.56–1.44 (m, 1 H, 5-H), 1.40 (s, 3 H, 3-CH_3), 0.98 (d, ^3J = 6.3 Hz, 3 H, 6-CH_3), 0.83 (d, ^3J = 7.2 Hz, 3 H, 9-CH_3) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 104.30 (C-3), 92.14 (C-10), 91.03 (C-12), 80.18 (C-12a), 51.91 (N-CH_2), 51.46 (C-6a), 47.02 (S-CH_2), 45.63 (C-8a), 37.49 (C-6), 36.19 (C-4), 34.22 (C-7), 29.07 (C-9), 25.91 (3-CH_3), 24.83 (C-5), 21.63 (C-8), 20.22 (6-CH_3), 13.45 (9-CH_3) ppm. The ^{13}C NMR spectrum is available as Supporting Information. There is a discrepancy between the ^{13}C data and those reported in ref.[15b] The ^{13}C NMR spectrum reported by Haynes et al. exhibits 18 signals for only 17 different carbon atoms in compound **3** (no assignment was provided); the signal at δ = 174.2 ppm (absent from our spectrum), cannot be likely assigned to a carbon atom of artemisone. Such a chemical shift is usual for a carbonyl function (acid, ester, amide) which does not exist in artemisone. In addition, we detected only two signals at δ = 90–92 ppm, and not three as reported by Haynes et al. These signals have been assigned by 2D correlations to C-10 and C-12 (δ = 92.14 and 91.03 ppm, respectively). The signal of C-12a, at the junction of four cycles, was detected at δ = 80.18 ppm. The article by Haynes et al. does not report any signal at this chemical shift.

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