Tetrahedron Letters 53 (2012) 1296–1299

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

A study of monoelectronic reduction of Artemisinin

Maria Luisa Navacchia, Massimo L. Capobianco, Mila D'Angelantonio, Giancarlo Marconi *

CNR-Istituto per la Sintesi Organica e la Fotoreattività (ISOF), Via P. Gobetti 101, 40129 Bologna, Italy

ARTICLE INFO

Article history:
Received 17 November 2011
Revised 21 December 2011
Accepted 4 January 2012
Available online 10 January 2012

Keywords:
Artemisinin
Malaria
γ-Radiolysis
Reduction
Alkoxyl radicals

ABSTRACT

The reaction of Artemisinin with solvated electrons (e^-_{solv}) generated by radiolytic methods led to the formation of acid **2** as the only detectable product. A possible mechanism leading to the formation of compound **2** is discussed.

Since its discovery in the late seventies of the last century, Artemisinin (ART), a metabolic compound produced by the plant *Artemisia annua* (Qinghao in Chinese), has imposed itself as the most effective drug to fight malaria.[1] Moreover, this molecule and its direct derivatives have been found promising in the cure of other serious diseases such as different kinds of solid cancer,[2] schistosomiasis,[3] and in the antiangiogenetic therapy.[4] At the moment, the cure for malaria suggested by WHO consists in a combination of ART derivatives with some other drugs aimed at prolonging its therapeutic effect (the so called ACT therapy).[5]

Despite the number of studies devoted to clarify the action mechanism of ART in vitro and in vivo, a univocal picture of its degradation has not been yet obtained. ART (Fig. 1) is a sesquiterpenic lactone that is conventionally divided into four rings. Among them, the pharmaceutical active part is located in the ring A, a six membered trioxanic ring containing an endoperoxidic bond, and B, the lactone ring, whereas the other two rings, that is the cyclohexane and the oxacycloheptane seem to play only a minor role in its action against the *Plasmodium* parasites. However not completely understood, the mechanism of action of this drug seems to involve free radicals generated during its catabolism. Specifically, induced de novo production of reactive hydroxyl moieties and superoxides within the malarial parasite has been reported to damage intracellular processes and to cause death. One of the mechanisms proposed involves a dissociative reduction of the peroxidic O–O bond by a not yet identified reductive agent present in the blood and, due the fact that the iron [Fe(II)] concentration is found to be particularly high in the parasitic food vacuole, great attention has been devoted to its involvement into the breaking of the peroxidic bond by direct electron transfer to the antibonding σσ* LUMO localized on this part of the molecule.[6] The two unstable distonic radicals oxyanions so generated, are claimed to undergo an intramolecular rearrangement into C-centered radicals through either a 1,5-hydrogen shift or a homolytic C–C bond cleavage. After this stage, the destiny of the secondary or primary carbon radicals produced by these intramolecular rearrangements is still not clear.

It was found that the ratio of products strongly depends on the nature of the counterion (Cl^-, SO_4^{2-}) present in the solution which contains iron as the main reducing agent.[5] It was also found that the presence of the carbonyl group, as well as the nature of substituents on C-12 (see Scheme 2 for numbering) plays a decisive role in orienting the deactivation mechanism of ART.[7] An alternative to the direct reduction is provided by the use of suitable photosensitizers with energy close enough to that of the antibonding LUMO of the endoperoxide, to allow a SET (Single Electron Transfer) leading to the formation of alkoxyl radicals.[8] Beyond the experimental studies, several theoretical investigations have been addressed to

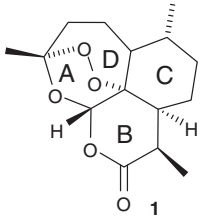


Figure 1. ART.

* Corresponding author. Tel.: +39 051 6399799; fax: +39 051 6399844.
E-mail address: marc@isof.cnr.it (G. Marconi).

0040-4039/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2012.01.007

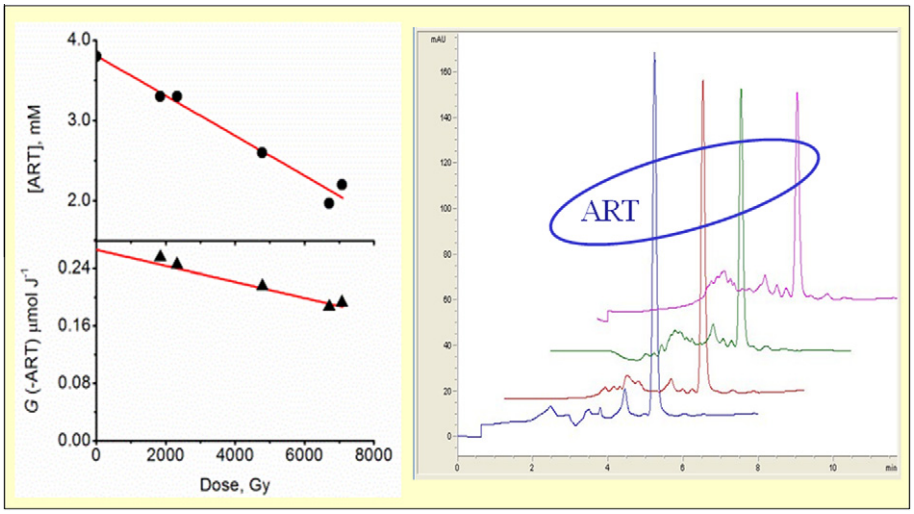


Figure 2. Left: top, plot of the linear consumption of ART as a function of dose; bottom, radiation chemical yield (G) as a function of dose for the consumption of ART G (−ART). Right: chromatogram profiles of irradiated mixtures (blue: dose = 0 Gy; red: dose = 2320 Gy; green: dose = 4780 Gy; pink: dose = 7080 Gy). HPLC analyses were performed using a Zorbax C8 column (4.6 × 150 mm, 5 µm), linear gradient H_2O/MeOH, flow rate 0.5 ml/min, detection at λ 220 nm.

the problem of identifying the most probable intermediates of the ART reductions, most of them adopting only partial models of the whole ART molecule.[9,10] A review of these studies has also recently appeared.[11]

Our knowledge in radiolysis techniques prompted us to study the reaction of solvated electrons (e^-_{solv}) with ART by radiolytic methods coupled with product studies.

The reaction of e^-_{solv} with ART was previously studied by some of us by pulse-radiolysis techniques in some detail.[12] Experiments of direct irradiation of a H_2O/EtOH (1:1 v/v) solution of ART revealed the initial formation of carbon centered radicals following the rapid intramolecular rearrangement of the initially produced oxyanion radicals in agreement with the reported data involving chemical reductants such as Fe(II).[13] However, in that case the proposed mechanism was not supported by product analysis.

In the present communication we report preliminary results on the gamma radiolysis of de-aerated solutions of ART (**1**) (ca. 3.5 mM, 15 mL) in H_2O/EtOH (1:1 v/v) irradiated under stationary-state conditions, using a ^{60}Co-Gammacell with a dose rate of ~6 Gy min^{-1}.

The following equations describe the chemical processes occurring during the irradiation:

$$H_2O/EtOH \rightsquigarrow e^-_{solv},\ CH_3{\cdot}CHOH,\ H_3O^+/CH_3CH_2{}^+OH_2 \quad (1)$$

$$\cdot OH \text{ or } \cdot H + EtOH \rightarrow CH_3{\cdot}CHOH + H_2O \text{ or } H_2 \quad (2)$$

$$ART + e^-_{solv} \text{ or } CH_3{\cdot}CHOH \rightarrow \text{radiolysis products} \quad (3)$$

$$CH_3{\cdot}CHOH + CH_3{\cdot}CHOH \rightarrow \text{products} \quad (4)$$

Radiolysis of neutral water/ethanol 1:1 v/v mixture leads to the species e^-_{solv} (0.2), $CH_3{\cdot}CHOH$ (0.34), $H_3O^+/CH_3CH_2{}^+OH_2$ (0.2), Eq. 1, [14,15] where the values in parenthesis represent the radiation chemical yields (G) expressed in µmol J^{-1}.[16] The dose absorbed by water/ethanol mixture was calculated by applying a correction factor of 0.7% to take into account the different electronic density of ethanol and water.[17] The use of water/ethanol mixtures for studying the radiolysis of water-insoluble species of biological importance has been discussed in the past.[14]

The presence of EtOH as solvent (8.7 M) guarantees that hydroxyl radicals and hydrogen atoms are scavenged efficiently ($k_{(EtOH + \cdot OH)} = 1.9 \times 10^9$ M^{-1} s^{-1}, and $k_{(EtOH + \cdot OH)} = 1.7 \times 10^7$ M^{-1} s^{-1}), Eq. 2. [18] As reported in Eq. 4, the bimolecular reaction of $CH_3{\cdot}CHOH$ with itself, k = 1.5 × 10^9 M^{-1} s^{-1}, must be taken into account during the stationary irradiation.[12]

The absorbed radiation dose was determined with the Fricke chemical dosimeter, by taking G (Fe^{3+}) = 1.61 µmol J^{-1}.[18] Samples were taken at different absorbed doses, analyzed by Reverse Phase-High Performance Liquid Chromatography/Mass Spectrometry (RP-HPLC/MS) and characterized by ^{1}H NMR after elimination of the solvent under reduced pressure. Samples irradiated at dose ca. 2320 and 7080 Gy were taken from repeated experiments and used to improve the quantitative analysis. Quantitative analysis for the consumption of ART was performed by RP-HPLC. The concentration values of ART taken from the different experiments reported in Figure 2 as a function of dose were normalised at the highest ART concentration used (3.8 mM).

The G data, calculated at increasing absorbed doses, were analyzed as a function of dose and linearly extrapolated to zero dose in order to obtain a G value unaffected by radiolytically induced side reactions that can reduce the calculated radiolysis yields.[17]

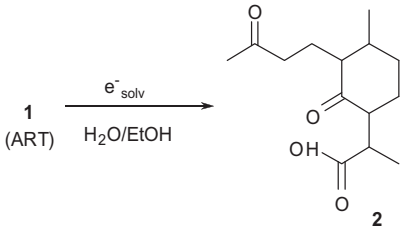
In Figure 2 (left, top) is reported the linear consumption of ART as a function of dose. In the same figure (left, bottom) G (−ART) was extrapolated to zero dose obtaining a value close to 0.27 µmol J^{-1} which is a slightly higher value than that reported in the literature for e^-_{solv}.[14] This finding indicates that only solvated electrons are responsible for the reduction of ART and excludes the involvement of $CH_3{\cdot}CHOH$ in the consumption of ART. In fact, the reaction of either e^-_{solv} and $CH_3{\cdot}CHOH$ with ART should have produced G = 0.54 µmol J^{-1}, that is the sum of solvated electrons and $CH_3{\cdot}CHOH$ yields.[14]

On this base Eq. 3 became:

$$ART + e^-_{solv} \rightarrow \text{radiolysis products} \quad (5)$$

The ^{1}H NMR analysis performed on the reaction mixtures indicates the presence of unreacted ART and only one reaction product identified as the carboxylic acid **2**. (Scheme 1). Compound **2** was characterized after purification on RP column chromatography of a large scale experiment reaction mixture by ^{1}H NMR and MS analyses.[19]

A quantitative analysis of the formation of acid **2** was performed by ^{1}H NMR of the reaction mixtures by comparing the integral of H11 signal of **2** (δ 2.85) with the integral of H11 signal of the unreacted ART **1** (δ 3.10) and plotted in Figure 3.



Scheme 1. γ-Radiolysis of ART.

As reported in Figure 3 (left, top), the formation of acid **2** is linear with the dose. The analysis, in terms of *G*, of the data related to the formation of the identified product (G(+Acid) = 0.097 μmol J^{-1}) shows that, comparing the *G* values at zero dose (Figs. 2 and 3 left, bottom), ~37% of reacted ART leads to the formation of this radiolysis product.

Acid **2** was previously reported in the literature as a minor product obtained by the treatment of ART with agents such as heme Iron(II) and (III).[14]

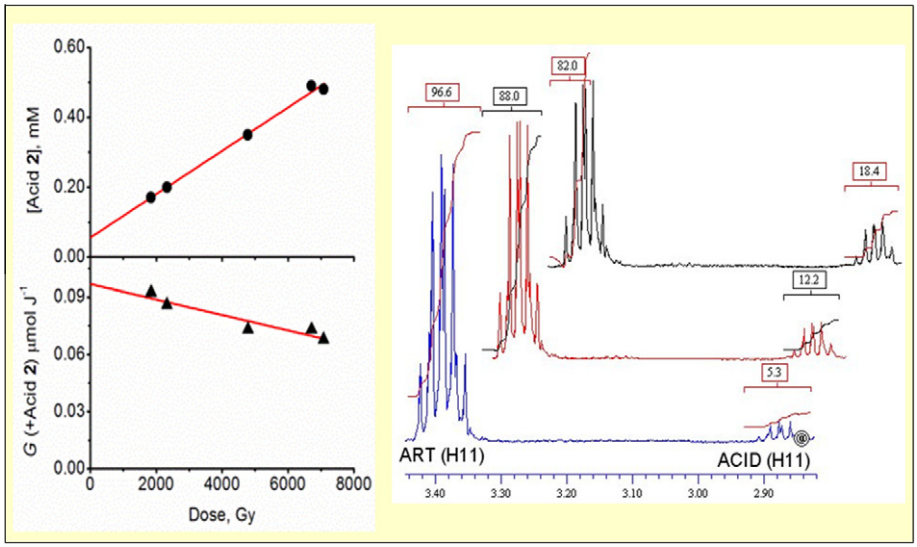
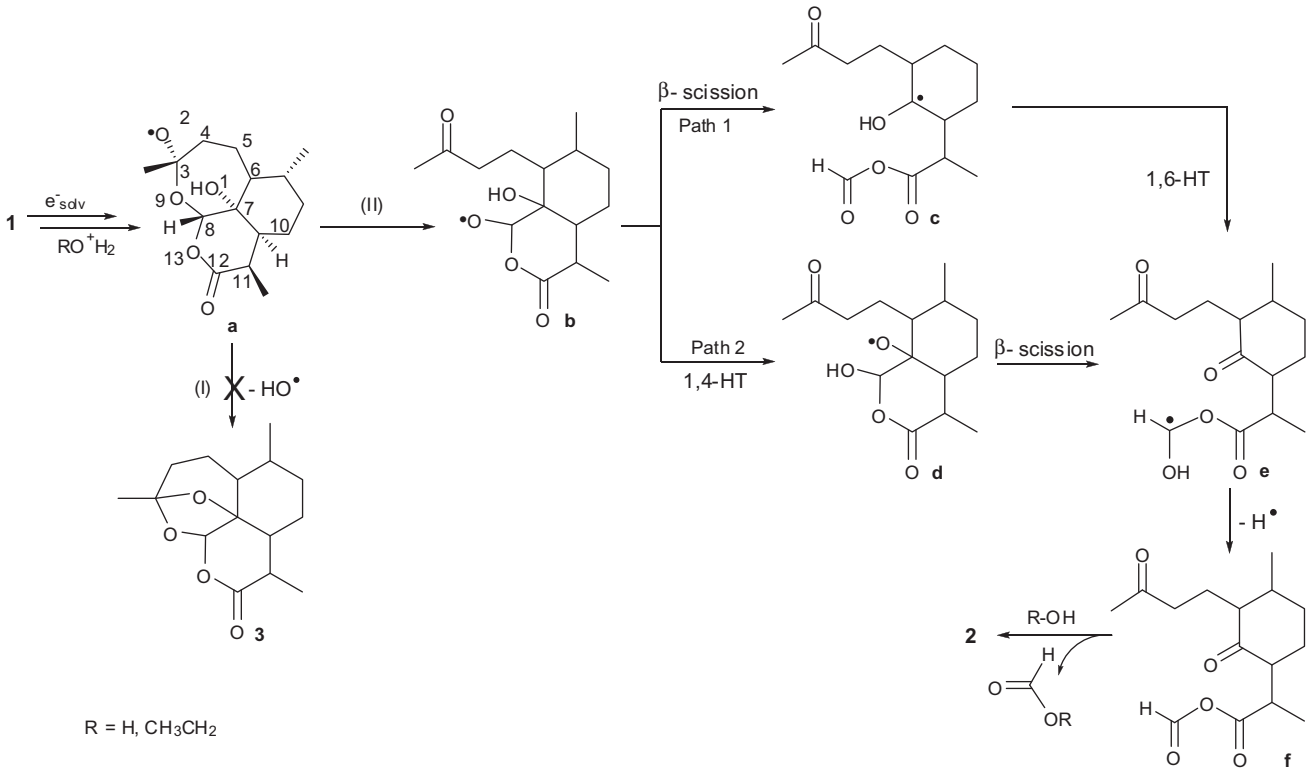


Figure 3. Left: top, plot of the linear formation of acid **2** as a function of dose; bottom, radiation chemical yield (*G*) as a function of dose for the formation of acid **2** *G* (+acid). Right: ^{1}H NMR profiles of irradiated mixtures (blue: dose = 2320 Gy; red: dose = 4780 Gy; black: dose = 7080 Gy).



Scheme 2. Proposed mechanisms for the formation of carboxylic acid **2**.

The formation of the detected product can be reasonably explained with the aid of the energy of the intermediates, quantum mechanically calculated by Moles et al.[10] Taking into account their simplified model of ART, we propose the mechanism drawn in Scheme 2. The initial distonic alkoxyl radical formed by the reductive cleavage of the peroxide bond is rapidly protonated to radical **a**. As depicted in Scheme 2 radical **a** can follow, in principle, the route (I) leading to the pharmacologically inactive deoxyartemisin **3** or the route (II), leading to the carboxylic acid **2**. The product analyses led us to exclude the route (I) since no deoxyartemisin **3** was found. Along the route (II), the first step of the decomposition of radical **a** starts with the scission of bond C3–O9 leading to O9 radical **b**. For this process the calculated energy barrier is around 14 kcal/mol, making this step particularly competitive with respect to other possible rearrangement processes within this route. From radical **b** to the final product **f** two different pathways are possible both of them involving two steps. The first pathway consists in breaking of the C7–C8 bond to give the secondary radical **c**, which in turn leads to radical **e** by intramolecular hydrogen transfer between O1 and O9. The second one, requires an immediate hydrogen shift from O1 to O9 to give radical **d**, followed by breaking of the C7–C8 bond leading to radical **e**. The accuracy of the theoretical methods used to describe these reactions is probably not sufficient to choose between the two mechanisms. The functional density B3LYP method in fact looks very favorable to the former but does not allow to find a stationary point for the second, while the HF/3–21G indicates a possible competition between the two. The final step is the formation of the anhydride **f** through the ·H release from O9, analogously to what is supposed to happen in the presence of Fe(II).[6] The energies required to overcome the transition states are 34.3, 24.7 kcal/mol for the processes **b–d** and **d**–**e**, respectively, and 26.5, 37.2 kcal/mol for the routes **b**–**c** and **c**–**e**, respectively, along the results of the HF/3–21G calculations.[10] The step **e**–**f** to reach the final product requires 49 kcal/mol. Beyond the reliable energies, the scheme proposed agrees with the description of the spin densities calculated on the atoms involved in the bond breakings and in the atom transfers, thus providing a further support to the mechanism proposed. It is worth noting that product **f** could not be isolated nor detected due to the reaction medium. It is well known in fact that anhydrides hydrolyze in the presence of water leading to the corresponding carboxylic acids and, as a consequence, it is not surprising that acid **2** is the only detected product.

In conclusion, we assessed that ART, under continuous γ-radiolysis, reacts quantitatively with e^-_{solv} leading to carboxylic acid **2** as the main and major reaction product. These preliminary results make the highly debated and controversial degradation mechanism of ART better understood supporting the route (II) over the route (I).

References and notes

1. Li, G. Q.; Guo, X. B.; Arnold, K.; Jian, H. X.; Fu, L. C. *Lancet* **1984**, *2*, 1360–1361.
2. Du, J. H.; Zhang, H. D.; Ma, Z. J.; Ji, K. M. *Cancer Chemother. Pharmacol.* **2009**, *65*, 895–902.
3. Xiao, S. H. *Acta Trop.* **2005**, *96*, 153–167.
4. Chen, H. H.; Zhou, H. J.; Wu, G. D.; Lou, X. E. *Pharmacology* **2004**, *71*, 1–9.
5. World Health Organization. World Health Organization: Geneva, 2010.
6. O'Neill, P. M.; Posner, G. H. *J. Med. Chem.* **2004**, *47*, 2945–2964.
7. Wu, W. M.; Wu, Y. K.; Wu, Y. L.; Yao, Z. J.; Zhou, C. M.; Li, Y.; Shan, F. *J. Am. Chem. Soc.* **1998**, *120*, 3316–3325.
8. Magri, D. C.; Donkers, R. L.; Workentin, M. S. *J. Photochem. Photobiol., A* **2001**, *138*, 29–34.
9. Gu, J. D.; Chen, K. X.; Jiang, H. L.; Leszczynski, J. *J. Phys. Chem. A* **1999**, *103*, 9364–9369.
10. Moles, P.; Oliva, N.; Safont, V. S. *J. Phys. Chem. A* **2006**, *110*, 7144–7158.
11. Denisov, E. T.; Solodova, S. L.; Denisova, T. G. *Russ. Chem. Rev.* **2010**, *79*, 981–1003.
12. Fuochi, P. G.; Marconi, G.; Mulazzani, Q. G. *Int. J. Radiat. Biol.* **2005**, *81*, 319–326.
13. Haynes, R. K.; Vonwiller, S. C. *Tetrahedron Lett.* **1996**, *37*, 253–256.
14. Jore, D.; Champion, B.; Kaouadji, N.; Jaygerin, J. P.; Ferradini, C. *Radiat. Phys. Chem.* **1988**, *32*, 443–448.
15. Leu, A. D.; Jha, K. N.; Freeman, G. R. *Can. J. Chem.* **1983**, *61*, 1115–1119.
16. Buxton, G. V.; Mulazzani, Q. G. In *Electron Transfer in Chemistry*; Balzani, V., Ed.; Wiley-VCH Verlag GmbH: Weinheim, 2001; pp 503–557.
17. Spinks, J. W. T.; Woods, R. J. *Introduction to Radiation Chemistry*, 3rd ed.; Wiley: New York, 1990.
18. Buxton, G. V.; Greenstock, C. L.; Helman, W. P.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1988**, *17*, 513–886.
19. ^{1}H NMR (400 MHz, $CDCl_3$), δ 11.90 (br s, COOH; disappeared on D_2O shake), 2.85 (1H, m, H, collapsing to d upon irradiation at δ 1.19, J = 5.6), 2.61 (1H, m, H4, collapsing to br d upon irradiation at δ 1.51), 2.54 (1H, m, H1), 2.54 (1H, m, H1), 2.39 (1H, m, H6), 2.12 3H, s, CH_3C), 1.90 (1H, m, H3, collapsing to br s upon irradiation at δ 1.51), 1.84–1.70 (2H, m, H2–H5), 1.51 (1H, m, H), 1.19 (3H, d, J = 6.8, CH_3, collapsing to s upon irradiation at δ 2.85), 1.06 (3H, d, J = 6.0, CH_3, collapsing to s upon irradiation at δ 1.51). Calculated mass ($C_{14}H_{22}O_4$): 254.15 found ESI-MS$^+$: 255 $[M+H]^+$; 277 $[M+Na]^+$; 531 $[2 \times M+Na]^+$; found ESI-MS$^-$: 253 $[M-H]^-$.