Tetrahedron Letters 54 (2013) 5257–5260

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

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

Monoelectronic reduction of dihydroartemsisinin (DHA): pH dependence and product analysis

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ARTICLE INFO

Article history:
Received 12 June 2013
Accepted 15 July 2013
Available online 23 July 2013

Keywords:
Dihydroartemisinin
Malaria
γ-Radiolysis
Reduction
Alkoxyl radicals

ABSTRACT

The reaction of dihydroartemisinin (DHA) with solvated electrons (e^-_{solv}) generated under radiolytic conditions showed high pH dependence. The reduction performed under neutral conditions led to the formation of compound **P1** deriving from the initial alkoxyl radical as the only detectable product.

Introduction

In the recent years many studies have been devoted to artemisinin (ART, Fig. 1), a secondary metabolite of *Artemisia annua*, a plant of the *Asteraceae* family of chinese origin now spread out also in the western countries.[1] This compound presents outstanding properties against the *Plasmodium* parasites responsible for malaria, but its very low content in the plant has stimulated a number of efforts in order to obtain, through semi-synthetic processes, more powerful, soluble, and in principle more available derivatives. Among them dihydroartemisinin (DHA, Fig. 1), obtained by reduction of ART with mild hydride-reducing agents,[2] presents an enhanced antimalarial activity with respect to ART and is claimed to be its main metabolite in the human body.[3] These remarkable properties against malaria have been joined to those of piperaquine in the new drug Eurartesim, which beyond the power of DHA, assures an increased degree of safety against resistance phenomena.[4] Moreover, the interest on this compound has been recently increased by the detection of a remarkable antitumor activity toward hepatocellular carcinoma in vitro and in vivo.[5] At the cellular level it is interesting to point out that, at variance with ART, DHA inhibits the cell growth, being even more selective than artemisone and artesunate.[6]

Several studies have been devoted to this molecule, which presents, instead of a lactone, a lactol moiety in position 10 responsible for the presence of two hemiacetal epimers rapidly interconverting. Recently, the thermodynamics of this process has been thoroughly studied.[7,8]

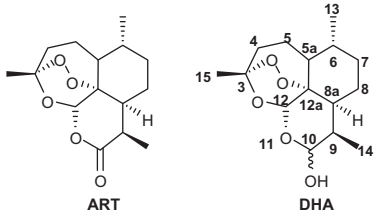

Figure 1.

The chemical reduction of DHA induced by $FeSO_4$ in aqueous acetonitrile was reported by Wu et al.,[9] and a possible radical cascade mechanism of the process was proposed. More recently, Haynes et al. extensively investigated the inhibition of parasite SERCA PfATP6 and the antimalarial activity of DHA and related derivatives such as the artemisone.[10–12] Moreover they investigated the Fe^{2+} mediated decomposition of DHA under several conditions and assessed that in aqueous media DHA furnished products deriving from the secondary C-4 radicals whereas in organic solvents led to products from alkoxyl radicals.[11]

In the last few years we dedicated some time to the study of the reduction of the parent ART under radiolytic conditions performing both pulse[13]- and γ-radiolysis[14] experiments.

γ-Radiolysis being usually performed in water solutions offers a quite interesting chance to study the reduction mediated by solvated electrons (e^-_{solv}) of bioactive compounds. In this Letter, we report a study on the radiolytic reduction of DHA completed

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0040-4039/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tetlet.2013.07.088

with the product study under experimental conditions similar to that reported in our previous work on ART.[14]

Results and discussion

Radiolysis of neutral water leads to e^-_{solv} (0.27), $HO^\cdot$ (0.28), and $H^\cdot$ (0.062) where the values in parentheses represent the radiation chemical yields (G) in units of μmol J^{-1}. The disappearance of the starting material or the appearance of the product (mol kg^{-1}) divided by the absorbed dose (1 Gy = 1 J kg^{-1}) gives the radiation chemical yield, that is, G (−DHA) or G (**P1**), respectively.

In the present work EtOH was used in 1:1 mixture with water in order to improve the solubility of DHA and to scavenge efficiently the $HO^\cdot$ radicals. Under these conditions the G value for the e^-_{solv} is 0.20 μmol J^{-1} as already reported.[14]

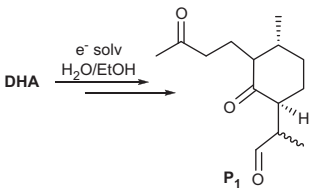
In a general experiment de-aerated solutions of DHA (3.0–3.5 mM, 10 mL) in H_2O/EtOH (1:1 v/v) were irradiated under stationary-state conditions, using a ^{60}Co-Gammacell with a dose rate ranging from of 4.8–4.6 Gy min^{-1} and samples taken at different absorbed doses were analyzed by HPLC–MS and ^{1}H NMR subject to elimination of the solvent under reduced pressure.[15]

In all cases only one reaction product was found that is compound **P1**, present as a mixture of α and β epimers in 50/50 ratio, characterized by ^{1}H NMR by comparison of the signals recorded with those reported in the literature[10] and HLPC–MS[16] (Scheme 1).

The quantitative analyses for the disappearance of starting material DHA were performed by HPLC–MS. The quantitative analyses of product **P1** were performed by ^{1}H NMR of the reaction mixtures by comparing the integral of CHO signals of **P1** (singlets at δ 9.729 and 9.731) with the integral of the H12 signals of the unreacted DHA (singlets at δ 5.390 and 5.601) (Fig. 2).

A preliminary experiment was performed in H_2O/EtOH (1:1 v/v) solution (**exp1**). Table 1 shows the consumption of DHA at each dose together with the pH value for each reaction mixture. HLPC–MS and ^{1}H NMR analyses of the reaction mixtures allowed to detect the formation of product **P1** only in trace amounts. These first data surprisingly indicated a very low consumption of DHA with respect to the dose.

After prolonged irradiation up to 44 h corresponding to a dose for the production of 120% of e^-_{solv} (120% dose), the consumption of DHA was still found very poor with respect to the dose. ^{1}H NMR analyses did not show a significant increasing of compound **P1** but the formation of numerous degradation products ascribable to the acidic conditions (pH up to 3.9) being already established the poor stability of DHA under both acidic and basic conditions.[7,10] Considering the lowering of pH during the γ-radiolysis the experiment was repeated starting from buffered solution at pH 7.0 (**exp2**). For this purpose a solution of DHA in phosphate buffer (100 mM)/EtOH (1:1 v/v) (pH 7.0) was irradiated with 10–25–40% dose. As reported in Table 1, in **exp2** the dose and the consumption of DHA were in good agreement. Maintaining the pH at the value of 7.0 allowed also the accumulation of compound **P1** up to 25% dose. At longer irradiation time the accumulation of **P1** was not linear anymore; reasonably, compound **P1** reacts with the e^-_{solv} in competition with DHA, as expected in continuous irradiation when the concentration of the radiolytic products increases with time. To confirm **exp1**, a solution of DHA in H_2O/EtOH (1:1 v/v) (pH 6.6) (**exp3**) was irradiated with 40% dose together with that of **exp2**. After 40% dose the pH was found 4.2, only 20% of DHA was consumed and only trace amounts of **P1** were detected in agreement with the previous **exp1**.



Scheme 1. γ-Radiolysis of DHA.

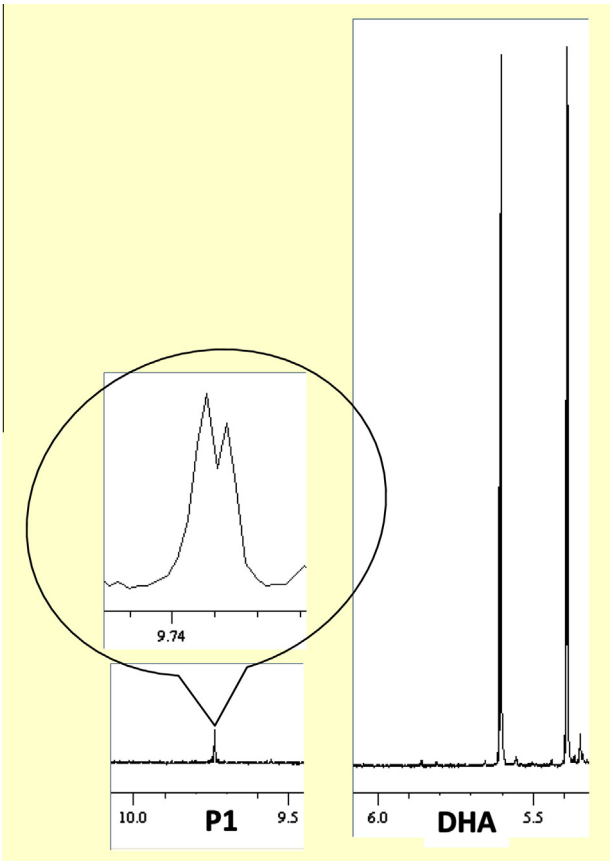


Figure 2. ^{1}H NMR profile of an irradiated mixture (dose = 4353 Gy).

We investigated also the γ-radiolysis of DHA in phosphate buffer (10 mM)/EtOH (1:1 v/v) at initial pH 7.0 (**exp4**) and 8.2 (**exp5**). As reported in Table 1 for **exp4** the lower concentration of the phosphate did not allow to buffer the reaction mixtures but the pH decreased slower with respect to the radiolysis performed in **exp1** and **exp3**. In the **exp4** case we found that the consumption of **DHA** was lower with respect to the dose in agreement with **exp1** and **exp3**, however considerable amount of compound **P1** was found with a G (**P1**) value comparable to that found in **exp2**.

The experiment performed at pH 8.2 (**exp5**) showed a higher consumption of **DHA** with respect to the irradiation dose in agreement with the poor stability of the molecule under basic conditions. An independent experiment showed that the decomposition of **DHA** in phosphate buffer (10 mM)/EtOH (1:1 v/v) at pH 8.2 after 24 h at room temperature occurred up to 30% (the quantitative analysis was performed by HPLC). As far as the formation of product **P1** is concerned, it was found in yield comparable to that of **exp2**.

The monoelectronic reduction of DHA in aqueous solution, different from that of ART,[14] showed a high sensitivity to pH. Our findings indicate that the reduction of DHA occurs at neutral pH whereas it seems to be inhibited at acidic pH (**exp1**, **exp3**, and **exp4**). At basic pH in the range of 8.2–7.7 (**exp5**) the hydrolysis of DHA strongly compete with the reduction process, as indicated by the higher consumption of DHA with respect to the dose, still leading to **P1** in comparable amount to that obtained at neutral pH (**exp2**).