Radiolytic study of α-tocopherol oxidation in ethanolic solution

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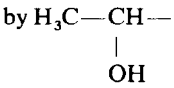
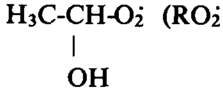
α-Tocopherol in aerated ethanolic solution is oxidized by $H_3C-\underset{OH}{\underset{|}{CH}}-O_2^{\cdot}$ radicals produced by γ-radiolysis.
The nature of the final product and the kinetic scheme are discussed.

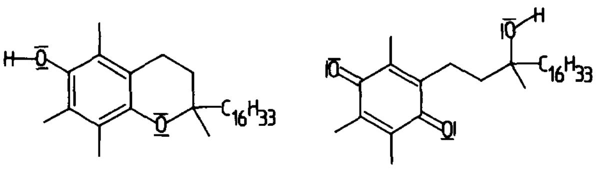


α-Tocopherol *Vitamin E* *Antioxidant effect* *γ-Radiolysis* *Peroxide radical*

1. INTRODUCTION

α-Tocopherol (αTH, fig.1), the most important component of vitamin E, plays biologically an antioxidant role by preventing the membranes from peroxidation [1,2]. It is generally believed that this activity is related to the scavenging of unsaturated fatty acid peroxide radicals in the membranes. Very little work has been done concerning the action of simple model peroxide radicals ([3,4] and references quoted therein). We projected to study the reaction of $H_3C-\underset{OH}{\underset{|}{CH}}-O_2^{\cdot}$ ($RO_2^{\cdot}$) radicals obtained by γ-radiolysis of aerated ethanol with dissolved α-tocopherol.





αTH

Fig.1. α-Tocopherol (αTH) and α-tocopheryl quinone.

Abbreviation: αTH, α-tocopherol

This study involves two main points: (i) the characterization of the final product; (ii) the mechanism of its formation.

2. MATERIALS AND METHODS

DL-α-Tocopherol was purchased from Merck. The absolute ethanol used was a Prolabo normapur analytical reagent. αTH titrations were made on a Beckman model 35 spectrophotometer with a 1 cm optical pathway. We established that Beer's law is verified up to [αTH] = 5.5×10^{-4} $mol \cdot l^{-1}$ ($\epsilon_{292} = 3.15 \times 10^3$ $mol^{-1} \cdot l \cdot cm^{-1}$). Water was triply distilled and its purity controlled by conductivity measurements ($\leqslant 10^{-6}$ $\Omega^{-1} \cdot cm^{-1}$). The vessels for irradiation were heated at 400°C for 4 h after washing. γ-irradiations were made in a ^{60}Co irradiator. Its dosimetry was determined by Fricke's method (radiooxidation of H_2SO_4 0.4 $mol \cdot l^{-1}$ ferrous sulfate solutions under air atmosphere) taking λ_{max} (Fe^{3+}) = 304 nm, ϵ_{304} = 2204 $mol^{-1} \cdot l \cdot cm^{-1}$ at 25°C and G = 15.6 molecules/100 eV. The doses were provided at a rate of $\simeq 2 \times 10^{18}$ $eV \cdot cm^{-3} \cdot h^{-1}$ (32 $krad \cdot h^{-1}$ or 320 $Gy \cdot h^{-1}$), and their values were used for calculation of the yields without any correction. The mass spectra of the final product were carried out on a Ribermag R 10-10 C device.

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3. RESULTS

3.1. *Irradiations*

Aerated αTH ethanolic solutions were irradiated for different concentrations $[\alpha TH]_0$ between 7.9×10^{-6} and 5.4×10^{-4} mol·l^{-1}.

Fig.2 shows as an example the differential absorption spectra obtained for some solutions ($[\alpha TH]_0 = 1.1 \times 10^{-4}$ mol·l^{-1}) irradiated in the conditions described above at different doses. The inset indicates some values of differential optical density versus irradiation dose. It can be observed that as the absorption at 292 nm due to αTH decreases, a new absorption maximum appears at 242 nm. Due to the two very clear isobestic points, it can be admitted that αTH is converted into a single product (P), whose molar absorption coefficient can therefore be calculated: $\epsilon_{242} = 8.6 \times 10^3$ mol^{-1}·l·cm^{-1}, knowing ϵ_{242} (αTH) $= 7 \times 10^2$ mol^{-1}·l·cm^{-1}. The initial yield G(P) can also be worked out: G(P) = 2 molecules/100 eV for the considered concentration $[\alpha TH]_0$. This was repeated for different values of $[\alpha TH]_0$ chosen in the considered range. Fig.3 shows the obtained yields plotted vs $[\alpha TH]_0$. This dilution curve points out a constant yield G(P) = 2.4 molecules/100 eV for $[\alpha TH]_0 \geqslant 2 \times 10^{-4}$ mol·l^{-1}.

The mass spectra attributed to P an M_r of 474.

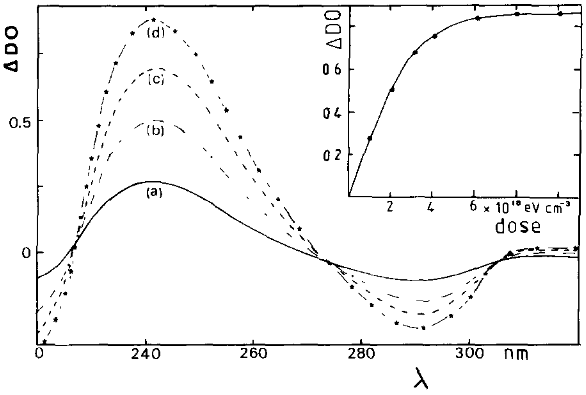


Fig.2. Differential absorption spectra of α-tocopherol ethanolic solutions irradiated under air. $[\alpha TH]_0 = 1.1 \times 10^{-4}$ mol·l^{-1}. Reference: initial solution. Curves: (a) 1.03×10^{18} eV·cm^{-3}, (b) 2.12×10^{18} eV·cm^{-3}, (c) 3.18×10^{18} eV·cm^{-3}, (d) 8.04×10^{18} eV·cm^{-3}. Dose rate: 2×10^{18} eV·cm^{-3}·h^{-1}. Inset: Differential optical density plotted vs dose for λ = 242 nm.

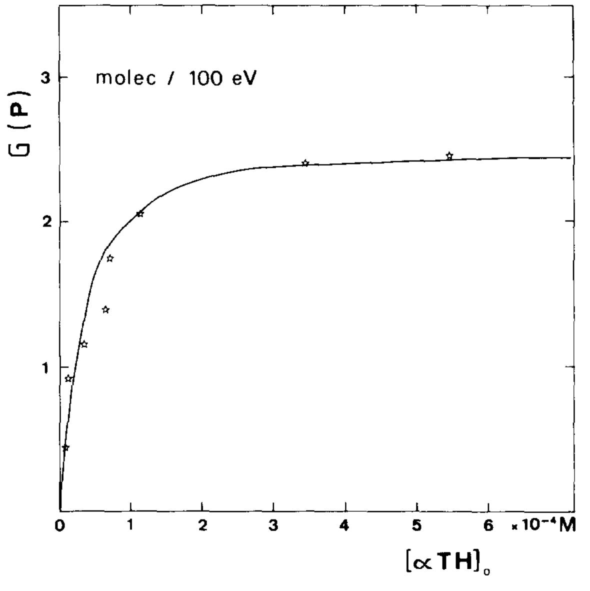


Fig.3. Dilution curve. Experimental points and calculated curve (see section 4 for kinetic scheme).

3.2. *Nature of the product obtained in acid medium*

To determine the nature of (P), the action of H_2SO_4 during and after irradiation was studied. We previously checked that H_2SO_4 did not react with αTH.

The αTH solutions ($[\alpha TH]_0 = 2.1 \times 10^{-4}$ mol·l^{-1}, $[H_2SO_4] = 1 \times 10^{-4}$ mol·l^{-1}) were irradiated. In this case, the solvent was a mixture, C_2H_5OH/H_2O with 30% water in volume.

Fig.4 shows as an example the differential spectra obtained for different doses. A maximum of absorption typical of αTH quinone (fig.1) appears between 262 and 269 nm ($\epsilon_{269} = 1.9 \times 10^4$ mol^{-1}·l·cm^{-1}) as described recently in analogous conditions [5], whereas in neutral medium, in the same solvent, the obtained product is identical to (P). (P) prepared by radiolysis in absolute ethanol was submitted to reaction with an equivalent quantity of H_2SO_4. As a result, fig.5 shows the evolution with respect to time of the absorption spectra of a mixture $[P]_0 = 1.3 \times 10^{-4}$ mol·l^{-1} and $[H_2SO_4] = 1.3 \times 10^{-4}$ mol·l^{-1} (solvent: C_2H_5OH/H_2O with 30% water in volume). The absorption at 242 nm decreases while the absorption at 262–269 nm increases.

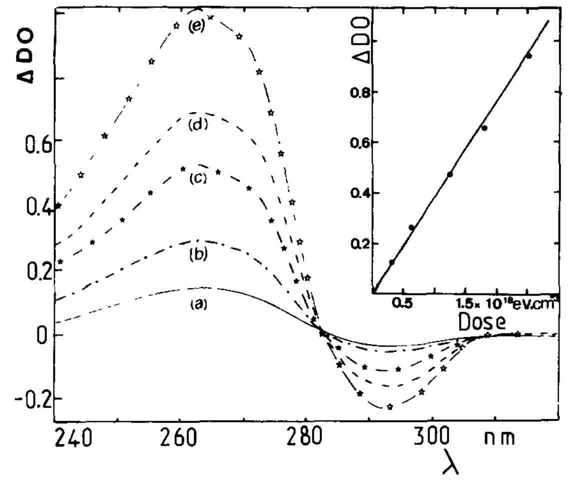


Fig.4. Differential absorption spectra of aerated α-tocopherol solutions irradiated with H_2SO_4. $[\alpha TH]_0 = 2.1 \times 10^{-4}$ mol·l^{-1}, $[H_2SO_4] = 1 \times 10^{-4}$ mol·l^{-1}. Solvent: ethanol/H_2O with 30% water in volume. Reference: initial solution. Curves: (a) 2.9×10^{17} eV·cm^{-3}, (b) 6.1×10^{17} eV·cm^{-3}, (c) 1.2×10^{18} eV·cm^{-3}, (d) 1.8×10^{18} eV·cm^{-3}, (e) 2.5×10^{18} eV·cm^{-3}. Dose rate: 1.8×10^{18} eV·cm^{-3}·h^{-1}. Inset: Differential optical density plotted vs dose for λ = 269 nm.

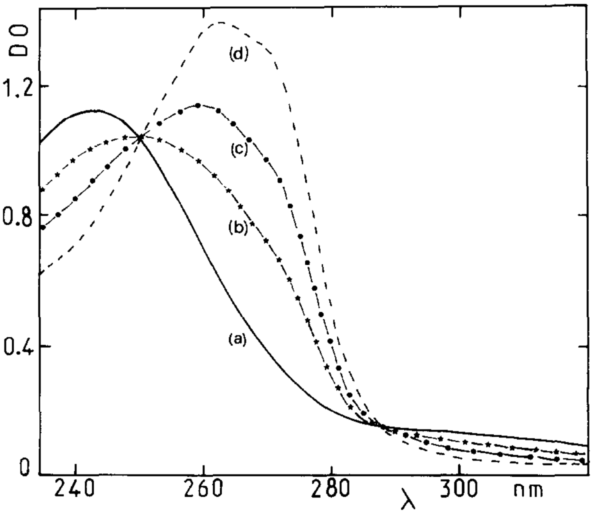


Fig.5. Evolution with respect to time of the absorption spectra of a mixture: $[P] = 1.3 \times 10^{-4}$ mol·l^{-1}, $[H_2SO_4] = 1.3 \times 10^{-4}$ mol·l^{-1}. Solvent: ethanol/water with 30% water in volume. Curves: (a) initial solution, (b) after 10 min, (c) after 20 min, (d) after 95 min.

4. DISCUSSION

γ-radiolysis of absolute ethanol provides H_3C-$\dot{C}H$-OH($R^{\cdot}$) radicals with a yield $G_{R^{\cdot}} \simeq G_{H^{\cdot}} + G_{e^-}$ (= 4.3 molecules/100 eV) [6,7]. Preliminary tests showed that the $R^{\cdot}$ radicals produced by radiolysis of N_2O-saturated ethanolic solutions do not react with αTH. Under air, the $R^{\cdot}$ radicals scavenge oxygen according to reaction 1 at a diffusion-controlled rate [8]:

$$R^{\cdot} + O_2 \longrightarrow RO_2^{\cdot} \qquad (1)$$

The observed value for the plateau of the dilution curve (fig.3) $G(P)$ = 2.4 molecules/100 eV, near to $G_{R^{\cdot}}/2$ leads us to propose the following mechanism for the oxidation of αTH: first, αTH reacts with $RO_2^{\cdot}$ radicals (eqn 2):

$$\alpha TH + RO_2^{\cdot} \longrightarrow \alpha T^{\cdot} + ROOH \qquad (2)$$

The obtained $\alpha T^{\cdot}$ radical may react with $RO_2^{\cdot}$ (eqn 3) and/or disproportionate (eqn 4):

$$\alpha T^{\cdot} + RO_2^{\cdot} \xrightarrow{H^+} \alpha T^+ + ROOH \qquad (3)$$

$$\alpha T^{\cdot} + \alpha T^{\cdot} \xrightarrow{H^+} \alpha T^+ + \alpha TH \qquad (4)$$

αT^+ is considered as the precursor of P and we will suppose that finally $[P] = [\alpha T^+]$ (see below).

These reactions explain the value of the observed plateau. For lower concentrations ($[\alpha TH]_0 < 2 \times 10^{-4}$ mol·l^{-1}), reaction 5:

$$RO_2^{\cdot} + RO_2^{\cdot} \longrightarrow \text{non-radical products} \qquad (5)$$

competes with reactions 2 and 3 and lowers the $G(P)$ value.

Taking $k_1 = 4.6 \times 10^9$ mol^{-1}·l·s^{-1} [8] and $2k_5 = (7 \pm 2) \times 10^8$ mol^{-1}·l·s^{-1} [9], the above kinetic scheme allows the calculation of $[\alpha T^+]$ at different times. Fig.6 shows, for various concentrations $[\alpha TH]_0$, the evolution of the calculated concentration $[\alpha T^+]$ with:

$k_2 = 9.1 \times 10^4$ mol^{-1}·l·s^{-1}

$k_3 = 2.5 \times 10^6$ mol^{-1}·l·s^{-1}

$k_4 = 1 \times 10^4$ mol^{-1}·l·s^{-1}

which is in the best agreement with the experimental results. It can be observed that $[\alpha T^+]$ varies linearly as a function of time when the steady

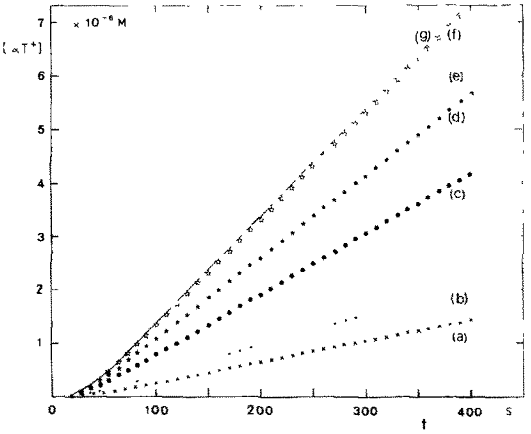


Fig.6. Evolution of calculated [αT^+] with respect to time for different concentrations [αTH]₀. Curves: (a) 7.9 × 10^{-6} mol·l^{-1}, (b) 1.3 × 10^{-5} mol·l^{-1}, (c) 3.5 × 10^{-5} mol·l^{-1}, (d) 6.5 × 10^{-5} mol·l^{-1}, (e) 1.1 × 10^{-4} mol·l^{-1}, (f) 3.4 × 10^{-4} mol·l^{-1}, (g) 5.4 × 10^{-4} mol·l^{-1}.

states ($\alpha T^\cdot$) and ($RO_2^\cdot$) are reached (t > 30 s). The slopes of the linear parts of these curves indicate the yields $G(\alpha T^+) = G(P)$ and were used to draw the dilution curve of fig.3 which, as can be seen, fits the experimental points perfectly.

The stable final product P corresponds neither to the quinone epoxide obtained by photolysis of αTH [10] (different M_r), nor to αTH quinone (λ_{max} is different), nor to unstable 8-hydroxy-α-tocopherone [11] (different M_r). The M_r (474) together with the results obtained with H_2SO_4 allow us to suggest for P the structures αTOC_2H_5 (fig.7).

P could be obtained by the fast and stoichiometrical reaction of αT^+ with ethanol:

$$\alpha T^+ + C_2H_5OH \longrightarrow \alpha TOC_2H_5 + H^+$$

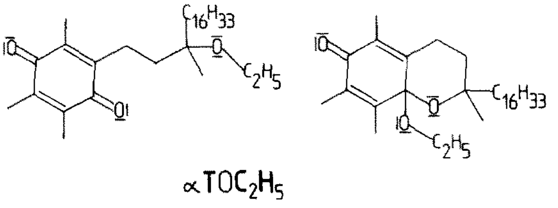
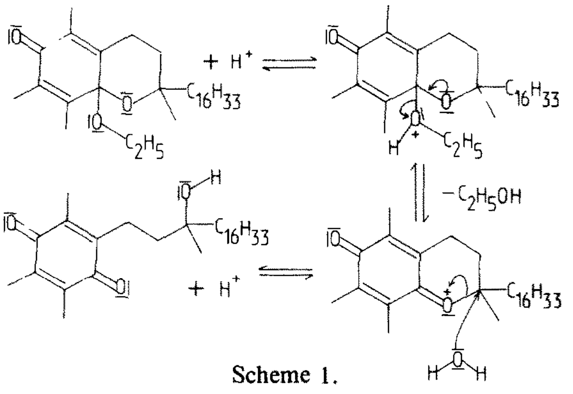


Fig.7. Proposed structures for P (αTOC_2H_5).



Scheme 1.

αTOC_2H_5 in aqueous acid medium is converted into α-tocopheryl quinone; the following mechanism may summarize this nucleophilic substitution (scheme 1).

In conclusion, this work proves that αTH does not react with the $R^\cdot$ radicals obtained radiolytically from ethanol, but does react with the $RO_2^\cdot$ radicals ($k_2 = 9.1 \times 10^4$ mol^{-1}·l·s^{-1}). The $\alpha T^\cdot$ obtained reacts in turn with $RO_2^\cdot$ ($k_3 = 2.5 \times 10^6$ mol^{-1}·l·s^{-1}) or disproportionates ($k_4 = 1 \times 10^4$ mol^{-1}·l·s^{-1}). This value of rate constant $k_{\alpha T^\cdot + \alpha T^\cdot}$ is in agreement with that determined by Bielski et al. [12], in a study of the reaction of $HO_2^\cdot$ with αTH in different conditions by the stopped-flow technique: $k_{\alpha T^\cdot + \alpha T^\cdot} = 3.6 \times 10^3$ mol^{-1}·l·s^{-1}. For each concentration [αTH]₀ studied, the values of [$\alpha T^\cdot$] and [$RO_2^\cdot$] could be calculated for the steady state: for example, if [αTH]₀ = 3.4 × 10^{-4} mol·l^{-1}, [$\alpha T^\cdot$] = 1.3 × 10^{-6} mol·l^{-1} and [$RO_2^\cdot$] = 1.2 × 10^{-9} mol·l^{-1}. This allows one to estimate in this case the rates of reactions 3 and 4: 3.9 × 10^{-9} mol·l^{-1}·s^{-1} and 1.7 × 10^{-8} mol·l^{-1}·s^{-1}, respectively. Therefore, it seems that $\alpha T^\cdot$ will rather disappear by disproportionation, but reaction 3 cannot be neglected completely. αT^+ reacts with ethanol and leads to αTOC_2H_5 (M_r 474), which can be easily converted into α-tocopheryl quinone by nucleophilic substitution in aqueous acid medium.

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