

# Photolysis of $\alpha$ -Tocopherol in Olive Oils and Model Systems

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The photolysis of  $\alpha$ -tocopherol (**I**) in olive oil (O) and in some model systems (*n*-hexane = H; anhydrous *n*-hexane = HA, and triolein = T) was studied under sunlight and under artificial light ( $\lambda > 290$  nm) by HPLC and GC/MS. In O and T, **I** disappeared linearly to 50% of the starting concentration, reached a constant value, and finally disappeared rapidly from the medium. In the model system, photolysis followed a pseudo-first-order kinetics. Although no peaks attributable to photoproducts were found in O, a main product identified by <sup>1</sup>H and <sup>13</sup>C NMR and GC/MS as 5-formyltocopherol (**II**) was found in the model systems. Irradiation of compound **II** led to species undetectable by HPLC in agreement with a slower consecutive kinetic process than that of **I**. In the HA and T systems, the formation of **II** occurred at lower levels than in H. The possible behavior of photodegradation is discussed.

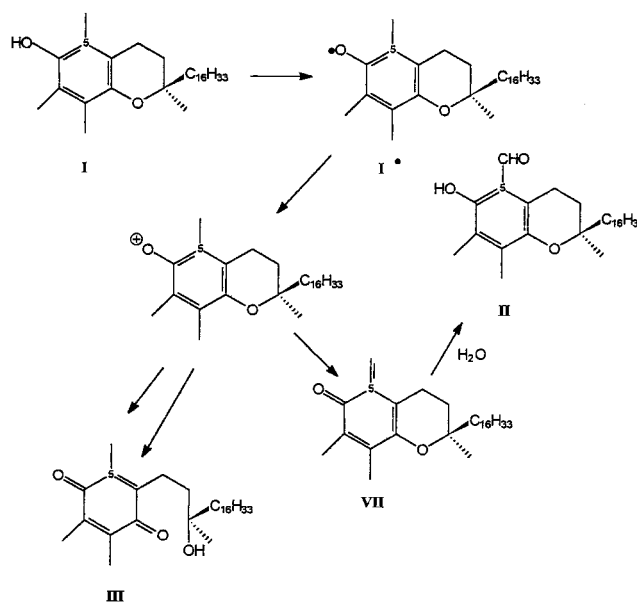
**Keywords:**  $\alpha$ -Tocopherol; photolysis; olive oil

## INTRODUCTION

Vitamin E is a well-known mixture of phenolic compounds with high antioxidative properties. Among these phenolic compounds,  $\alpha$ -tocopherol (**I**, Figure 1) is the main component (Burton and Ingold, 1986).

Many foodstuffs (cereals, late leaves vegetables, oil-seeds, and fruits, and, of course, their oils) contain **I** in significant amount for the human diet. The original amount of **I** in foodstuffs could be reduced significantly by a reforming process, whereas cooking and freezing have only a small influence (*Encyclopedia of Food Science and Technology*, 1992).

The reduction of **I** mainly by reaction with hydroperoxides generated from polyunsaturated fatty acids (PUFA) has been well studied in model systems (Yamauchi et al., 1995). Its reactivity and that of some of its model compounds toward chemical oxidants have also been reported (Suarna et al., 1988). The influence of other physical factors on **I**, such as  $\gamma$ -rays, was studied by several authors (Knapp and Tappel, 1961; Jore and Ferradini, 1985; Molnar and Koswig, 1992). Surprisingly, data on the photolysis of **I** under domestic conditions (room temperature, sunlight) are not reported in the literature, although some foodstuffs (e.g., oils) could easily be injured by light. In past years the only studies on this topic were a dye-sensitized photooxidation (Grams et al., 1972, which reported that **I** was "insensitive to incandescent light. . . at 25 °C for 16 h") and a photolysis at -30 °C (Clough et al., 1979). Recently, the photolysis of **I** in liposomes by UVB rays (ultraviolet beta, 290–320 nm) was reported by Kramer and Liebler (1997), and Servili et al. (1996) have studied



**Figure 1.** Formulas of **I**, its rearrangement compounds, and **II**.

under sunlight the interaction of **I** with phenolic compounds of antioxidant activity. In this paper we report data on the photolysis of **I** under sunlight and under artificial light ( $\lambda > 290$  nm) in virgin olive oils and in model systems of *n*-hexane (H), anhydrous *n*-hexane (HA), and triolein (T).

## MATERIALS AND METHODS

**Chemicals.** *n*-Hexane, chloroform, dichloromethane, methanol, and acetonitrile were of HPLC solvent grade (Merck, Darmstadt, Germany).  $\alpha$ - and  $\gamma$ -tocopherol, triolein (Aldrich or Sigma, Milan, Italy),  $K_2CrO_4$ ,  $Na_2CO_3$  (Carlo Erba, Milan), and anhydrous  $Na_2SO_4$  (Merck) were analytical grade reagents (>98%).

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$\gamma$ -Tocopherol was used as an internal standard (i.s.) at 200 ppm in  $\text{CHCl}_3$  or in hexane. The *n*-hexane was anhydriated with metallic Na.

Two olive oils were employed. They were obtained from cv. Bosana olives by mill hammer crushing, beating, and cold extraction with continuous oil centrifuge. The sample employed in sunlight experiments shows the following parameters: free acidity, 0.33% (as oleic acid); peroxides, 6.66 mequiv of  $\text{O}_2/\text{kg}$ ;  $K_{270} = 0.180$ ;  $K_{232} = 2.100$ ;  $\alpha$ -tocopherol, 188 ppm. The sample used in artificial light had the following parameters: free acidity, 0.28% (as oleic acid); peroxides, 6.5 mequiv of  $\text{O}_2/\text{kg}$ ;  $K_{270} = 0.173$ ;  $K_{232} = 1.630$ ;  $\alpha$ -tocopherol, 281 ppm. The latter sample was also used in other lamp experiments after saturation with water.

**Irradiation of I and Its Photoproducts.** In all experiments nonirradiated samples were held in the dark as control. The photolysis performed experiments were as follows: olive oil under sunlight ( $\text{O}_S$ ) and artificial light ( $\text{O}_L$ ); *n*-hexane under sunlight ( $\text{H}_S$ ) and artificial light ( $\text{H}_L$ ); triolein ( $\text{T}_L$ ) and anhydrous *n*-hexane under artificial light ( $\text{HA}_L$ ). This latter system was employed to determine the water content effect on the process. HPLC and/or GC/MS were performed, as reported above, and irradiation behavior was checked. With the latter technique the samples were injected directly without any further preparation.

**$\text{O}_S$  Conditions.** Eighteen 100 mL screw-capped bottles, 9 dark (DBS, dark bottles sunlight) and 9 white (WBS), were filled with a virgin olive oil, hermetically sealed, and exposed to sunlight at latitude  $39^\circ 12'$  north and longitude  $9^\circ 07'$  east from Greenwich, between February 1 and November 12, 1996. Another 18 glass bottles, 9 dark (DBI, dark bottle) and 9 white (WBI), were exposed to indirect sunlight in our laboratory. On May 25, September 2, and November 11, 1996, three replicates from each series were collected and analyzed by HPLC.

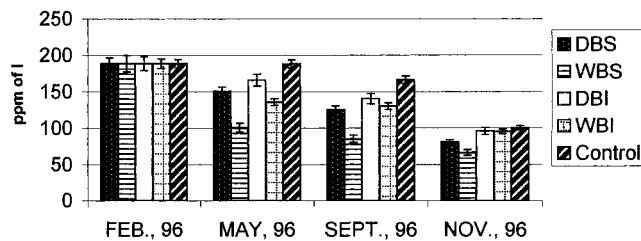
**$\text{O}_L$  Conditions.** The oil was placed into a 500-mL cylindrical flask containing a mercury lamp. At selected times three replicates (each of 200  $\mu\text{L}$  of oil) were withdrawn, dissolved in 1.0 mL of chloroform containing the internal standard, and analyzed.

**$\text{H}_S$  Conditions.** In these experiments the compounds I and its derivative (II) were dissolved in *n*-hexane in 2 mL screw-capped borosilicate vials at the concentration of  $\approx 200$  ppm and exposed to direct sunlight. At selected times three samples were withdrawn and analyzed. Each experiment was replicated four times.

**$\text{H}_L$ ,  $\text{HA}_L$ , and  $\text{T}_L$  Conditions.** Solutions containing  $\approx 200$  ppm of I (about the same concentration of the oil samples) were placed in cylindrical flasks as described above. At selected times samples were withdrawn and analyzed. The experiments in  $\text{T}_L$  were carried out as follows: an aliquot of stock solution (solvent anhydrous *n*-hexane) of I was placed in each of 10 2 mL screw-capped borosilicate vials to reach the final concentration of  $\approx 200$  ppm (0.46 M) in 0.2 mL. After the solvent had been evaporated with a gentle nitrogen stream, 0.2 mL of a solution obtained by dissolving 1.0 g of T (0.56 M) in 2.0 mL of anhydrous *n*-hexane was added to each vial. The vials were sonicated for 5 min, capped, and irradiated with the lamp. At selected times the vials were withdrawn, frozen at  $-25^\circ\text{C}$  for 5 min, evaporated under  $\text{N}_2$ , and recovered with 0.2 mL of  $\text{CHCl}_3$  containing the i.s. The solution was finally injected and analyzed.

**Artificial Light.** A water-cooled high-pressure mercury lamp with a wavelength emittance of  $\lambda > 290$  nm with a maximum wavelength emittance at 360 nm (125 W,  $I_\lambda = 3.97 \times 10^{-7}$  E L $^{-1}$  s $^{-1}$ , Helios Italquartz, Milan) was used.

**Apparatus and Chromatography.** HPLC. A Varian 5020 HPLC pump (Varian, Palo Alto, CA) was employed. The pump was equipped with a Hewlett-Packard 1050 automatic injector (Hewlett-Packard, Avondale, PA), an LC-235 diode array, and an LCI-100 integrator (Perkin-Elmer, Norwalk, CT). The samples (25–100  $\mu\text{L}$ ) were injected in Spherisorb ODS2 and  $\text{C}_8$  columns (250  $\times$  4.0 mm i.d., 5  $\mu\text{m}$ , Waddinxveen, The Netherlands). The operating conditions were as follows: eluent mixture, methanol/acetone/nitrate (50:50, v/v); flow, 1.5 mL/min; program of  $\lambda$ , at 295 nm from 0 to 9 min and  $\lambda = 286$  nm



**Figure 2.** Histogram of the concentration of I in the oil exposed to sunlight during long-time experiment.

from 9 to 20 min. In the  $\text{O}_S$  and  $\text{O}_L$  experiments the injected samples were made by dissolving 200  $\mu\text{L}$  of oil in 1.0 mL of  $\text{CHCl}_3$  containing the i.s. In  $\text{H}_S$ ,  $\text{H}_L$ , and  $\text{HA}_L$  experiments the samples were prepared as follows: 200  $\mu\text{L}$  of the hexane solution was evaporated under  $\text{N}_2$ , dissolved with an equal amount of eluent mixture containing the i.s.

Calculation of the compound concentration in the chromatograms was made by the i.s. method.

**GC/MS.** An HP-5890 GC (Hewlett-Packard) equipped with an HP-5971 MS detector (Hewlett-Packard) was used. The column was a Durabond fused silica (30 m  $\times$  0.25 mm i.d., J&W Scientific, Folsom, CA) with DB-5 liquid phase (5% phenyl, 95% dimethylpolysiloxane; film thickness, 0.25  $\mu\text{m}$ ). The sample (2  $\mu\text{L}$ ) was injected in the split mode (1:50). Detector and injector operating conditions were, respectively, 280 and 250  $^\circ\text{C}$ ; the oven temperature was programmed as follows: 80  $^\circ\text{C}$  (1 min) raised to 250  $^\circ\text{C}$  (10  $^\circ\text{C}/\text{min}$ ), and held for 25 min. Helium was the carrier gas at 1.8 mL/min (45 kPa). Mass spectrometer operating conditions were as follows: electron ionization, 70 eV; ion source 180  $^\circ\text{C}$ ; scan mass, range 50–550; scan interval, 1.5 s; solvent delay, 6 min.

**IR.** Infrared spectrophotometric analysis was performed on a Perkin-Elmer 1310 infrared spectrophotometer, using a scan time of 12 min in liquid phase with chloroform as a solvent.

**NMR.** Proton and  $^{13}\text{C}$  NMR spectra were recorded at 500 and 125 MHz, respectively, in  $\text{CDCl}_3$  with a Bruker spectrometer. The temperature was fixed at 300 K. All values are quoted in  $\delta$  (ppm) with respect to the internal standard tetramethylsilane (TMS).

**Separation of Photoproducts.** The separation of photoproducts was performed by column chromatography (glass column, 400  $\times$  25 mm i.d.). The column was filled with silica gel (particle size = 0.05–0.20 mm) activated in a stove (12 h at 110  $^\circ\text{C}$ ). The mobile phase was a mixture of hexane and dichloromethane (60:40, v/v). A 1.2 g aliquot of I was irradiated to sunlight in hexane solution; the crude photodegradation mixture was evaporated to dryness under reduced pressure and chromatographed. Fractions of 10 mL were collected and analyzed by TLC (silica gel plates 60-F $_{254}$ , 0.2  $\mu\text{m}$ ; Merck) using the same eluents as in chromatography column. The fractions containing the spots with the same  $R_f$  were collected in six samples (0.410 g of II; 0.260 g of unreacted I; and four fractions of a total weight of 0.200 g) and analyzed by HPLC and/or GC/MS. The total final weight was 0.870 g, corresponding to 73% of starting vitamin E.

## RESULTS AND DISCUSSION

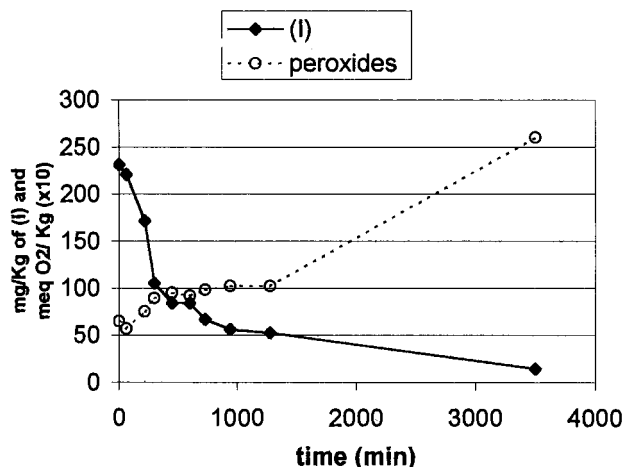
**Experiments in Olive Oil. Sunlight.** In the long-time sunlight experiment, the content of I showed a peculiar behavior within time (Figure 2), which could have been enhanced by the transmittance features of the glass. From a comparison of the white bottles with the dark ones, the following was observed:

1. In the sampling of May and September the content of I in DBS was  $\approx 26\%$  higher than in WBS, whereas that in DBI was  $\approx 16\%$  higher than in WBI.
2. In the WBS sample the maximum percent reduction occurred at the first sampling time.
3. Indirectly exposed bottles always showed a lower reduction than directly exposed ones.

**Table 1.** Chemical Parameters of the Oil Exposed to Sunlight during Long-Time Experiment

bottle	Feb				May				Sept				Nov			
	A <sup>a</sup>	P <sup>b</sup>	K <sub>270</sub>	K <sub>232</sub>	A	P	K <sub>270</sub>	K <sub>232</sub>	A	P	K <sub>270</sub>	K <sub>232</sub>	A	P	K <sub>270</sub>	K <sub>232</sub>
DBS	0.33	6.66	0.18	2.10	0.34	9.8	0.24	2.05	0.39	8.22	0.24	2.45	0.38	8.5	0.29	2.25
WBS	0.33	6.66	0.18	2.10	0.34	8.7	0.22	2.16	0.36	7.0	0.18	2.14	0.39	7.2	0.30	2.16
DBI	0.33	6.66	0.18	2.10	0.34	10.7	0.20	2.10	0.37	10.4	0.18	2.15	0.36	11.1	0.22	2.16
WBI	0.33	6.66	0.18	2.10	0.34	10.2	0.22	2.23	0.35	9.8	0.22	2.20	0.35	9.8	0.29	2.26
control	0.33	6.66	0.18	2.10	0.33	11.6	0.19	2.08	0.37	13.3	0.20	2.45	0.37	16.4	0.20	2.46

<sup>a</sup> A = acidity as percent oleic acid. <sup>b</sup> P = peroxides as mequiv of O<sub>2</sub>/L.

**Figure 3.** Variation of the concentration of I and of peroxides in olive oil irradiated with  $\lambda > 290$  nm.

4. The sample stored in the dark (cylindrical stainless steel tank of 50 L) shows a high reduction of the content of I only in the last sampling. This could be a consequence of the headspace in the tank (~15 cm).

Thus, in the period February–September, the differences among the samples exposed directly to sunlight, those exposed indirectly in the laboratory, and the control show that light plays a important role in the disappearance of I.

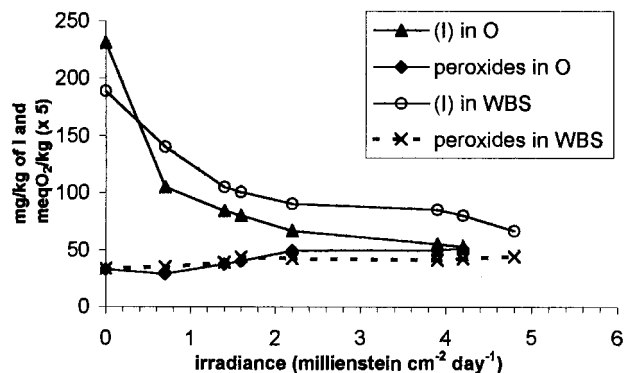
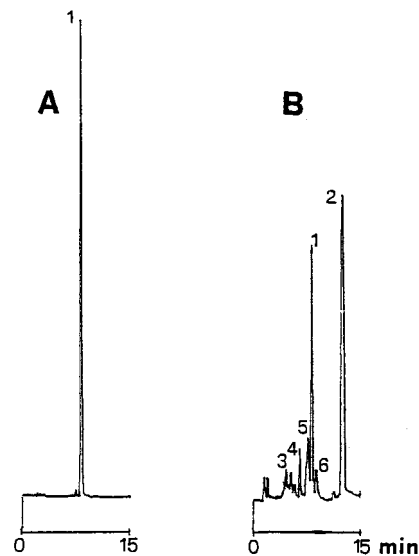
The main chemical parameters of the oil during the experiment are reported in Table 1. From these data it was clear that at the first sampling time the K<sub>270</sub> parameters of the oil sample already exceeded the limits for the virgin olive oil (Directive CEE 2568/91).

The number of peroxides increased up to May and later remained constant.

The coefficients of variation (CV) showed values <10%.

**Artificial Light.** The same experiment was repeated by lamp irradiation ( $\lambda > 290$  nm). In these conditions no degradation was observed in the blanks stored in the dark. In both cases the concentration of I in the oil decreases to 50% rapidly and linearly ( $r = -0.9788$ ) in 500 min; it then remained constant for about the same time and later decreased to 17% of the starting concentration in 2500 min. The peroxides showed an increase up to I and a decrease to 50%, then remained constant for another 500 min, and later progressively increased (Figure 3). When the irradiance emitted from the lamp was compared to the oil with solar actinic irradiance received under sunlight by the WBS, similar behaviors were found (Figure 4). During these experiments new peaks never appeared in the HPLC chromatograms when I disappeared from the oil. The peroxide behaviors were similar in the two experiments.

**Irradiation in Model Systems and Structure of Photoproducts.** H Conditions. Experiments per-

**Figure 4.** Variation of the concentration of I and peroxides versus the irradiance received by an olive oil irradiated under lamp (O<sub>L</sub>) and sunlight (WBS).**Figure 5.** HPLC chromatograms of  $\alpha$ -tocopherol (A) and photodegradation mixture (B).

formed in the semipreparative scale under sunlight and artificial light showed the same behaviors. I disappeared from the solution, yielding a main photoproduct (2,  $t_R = 12.88$  min;  $\lambda_{max} = 286$  nm). After the appearance of this signal, other signals at 6.23 min (3,  $\lambda_{max} = 266$  nm), 6.95 min (4,  $\lambda_{max} = 300$  nm), 8.04 min (5,  $\lambda_{max} = 300$  nm), and 9.29 min (6,  $\lambda_{max} = 300$  nm) were present in the HPLC chromatograms (Figure 5). The HPLC analysis of the six main fractions obtained by column chromatography showed the following: unreacted I mixed with some minor unidentified peaks; in the other four fractions, mixtures (in different ratios) of signals 3–6 (I and signal 2 were present as impurities); the sixth fraction contains peak 2 alone. Preliminary UV and GC/MS data of the main peak of each fraction seem to suggest the structure of the tocopheryl quinone III (Figure 1) for peak 3 ( $M^+ = 430$ ; UV

**Table 2.**  $^{13}\text{C}$  NMR Data of **I**<sup>a</sup>

11.0 (CH <sub>3</sub> ); 13.1 (CH <sub>3</sub> ); 18.4 (CH <sub>2</sub> ); 19.6 (CH <sub>3</sub> ); 19.7 (CH <sub>3</sub> ); 20.9 (CH <sub>2</sub> ); 22.6 (CH <sub>3</sub> ); 22.7 (CH <sub>3</sub> ); 23.6 (CH <sub>3</sub> ); 24.4 (CH <sub>2</sub> ); 24.8 (CH <sub>2</sub> ); 28 (CH); 29.7 (CH <sub>2</sub> ); 32.6 (CH); 32.7 (CH); bs37.3–37.4 (4 × CH <sub>2</sub> ); 39.4 (CH <sub>2</sub> ); 39.6 (CH <sub>2</sub> ); 68.9 (C); 114.5 (C); 117.5 (C); 122.2 (C); 138.4 (C); 144 (C); 155.8 (C); 193.9 (CH)
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<sup>a</sup>  $\delta$ ; the multiplicity was from DEPT experiments.

**Table 3.** Pseudo-First-Order Constants of the Photodegradation of **I** and **II** in Different Conditions

conditions	<b>I</b>			<b>II</b>		
	$k$ $10^{-4} \text{ s}^{-1}$	$t_{1/2}$ (min)	$r^2$	$k$ $10^{-4} \text{ s}^{-1}$	$t_{1/2}$ (min)	$r^2$
H <sub>S</sub>	1.03	112	-0.96711	0.03	4119	-0.9792
H <sub>L</sub>	1.86	62	-0.9985	0.49	393	-0.9446
HA <sub>L</sub>	1.41	82	-0.9719			
T <sub>L</sub>	0.98	117	-0.9836			

spectrum totally overlapping with that of an authentic specimen). For the peaks 4–6 we were unable to propose a structure, because only few milligrams (0.5–0.8) of each was isolated and the spectroscopic data were scanty. Compound **2** was similar to a yellow oil, and its elemental analysis agreed with the formula C<sub>29</sub>H<sub>48</sub>O<sub>3</sub>. The IR spectrum showed the presence of a carbonyl group at 1680 cm<sup>-1</sup>, and the GC/MS analysis showed the molecular ion at  $m/z$  444, the base peak at  $m/z$  201, and another two important ions at  $m/z$  220 and 179.

The GC/MS spectrum of **I** shows the molecular ion at  $m/z$  430 and other important peaks at  $m/z$  165 and 205. The mass spectra of **I** and peak **2** exhibit similar behaviors. The molecular ion at  $m/z$  444 of peak **2** could correspond to an  $\alpha$ -tocopherol-like structure, with a mass higher than the molecular ion of **I** by  $\approx 14$ . Moreover, the ions at  $m/z$  220 and 179 of peak **2** could be considered "derivatives" of the ions at  $m/z$  205 and 165 of **I**, plus a  $m/z = 14$ , respectively. The ion at  $m/z$  201 does not have a corresponding ion in **I**. <sup>1</sup>H and <sup>13</sup>C NMR analyses have provided the necessary information to identify compound **2** as 5-formyl- $\alpha$ -tocopherol (**II**) (Figure 1).

The <sup>1</sup>H spectrum of compound **II** is different from that of compound **I** in the following: (a) one singlet at  $\delta$  10.12, indicating the presence of a CHO group with a strong intramolecular hydrogen bonding with an OH ( $\delta$  12.05, exchangeable hydrogen with D<sub>2</sub>O); (b) only two singlets attributable to CH<sub>3</sub> on the aromatic ring of **I**.

The above results and NOE experiment (strong negative Overhauser effect of the CH<sub>2</sub> group in position 4 on the aldehyde signal) suggest that the CH<sub>3</sub> group in the 5-position (in **I**) of the aromatic ring has been oxidized into a CHO group. Further <sup>13</sup>C NMR data (Table 2) confirm the identification.

Several authors report the formation of formyl derivatives from **I** during the autoxidation of vitamin E (Fujimaki et al., 1970 and, after irradiation with  $\gamma$ -rays in two different solvents, *n*-hexane and CHCl<sub>3</sub> (Molnar et al., 1992).

**Kinetic Trials.** *H<sub>S</sub> and H<sub>L</sub> Conditions.* Under sunlight and artificial light **II** and **I** showed similar behaviors. **I** disappeared from the irradiated solutions according to a pseudo-first-order kinetics (Table 3) and yielded photoproducts. Experiments over a long period ( $> 3t_{1/2}$ ) showed an increase of compound **II** for up to  $2t_{1/2}$ . Compound **II** then slowly disappeared, and no new signals appeared in the chromatograms. The other photoproducts, peaks 3–6, reached a constant value soon after they appeared and were stable until **I** disappeared completely.

Photodegradation experiments of **II** alone were carried out to understand the following: (1) whether signals 3–6 could have **II** as a parent compound and (2) how the global reaction proceeded kinetically.

The photodegradation of **II** was very slow (Table 3). Because the HPLC chromatograms of **II** did not give signals 3–6, a parallel kinetic process could be hypothesized, which gave five species contemporaneously. However, according to Frost and Pearson (1961), for such a hypothesis to be valid, the formed products must be in constant ratio with each other irrespective of the time. Because this condition did not occur, such a hypothesis must be rejected.

We should therefore assume that **I** was photodegraded with a pseudo-first-order consecutive kinetics (eq 1), where **II** disappeared with  $K_2 < K_1$  to give compounds undetectable by HPLC.



This hypothesis has been confirmed by a long-time photodegradation of **I**, where, when the concentrations of other photoproducts were considered to be negligible, the concentrations of **I** and **II** were experimentally found to be in good accordance with those calculated by eq 2 of the consecutive process

$$[\text{II}] = \frac{[\text{I}_0]k_1}{k_2 - k_1} (e^{-k_1 t} - e^{-k_2 t}) \quad (2)$$

where  $K_{\text{obs}} = k_1$  and  $[\text{I}_0]$  is the starting concentration of the  $\alpha$ -tocopherol (Frost and Pearson, 1961).

*HA<sub>L</sub> Conditions.* Also in this case the disappearance of **I** followed a pseudo-first-order kinetics. Some differences occurred compared to the behaviors in *H<sub>S</sub>* and *H<sub>L</sub>* conditions. When the content of unreacted **I** was of the same order in both experiments, the  $K_{\text{obs}}$  values were lower and **II** was formed in very small concentrations (<5%). Furthermore, the HPLC chromatograms showed neither signals of 3–6 nor other compounds.

*T<sub>L</sub> Conditions.* Under these conditions the disappearance of **I** followed a pseudo-first-order kinetics with a slower constant rate than in *H<sub>S</sub>*, *H<sub>L</sub>*, and *HA<sub>L</sub>* conditions. Moreover, the appearance of **II** also occurred as in *HA<sub>L</sub>*.

In all experiments the  $K_{\text{obs}}$  values of the disappearance of **I** show CV ranging between 8.4 and 11.0%.

## CONCLUSIONS

From these data it can be concluded that in the model systems employed the photodegradation of **I** followed the consecutive kinetic process summarized in eq 2, where **II** was the main photoproduct. Under *H<sub>S</sub>* and *H<sub>L</sub>* conditions, the unknown species present in very low amounts might be due to secondary reactions of the radical species, **I**\*, the intermediate product that led to all photocompounds. Thus, eq 1 can be rewritten as follows:



Of course  $K_I \gg K_1$ , and the overall kinetic process depends on  $K_1$  (Burton and Ingold, 1986). Thus, from **I**\*, via rearrangements, the quinone **III** could be generated and, from it, compound **VII**. The latter species produced **II** by Michael addition of H<sub>2</sub>O (Fujimaki et al., 1970; Suarna et al., 1988). Under *H<sub>S</sub>* and *H<sub>L</sub>* conditions, **II** was produced because *n*-hexane contains 0.01% water (corresponding to  $\sim 5 \times 10^{-3}$  M), which is

of the same order as that of **I**. The nature of the further degraded photoderivatives is unknown. The presence of **II** in HA<sub>L</sub> and T<sub>L</sub> experiments in low concentrations (<5%) could be due to impurities of water contained in the hexane and in triolein. On the contrary, the absence of **II** in O<sub>S</sub> and O<sub>L</sub> experiments could be explained by assuming that **I'** mostly reacts with the peroxy radicals generated from the fatty acids during irradiation. This should explain the constancy in milliequivalents of O<sub>2</sub> per kilogram of the oil during the time. Thus, when the concentration of **I** was <20 mg/kg, the number of peroxides increased rapidly. This finding agrees with the data of Servili et al. (1996).

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