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A Quantitative Approach to the Recycling of α-Tocopherol by Coantioxidants

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A systematic investigation is reported on the regeneration of α-tocopherol (α-TOH) in homogeneous solution by coantioxidants in order to better understand the mechanism and the factors responsible for the effectiveness of this process. The current availability of thermochemical data concerning the reactants involved in the regeneration reactions, as well as a large number of the kinetic constants for the various reactions involved, allowed us to rationalize the experimental observations collected so far. Three limiting cases have been considered. The first case is that of a coantioxidant irreversibly regenerating α-TOH, where the effectiveness of the recycling process depends on the magnitude of the rate constant k_r. The second case is that of a coantioxidant reversibly recycling α-TOH, where regeneration can only be observed if the bond dissociation enthalpy value of the coantioxidant is lower or at least close to that of the O−H bond of α-tocopherol. The third case is that of a catechol derivative (chosen as a model compound for polyphenolic antioxidants), where recycling of α-TOH is feasible even though the BDE value is significantly higher than that of vitamin E. In this case, the driving force for the recycling process is the removal of the semiquinone radical from the catechol derivative by the α-tocopheroxyl radical, which makes the regeneration of α-TOH practically irreversible.

Introduction

The observation that the antioxidant activity of vitamin E (tocopherols) is enhanced by the presence in the system of ascorbic acid (vitamin C) was reported for the first time by Columbic and Mattill in 1941.[1] Since then, several investigations have demonstrated that these two vitamins show a synergistic behavior and their mixtures display much better antioxidant properties than expected on the basis of the activity of the single components. Exhaustive studies have been reported by Niki et al.[2] in homogeneous solutions, by Ingold and co-workers and by Niki et al.[3] in phospholipid liposomes, and by Barclay et al.[4] in micelles. Synergism with α-tocopherol has also been reported for ubiquinol-10 in liposomes,[5] for ubiquinol-3 in homogeneous solution and in liposomes,[6] and for flavonoids such as epicatechin, epigallocatechin, epicatechin gallate, etc. both in solution and micelles.[7,8]

Despite the large number of experimental reports, the regeneration mechanism of vitamin E by other coantioxidants has not yet been fully clarified since the efficiency of the recycling process has been found to depend not only on the structure of the coantioxidant but also on its relative concentration and on the microenvironment of the reaction medium. We have therefore undertaken a systematic investigation of the regeneration of α-tocopherol in homogeneous solution in order to better understand the mechanism and factors responsible for the effectiveness of this process, following a first attempt to rationalize the synergistic behavior of two antioxidants reported by Mahoney some time ago.[9] The current availability of thermochemical data concerning the reactants involved in the regeneration reactions, as well as the pertinent kinetic constants, allowed us to rationalize the experimental observations collected so far.

Results and Discussion

These studies were performed by investigating the thermally initiated autoxidation of styrene either in the

(1) Golumbic, C.; Mattill, H. A. *J. Am. Chem. Soc.* **1941**, *63*, 1279−1280.
(2) Niki, E.; Saito, T.; Kawakami, A.; Kamiya, Y. *J. Biol. Chem.* **1984**, *259*, 4177−4182.
(3) Doba, T.; Burton, G. W.; Ingold, K. U. *Biochim. Biophys. Acta* **1985**, *835*, 298−303. Niki, E.; Kawakami, A.; Yamamoto, Y.; Kamiya, Y. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 1971−1975.
(4) Barclay, L. C. R.; Locke, G. J.; MacNeil, J. M. *Can. J. Chem.* **1985**, *63*, 366−374.
(5) Frei, B.; Kim, M. C.; Ames, B. N. *Proc. Natl. Acad. Sci.* **1990**, *87*, 4879−4883.
(6) Landi, L.; Cabrini, L.; Fiorentini, D.; Stefanelli, C.; Pedulli, G. F. *Chem. Phys. Lipids* **1992**, *61*, 121−130.
(7) Jia, Z. S.; Zhou, B.; Yang, L.; Wu, L. M.; Liu, Z. L. *J. Chem. Soc., Perkin Trans. 2* **1998**, 911−915. Zhou, B.; Jia, Z. S.; Chen, Z. H.; Yang, L.; Wu, L. M.; Liu, Z. L. *J. Chem. Soc., Perkin Trans. 2* **2000**, 785−791. Chen, Z. H.; Zhou, B.; Yang, L.; Wu, L. M.; Liu, Z. L. *J. Chem. Soc., Perkin Trans. 2* **2001**, 1835−1839.
(8) Pedrielli, P.; Skibsted, L. H. *J. Agric. Food Chem.* **2002**, *50*, submitted for publication.
(9) Mahoney, L. R. *Angew. Chem., Int. Ed. Engl.* **1972**, *8*, 547.

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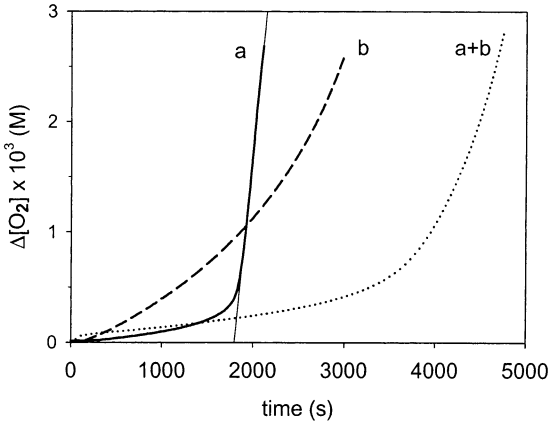
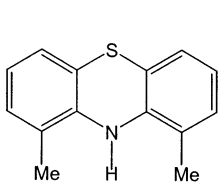


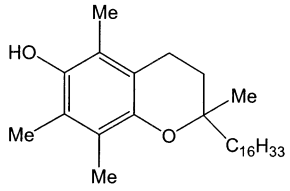
FIGURE 1. Oxygen consumption observed during the AMVN (0.046 M) initiated autoxidation at 30 °C of styrene (7.5 M) in chlorobenzene in the presence of (a) 5×10^{-5} M α-TOH, (b) 1.05×10^{-4} M MPTZ, and (a + b) a mixture of 5×10^{-5} M α-TOH and 1.05×10^{-4} M MPTZ.

absence or in the presence of the antioxidants. The reaction was carried out in a closed system under controlled conditions at 30 °C in air-saturated solutions of the oxidizable substrate in chlorobenzene. The autoxidations were initiated with 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN) or 2,2′-azobis(isobutyronitrile) (AIBN) and followed by monitoring the oxygen consumption with an automatic recording gas absorption apparatus using a differential pressure transducer.[10]

At first we investigated the sparing effect on α-tocopherol (α-TOH) of 1,9-dimethylphenothiazine (MPTZ), which is a coantioxidant characterized by a bond dissociation energy (BDE) at the N−H bond (77.7 kcal/mol)[11] very close to that measured at the O−H bond of TOH (78.2 kcal/mol),[12] although much less reactive than α-tocopherol toward peroxyl radicals because of the steric crowding about the N−H group due to the presence of the methyl substituents in positions 1 and 9.



MPTZ



α-TOH

The rate of oxygen consumption determined during the initiated autoxidation of styrene in PhCl in the presence of α-TOH (5.0×10^{-5} M) and MPTZ (1.05×10^{-4} M), i.e., a concentration twice as large as that of α-TOH and equal concentrations of both antioxidants, is plotted in Figure 1.

It appears that the autoxidation reaction is strongly inhibited by α-TOH (trace a) for an initial period (τ_{AH})

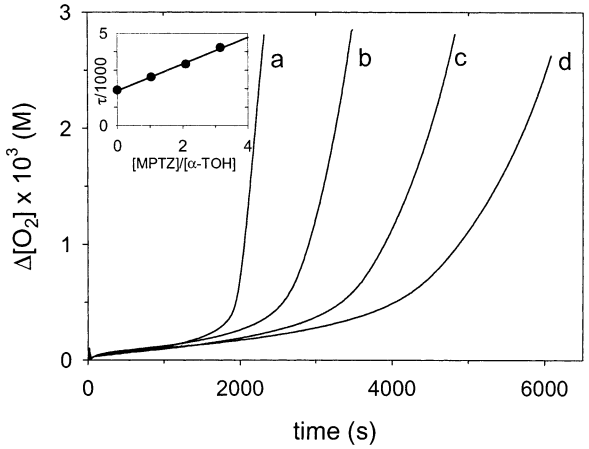


FIGURE 2. Oxygen consumption traces observed during the AMVN (0.023 M) initiated autoxidation at 30 °C of styrene (7.5 M) in chlorobenzene in the presence of 2.5×10^{-5} M α-TOH and 1,9-dimethylphenothiazine (MPTZ) at concentrations of (a) 0 M, (b) 2.6×10^{-5} M, (c) 5.25×10^{-5} M, and (d) 7.9×10^{-5} M. Insert reports the length of the induction period (see text) as a function of the MPTZ concentration.

after which the slope of the oxygen uptake trace increases suddenly reaching the same value observed in the absence of any antioxidant (not shown) and indicating that α-TOH has been completely consumed. With MPTZ, (trace b) no clear induction period can be observed so that the methylated phenothiazine behaves as a retardant[13] rather than as an inhibitor. The mixture α-TOH/MPTZ, in a 1:2 ratio (trace c), inhibits the autoxidation for a period ($\tau_{AH + CoAH}$), which is about twice as long as that observed with only α-TOH, and then retards the oxygen consumption for some more time.

The plots of Figure 1 can be interpreted in terms of partial regeneration of α-tocopherol by the methylated phenothiazine, so that the period where the autoxidation is strongly inhibited is longer than that observed in the presence of α-TOH alone. Since, in the absence of a coantioxidant, each molecule of α-TOH as well as of MPTZ[11] is known to trap approximately two peroxyl radicals and since the length of the induction period is nearly doubled by the 1:2 mixture, about one-half of the MPTZ molecules are capable of regenerating α-tocopherol under the present conditions. This conclusion is further supported by the plots of Figure 2 showing the oxygen uptake traces observed when inhibiting the autoxidation of styrene with α-TOH and with 1:1, 1:2, and 1:3 α-TOH/MPTZ mixtures. The insert shows that the induction period increases linearly with the phenothiazine concentration, the slope being consistent with a regeneration of about 40%. It is also important to point out that the retarding effect at the end of the induction period increases with the MPTZ concentration, this indicating that after α-TOH is completely consumed, a remarkable amount of the coantioxidant is still present in solution.

These results can be expressed quantitatively by means of eq 1, which gives the stoichiometric factor n_{CoAH} of the coantioxidant in the mixture with AH. The value of n_{CoAH} obtained for MPTZ from the data reported in

(10) Lucarini, M.; Pedulli, G. F.; Valgimigli, L.; Amorati, R.; Minisci, F. *J. Org. Chem.* **2001**, *66*, 5456−5462.

(11) Lucarini, M.; Pedrielli, P.; Pedulli, G. F.; Valgimigli, L.; Gigmes, D.; Tordo, P. *J. Am. Chem. Soc.* **1999**, *121*, 11546−11553.

(12) (a) Lucarini, M.; Pedrielli, P.; Pedulli, G. F.; Cabiddu, S.; Fattuoni, C. *J. Org. Chem.* **1996**, *61*, 9259. (b) Lucarini, M.; Pedulli, G. F.; Cipollone, M. *J. Org. Chem.* **1994**, *59*, 5063.

(13) Waters, W. A.; Wickham-Jones, C. T. *J. Chem. Soc.* **1951**, *51*, 812. Howard, J. A.; Ingold, K. U. *Can. J. Chem.* **1964**, *42*, 2324.

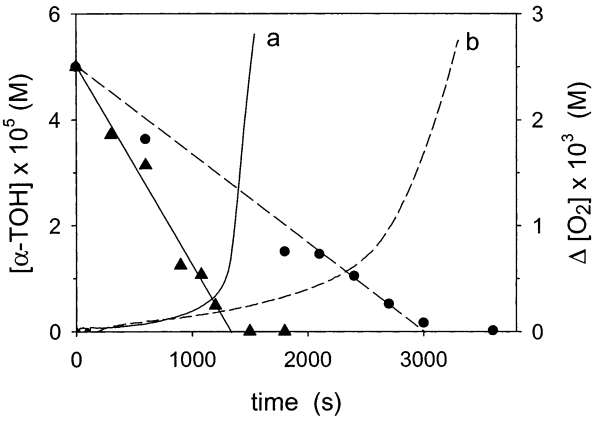


FIGURE 3. α-Tocopherol (α-TOH) consumptions observed at 60 °C during the thermal decomposition of 5×10^{-3} M AIBN in chlorobenzene in the presence of (▲) 5×10^{-5} M α-TOH and (●) 5×10^{-5} M α-TOH and 1×10^{-4} M 1,9-dimethylphenothiazine. Plot shows also the oxygen uptake traces obtained under similar conditions except for the presence of 4.3 M styrene added to amplify the oxygen consumption after the end of the induction period (traces a and b refer to the experiments carried out in the presence of only α-TOH and the mixture α-TOH/MPTZ, respectively).

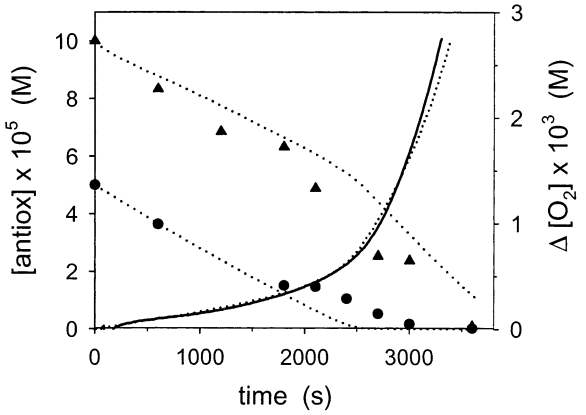


FIGURE 4. α-Tocopherol (●) and 1,9-dimethylphenothiazine (▲) consumptions observed at 60 °C during the thermal decomposition of 5×10^{-3} M AIBN in chlorobenzene in the presence of 5×10^{-5} M α-TOH and 1×10^{-4} M MPTZ. The oxygen (solid line) trace has been obtained in the presence of 4.3 M styrene under otherwise identical conditions. Dotted lines represent computer simulations (see text).

Figure 2 is approximately 0.76, i.e., much smaller than the stoichiometric factor of α-TOH ($n_{AH} = 2$) and that of MPTZ alone ($n_{AH} = 1.7$) measured by studying the inhibited autoxidation of cumene.[11]

$$n_{CoAH} = R_i (\tau_{AH + CoAH} - \tau_{AH}) / [CoAH] \qquad (1)$$

The occurrence of partial regeneration of α-tocopherol by MPTZ is also supported by the time dependence of the concentrations of the two antioxidants, measured by HPLC-MS using electrospray ionization (ESI-MS), during the thermal decomposition of AIBN at 60 °C (Figure 3), i.e., under experimental conditions similar to those used when carrying out the autoxidation reactions. In these experiments, no styrene was introduced to avoid interference with the phenol analytes in the ESI-MS analysis due to matrix effects, and the temperature was kept higher than 30 °C to accelerate the decomposition of the azo initiator. Parallel autoxidation experiments performed in the presence of 4.3 M styrene (see Figure 3) show induction periods identical to those obtained from the disappearance of α-TOH. The plots obtained in the presence of both antioxidants indicate that the lengthening of the induction period is due to a slower net consumption of α-tocopherol. From the data of Figures 4, which report also the disappearance of the coantioxidant MPTZ, it appears that both antioxidants are consumed at similar rates and that when α-TOH has reacted completely, about 50% of MPTZ is still present, this being the reason for the retarding effect observed in the oxygen consumption traces after the end of the induction period.

As already pointed out in the Introduction, other important examples of coantioxidants showing a recycling effect toward α-tocopherol are those of flavonoids, a class of natural compounds found in nearly every plant, whose antioxidant properties are mostly due to the presence in their structure of a catechol ring and which are almost invariably accompanied by some vitamin E. With the aim of better understanding the synergistic behavior of flavonoids/vitamin E blends, we have investigated the initiated autoxidation of styrene inhibited by antioxidant mixtures containing α-tocopherol and, as a model compound for flavonoids, 4-*tert*-butylcatechol (BC).

Figure 5 shows the effect on the oxygen uptake traces of the addition to the reaction medium of α-TOH (5×10^{-5} M), BC (1×10^{-4} M), and both α-TOH (5×10^{-5} M) and BC (5×10^{-5}, 1×10^{-4}, and 1.5×10^{-4} M). It appears from the dashed trace that the antioxidant activity of BC is not as good as that of α-TOH; actually, the inhibition rate constant k'_{inh} has been measured in the present work as 5.6×10^5 M^{-1} s^{-1} from inhibited autoxidation studies of styrene in chlorobenzene at 30 °C. This value is six times lower than that of α-tocopherol (3.2×10^6 M^{-1} s^{-1}) under the same experimental conditions. However, when using the two inhibitors together, the antioxidant activity of the mixtures is much better than expected on the basis of a purely additive effect, since the rate of oxidation during the induction period is the same as the inhibited rate observed when only vitamin E was used. This behavior indicates that some vitamin E is still present in the system until the end of the induction period and, thus, that α-TOH is recycled by 4-*tert*-butylcatechol during the autoxidation reaction.

We also measured by HPLC-ESI-MS the rate of consumption of both α-TOH and 4-*tert*-butylcatechol observed during the thermally induced decomposition of AIBN under air (in the absence of styrene, see above). The results, reported in Figure 5, show that the first antioxidant to be consumed is the catechol derivative even though its reaction with peroxyl radicals is 6 times slower than that of α-tocopherol. Only after the coantioxidant has been completely consumed is the disappearance of α-tocopherol observed; thus, at the end of the induction period, no coantioxidant is present anymore in the reaction mixture. Therefore, unlike the system α-TOH/MPTZ, with BC, no additional retarding of the autoxidation seems to be observed at the end of the induction period $\tau_{AH + CoAH}$.

Mechanism of Regeneration. To explain the experimental results, the mechanism of the inhibited autoxi-

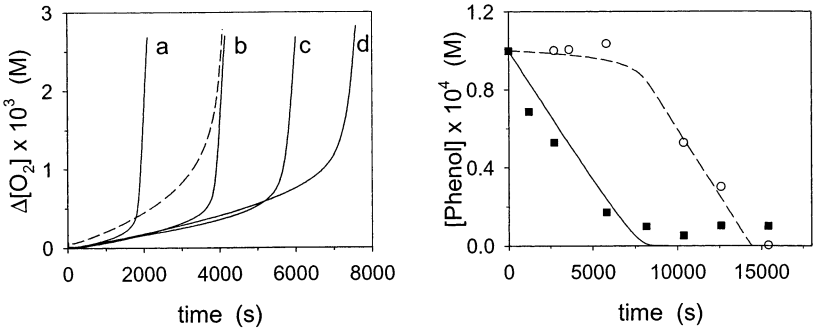


FIGURE 5. (Left) Oxygen consumption during the AMVN (0.046 M) initiated autoxidation at 30 °C of styrene (7.5 M) in chlorobenzene in the presence of 1×10^{-4} M 4-*tert*-butylcatechol (BC) (dashed line). Solid lines have been recorded in the presence of 5×10^{-5} M α-TOH and BC concentrations of (a) 0 M, (b) 5×10^{-5} M, (c) 1×10^{-4} M, and (d) 1.5×10^{-4} M. (Right) Disappearance of α-TOH (○) and BC (■) observed during the thermal (60 °C) decomposition under air of AIBN (0.002 M) in chlorobenzene containing both antioxidants at initial concentrations of 1×10^{-4} M. Full and dashed lines represent computer simulations (see text).

dation of an hydrocarbon, RH, should be considered. The overall reaction scheme is given by the following series of equations, where eqs 2 and 3 represent the initiation and eqs 4 and 5 the propagation, and eq 6 represents the termination steps.[14]

$$\xrightarrow{R_i} \mathrm{InOO^\bullet} \quad (2)$$

$$\mathrm{InOO^\bullet + RH \rightarrow InOOH + R^\bullet} \quad (3)$$

$$\mathrm{R^\bullet + O_2 \rightarrow ROO^\bullet} \quad (4)$$

$$\mathrm{ROO^\bullet + RH \xrightarrow{k_p} ROOH + R^\bullet} \quad (5)$$

$$\mathrm{2ROO^\bullet \xrightarrow{2k_t} products} \quad (6)$$

In the presence of a chain-breaking antioxidant, AH, such as α-tocopherol and a coantioxidant CoAH, chain-propagating peroxyl radicals can be trapped by either or by both of them (eqs 7, 8) depending on the relative rate constants k_{inh} and k'_{inh}.

$$\mathrm{ROO^\bullet + AH \xrightarrow{k_{inh}} ROOH + A^\bullet} \quad (7)$$

$$\mathrm{ROO^\bullet + CoAH \xrightarrow{k'_{inh}} ROOH + CoA^\bullet} \quad (8)$$

$$\mathrm{A^\bullet + CoAH \underset{k_{-r}}{\overset{k_r}{\rightleftharpoons}} AH + CoA^\bullet} \quad (9)$$

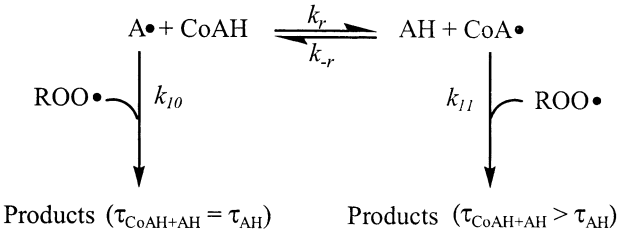
$$\mathrm{ROO^\bullet + A^\bullet \rightarrow products} \quad (10)$$

$$\mathrm{ROO^\bullet + CoA^\bullet \rightarrow products} \quad (11)$$

The resulting α-tocopheroxyl radical can react with a molecule of the coantioxidant to regenerate α-tocopherol (eq 9) with a rate constant k_r or react with a second peroxyl radical to give termination products (eq 10). Similarly, the radical from the coantioxidant, CoA•, can either give the hydrogen exchange reaction with α-TOH (eq −9) or terminate with ROO• (eq 11).

In the absence of the hydrogen atom exchange reaction (eq 9), α-tocopherol and the coantioxidant would be consumed with rates only depending on the k_{inh}/k'_{inh} ratio and on the relative concentrations of the two species, so that the inhibition effect of the mixture would be purely additive without any synergism between the two antioxidants. If, however, hydrogen exchange takes place at a rate larger or comparable to that of the termination reaction (eq 10) leading to the disappearance of the α-tocopheroxyl radicals, the latter ones will abstract a hydrogen atom from the coantioxidant, thus regenerating α-TOH. As a result, α-tocopherol will be consumed at a lower rate, while the coantioxidant will disappear at a rate higher than predicted on the basis of the inhibition rate constants k_{inh} and k'_{inh}. At the same time, the oxygen uptake will show an inhibition period longer than expected on the basis of the initial α-tocopherol concentration, followed by a second period where the autoxidation is retarded by the residual coantioxidant. The apparent stoichiometric factor *n* of α-tocopherol will appear larger than 2, its value depending on the relative rates of reactions (reactions 8−11). The overall behavior can be conveniently visualized on the basis of the simplified Scheme 1.

SCHEME 1



In practice, regeneration of α-tocopherol by a coantioxidant can only be observed under the experimental conditions where the rate of reaction 9 is larger or at least comparable to that of reaction 10. If the regeneration reaction is irreversible, i.e., $k_{-r} \ll k_r$ or $k_{11}[\mathrm{ROO^\bullet}] \gg k_{-r}[\mathrm{AH}]$, this condition can be simply expressed by eq 12 or 13.

$$k_{10}[\mathrm{ROO^\bullet}][\mathrm{A^\bullet}] \leq k_r[\mathrm{CoAH}][\mathrm{A^\bullet}] \quad (12)$$

$$[\mathrm{CoAH}] \geq (k_{10}/k_r)\,[\mathrm{ROO^\bullet}] \quad (13)$$

This means that recycling of vitamin E will be more easily observed at large coantioxidant concentrations or,

(14) Denisov, E. T.; Khudyakov, I. V. *Chem. Rev.* **1987**, *87*, 1313. Howard, J. A. In *Free Radicals*; Kochi, J. K., Ed.; Wiley & Sons: New York, 1973; Vol. 2, pp 3−62.

since $[ROO^{\bullet}] = R_i/(2k_{inh}[AH] + 2k'_{inh}[CoAH]) \cong R_i/2k_{inh}[AH]$, at low rates of initiation and at high α-TOH content.

α-*Tocopherol/Vitamin C.* A coantioxidant for which the condition $k_{-r} \ll k_r$ holds is vitamin C. Actually, the pertinent rate constants measured in *tert*-butyl alcohol are $k_{inh} = 5.6 \times 10^5$ M^{-1} s^{-1} (eq 7; AH = TOH)[15] and $k'_{inh} = 7.5 \times 10^4$ M^{-1} s^{-1} (eq 8; CoAH = vitamin C).[2] Despite the absence of experimental data for the rate constant k_{-r} of the reverse reaction (eq −9), this can be evaluated from the free energy change for the reaction of the α-tocopheroxyl radical with ascorbate to regenerate vitamin E[16] that has been estimated as −5.0 kcal/mol, on the basis of the standard one-electron reduction potentials of the involved species[17] and calculated as −7.7 kcal/mol in a recent theoretical paper.[18] Thus, the value of k_{-r} must be about 4−5 orders of magnitude lower than $k_r = 1.55 \times 10^6$ M^{-1} s^{-1} (eq 9; AH = TO$^{\bullet}$ and CoAH = vitamin C),[19] reaction 9 can be considered to be practically irreversible, and eq 12 should hold. To check if the condition expressed by eq 13 is also fulfilled, the rate constant k_{10} for the combination of peroxyl with α-tocopheroxyl radicals should be known. Since most of the reported kinetic constants for the reaction of peroxyl and phenoxyl radicals are of the order of 10^8 M^{-1} s^{-1},[20−22] we may assume for k_{10} the reasonable value of 1×10^8 M^{-1} s^{-1}. From the rate of initiation reported by Niki et al.,[2] $R_i = 2.58 \times 10^{-8}$ M s^{-1} at 37 °C, the minimum concentration of the coantioxidant required to observe synergism can be calculated as 3×10^{-8} M, a value much smaller than the vitamin C concentration of 5.5×10^{-5} M used in these regeneration experiments.[2]

Thus, on the basis of these data it is expected that, when α-TOH and vitamin C are present in solution in similar amounts, the peroxyl radicals will react first with the former to give an α-tocopheroxyl radical that immediately reacts with vitamin C to regenerate α-TOH. This is in complete agreement with the behavior reported in homogeneous solution by Niki et al.,[2] who observed complete disappearance of vitamin C before α-tocopherol started to be consumed and 100% regeneration of the latter antioxidant.

α-*Tocopherol/1,9-Dimethylphenothiazine*. In the case of the mixture α-tocopherol/MPTZ, the pertinent rate constants of the various reactions involved are $k_{inh} = 3.2 \times 10^6$ M^{-1} s^{-1} for α-TOH[15,23] at 30 °C and $k'_{inh} = 1.4 \times 10^5$ M^{-1} s^{-1} for MPTZ at 50 °C.[11] Thus, in the absence of the hydrogen atom exchange reaction (eq 9), since the ratio $k_{inh}/k'_{inh} \cong 23$ and [AH]/[CoAH] = 0.5, α-tocopherol should be consumed 12.5 times faster than MPTZ at the beginning of the oxidation reaction. If, however, hydrogen exchange takes place at a rate greater than or comparable to that of the termination reaction 10, α-tocopherol will be regenerated by MPTZ and the mixture of the two antioxidants will show synergism similar to that observed when vitamin C is the coantioxidant. To check this condition, the values $R_i = 5.6 \times 10^{-8}$ M s^{-1} and $[ROO^{\bullet}] = 1.75 \times 10^{-10}$ M were extracted from the plots of Figure 1, while k_r and k_{-r} were determined by EPR, as described in the following section, as 1.5×10^3 M^{-1} s^{-1} and 6.5×10^2 M^{-1} s^{-1}, respectively, at 25 °C in benzene solution. The minimum concentration of the coantioxidant, $(k_{10}/k_r)[ROO^{\bullet}]$, required to observe some regeneration according to eq 12, is 1.2×10^{-5} M, i.e., a value much lower than the experimental concentration (1.0×10^{-4} M) of MPTZ employed. Therefore, although the absolute rate constant for hydrogen transfer from 1,9-dimethylphenothiazine to α-TO$^{\bullet}$, k_r, is much smaller than that with ascorbate, partial regeneration of α-tocopherol can take place.

However, there is an important difference with respect to vitamin C, since the hydrogen exchange reaction (eq 9) with MPTZ is almost thermoneutral and thus reversible ($\Delta G_r^{\circ} = -0.5$ kcal/mol at 298 K from the known N−H[11] and O−H[12] BDE values in MPTZ and α-TOH). If the hydrogen exchange reaction (eq 9) is faster than the termination reactions 10 and 11, the concentrations of the two antioxidants AH and CoAH and those of the corresponding radicals are determined by the equilibrium constant K_r (eq 14).

$$K_r = k_r/k_{-r} = \frac{[AH][CoA^{\bullet}]}{[A^{\bullet}][CoAH]} \qquad (14)$$

From the simultaneous equations describing the evolution of the species appearing in eqs 2−11, the rate laws for the disappearance of α-tocopherol, coantioxidant, and oxygen (in a closed system) can be straightforwardly derived under the steady-state approximation for the radical species ROO$^{\bullet}$, A$^{\bullet}$, and CoA$^{\bullet}$ and are given by eqs 15−17.

$$-\frac{d[AH]}{dt} = \frac{R_i}{2\left(1 + \frac{K_r k_{11}[CoAH]}{k_{10}[AH]}\right)} \qquad (15)$$

$$-\frac{d[CoAH]}{dt} = \frac{R_i}{2\left(1 + \frac{k_{10}[AH]}{K_r k_{11}[CoAH]}\right)} \qquad (16)$$

$$-\frac{d[O_2]}{dt} = R_i\left[1 + \frac{k_p[RH]}{2(k_{inh}[AH] + k'_{inh}[CoAH])}\right] \qquad (17)$$

From the last equation it can be inferred that the oxygen consumption plots during the inhibition period should display a slope slightly lower than that observed in the presence of only α-tocopherol, which depends on the value of k'_{inh} and on the concentration of CoAH. Equation 15 can be used to obtain the induction time, τ, for the AH and CoAH mixture, which is the time at which a sudden change of slope will be observed in the oxygen

(15) Valgimigli, L.; Banks, J. T.; Lusztyk, J.; Ingold, K. U. *J. Org. Chem.* **1999**, *64*, 3381−3383.
(16) Although reaction 9 could take place via an initial electron transfer followed by proton transfer, a recent computational study suggests the direct hydrogen transfer mechanism as a more likely pathway.[17]
(17) Buettner, G. R. *Archiv. Biochem. Biophys.* **1993**, *300*, 535−543.
(18) DiLabio, G. A.; Wright, J. S. *Free Rad. Biol. Med.* **2000**, *29*, 480−485.
(19) Packer, J. E.; Slater, T. F.; Willson, R. L. *Nature* **1979**, *278*, 737−738.
(20) Griva, A. P.; Denisov, E. T. *Int. J. Chem. Kinet.* **1973**, *5*, 869.
(21) Mahoney, L. R.; DaRooge, M. A. *J. Am. Chem. Soc.* **1975**, *97*, 4722.
(22) Bowry, V. W.; Ingold, K. U. *J. Org. Chem.* **1995**, *60*, 5456−5467.
(23) Burton, G. W.; Doba, T.; Gabe, E. J.; Hughes, L.; Lee, F. L.; Prasad, L.; Ingold, K. U. *J. Am. Chem. Soc.* **1985**, *107*, 7053−7065.

consumption plots. Since this will occur when α-tocopherol is completely consumed, $\tau = \tau_{AH} + \tau_{CoAH}$ will be given by eq 18.

$$\tau = \frac{[AH]}{-d[AH]/dt} = \frac{2[AH]}{R_i}\left(1 + \frac{K_r k_{11}[CoAH]}{k_{10}[AH]}\right) \quad (18)$$

Therefore, under equilibrium conditions for the hydrogen exchange reaction 9, the plot of the measured induction period as a function of the concentration ratio of the two antioxidants [CoAH]/[AH] should be linear with a slope α equal to $K_r k_{11}/k_{10}$. On the basis of the definition of the stoichiometric coefficient n_{CoAH} of the coantioxidant in the mixture, given in eq 1, it can be easily shown that for coantioxidants capable of scavenging two peroxyl radicals such as phenols and aromatic amines, eq 19 holds.

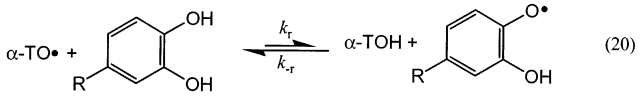
$$n_{CoAH} = 2\,\alpha \quad (19)$$

In the case of a coantioxidant such as vitamin C, which can scavenge only one peroxyl radical, eq 19 becomes $n_{CoAH} = \alpha$, where α represents in both cases a measure of the effectiveness of the regeneration process.

Good linearity, with slope $\alpha = 0.38$, has actually been observed (see the insert of Figure 2) when plotting the induction times measured during the initiated autoxidation of styrene against the ratio [1,9-dimethylphenothiazine]/[α-tocopherol]. Since the equilibrium constant for reaction 9 is 2.3 for the couple AH = α-TOH and CoAH = MPTZ, the plot provides also the ratio k_{11}/k_{10} as 0.17. Thus, the reaction of peroxyl with α-tocopheroxyl radicals is ca. 6 times faster than that with dimethylphenothiazinyl radicals. To check whether the k_{11} value obtained by α is reasonable, we tried to fit the oxygen and antioxidants consumption traces obtained in the experiments carried out in the presence of the MPTZ/α-TOH mixtures by using a method of chemical reaction kinetics simulation (see experimental). These simulations are shown in Figure 4 as dotted lines for the traces of the calculated time dependence of the concentrations of α-tocopherol, 1,9-dimethylphenothiazine, and oxygen, superimposed to the experimental measurements. The good agreement with the experimental results indicates that the assumed kinetic model is satisfactory.

α-*Tocopherol/4-tert-Butylcatechol (BC).* The mixture α-TOH/BC provides another example of regeneration of vitamin E through a mechanism characterized by some important differences with respect to the previous ones. On the basis of the known values of the rate constants $k_{inh} = 3.2 \times 10^6$ M^{-1} s^{-1} for α-TOH and $k'_{inh} = 5.6 \times 10^5$ M^{-1} s^{-1} for BC (vide infra) and of the estimated value of the equilibrium constant K_r for the hydrogen transfer reaction between the substituted catechol and α-tocopheroxyl radicals, the regeneration factor α should be negligibly small, as it is shown below, while experimentally it is found to be close to 1 (i.e., 100% regeneration). To evaluate K_r we have used the recently measured O−H BDE value[24] of 3,5-di-*tert*-butylcatechol (79.3 kcal/mol), which differs from 4-*tert*-butylcatechol only for the presence of an additional *tert*-butyl group. Being the substituent effect of this group −1.75 kcal/mol,[25] the BDE value for BC can be calculated as 81.1 kcal/mol; thus, the free energy change for reaction 9 (with CoAH = BC) and its equilibrium constant K_r at 303 K can be estimated as 2.9 kcal/mol and 8.1×10^{-3}, respectively. Assuming that the rate constants for the termination with peroxyl radicals of α-TO• and of the semiquinone radical from the catechol derivative are the same ($k_{10} \cong k_{11}$), the regeneration factor α should be equal to K_r. Since the calculated value of K_r is too small to justify the observed complete recycling of α-TOH by 4-*tert*-butylcatechol (α = 1), some other reaction not considered so far must be taken into account in the system α-TOH/BC. This conclusion is supported by the observation that no regeneration at all was found[27] when investigating the antioxidant activity of a 1:1 mixture of α-TOH and a 2,4,6-tri-*tert*-butylphenol having nearly the same BDE (81.2 kcal/mol)[26] and K_r values of BC.

The complete recycling of α-TOH by catechol derivatives can be explained in terms of the two-step mechanism, exemplified in eqs 20 and 21, similar to that previously suggested in the case of the reduced form of ubiquinone Q_3, which is also able to recycle vitamin E.[5] In the first step (the analogue of reaction 9), reversible hydrogen transfer takes place between BC and α-TO• to give a semiquinone radical, while in the second step (eq 21) the resulting semiquinone reacts with another α-tocopheroxyl radical to give α-TOH and 4-*tert*-butyl-*ortho*-quinone.[28]



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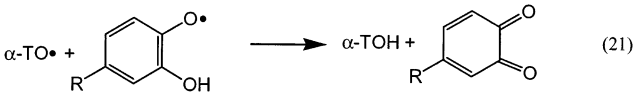

(21)

On the basis of the known heat of formation ΔH_f° of catechol (−63.9 kcal/mol)[30] and that calculated for *ortho*-quinone (−30.0 kcal/mol) by using the Benson additivity rules[31] and the estimated O−H BDE value in catechol (83.0 kcal/mol),[24] the heats of reactions 20 and 21, with

(24) Lucarini, M.; Mugnaini, V.; Pedulli, G. F. *J. Org. Chem.* **2002**, *67*, 928−931.

(25) Lucarini, M.; Pedrielli, P.; Pedulli, G. F. In *Free Radicals in Chemistry, Biology and Environment*; Minisci, F., Ed.; Kluwer Academic Publishers: Norwell, MA, 1997; pp 169−179.

(26) Mahoney, L. R.; Ferris, F. C.; DaRooge, M. A. *J. Am. Chem. Soc.* **1969**, *91*, 3883−3889.

(27) Amorati, R.; Calabrese, V.; Lucarini, M.; Pedulli, G. F., Valgimigli, L. Results to be published.

(28) Evidence for the formation of 4-*tert*-butylquinone (4-BQ) as the reaction product of the regeneration of α-tocopherol by 4-*tert*-butylcatchol was obtained by analyzing aliquots of a typical autoxidation mixture (inhibited by 1×10^{-4} M α-TOH and 1×10^{-4} M BC) by a specific spectrophotometric assay recently developed by some of us[29] based on the reaction of 4-BQ with ADA (4-diethylaminoaniline) to yield an intensely blue adduct ($\lambda_{max} = 625$ nm, $\epsilon = 11\,120$ $M^{-1}cm^{-1}$ in CH_2Cl_2). By this assay, an amount of 4-BQ of about 10% the value expected from complete conversion of BC was observed toward the end of the inhibited period. The lack of quantitative recovery of 4-BQ will be a matter of further work.

(29) Rescigno, A.; Sanjust, E.; Pedulli, G. F.; Valgimigli, L. *Anal. Lett.* **1999**, *32*, 2007−2017. Valgimigli, L.; Pedulli, G. F.; Cabiddu, S.; Sanjust, E.; Rescigno, A. *Tetrahedron* **2000**, *56*, 659−662.

(30) Ribeiro da Silva, M. D. M. C.; Ribeiro da Silva, M. A. V. *J. Chem. Thermodynam.* **1984**, *16*, 1149−1155.

(31) Estimated on the basis of the Benson additivity rules[32] assuming that CO − (C_d)(CO) = CO − (C_B)(CO).

R = H, are calculated to be 4.8 and −23.1 kcal/mol, respectively. Given its high exothermicity (due to the very low O−H bond strength in the semiquinone radical, ca. 55 kcal/mol), reaction 21 is expected to be fast enough to favorably compete with termination reaction 11. Therefore, the semiquinone radicals produced by reaction with either peroxyl radicals (eq 8) or α-tocopheroxyl radicals (eq 20) will be continuously subtracted from the reaction medium making the regeneration of α-TOH much more efficient than predicted on the basis of the K_r value. This mechanism can explain the experimental results shown in Figure 5 reporting the disappearance of α-TOH and 4-*tert*-butylcatechol during the thermal decomposition of AIBN in chlorobenzene.

To estimate the unknown rates of the reactions involved we have simulated the time dependence of the antioxidant concentrations of Figure 5. To do so, we first determined by EPR the rate constant for the hydrogen transfer from 4-*tert*-butylcatechol to α-tocopheroxyl radicals in benzene at 298 K (vide infra). By assuming that reaction 20 is the rate-limiting step of the sequence described by eqs 20 and 21, we obtained $k_r = (2.8 \pm 1.2) \times 10^3$ M^{-1} s^{-1}. From the calculated equilibrium constant $K_r = 8.1 \times 10^{-3}$, the value of k_{-r} is obtained as 3.5×10^5 M^{-1} s^{-1}.

Simulations (see Figure 5) were performed using the same method previously described by leaving unchanged the experimentally determined rate constants as well as $k_{10} = 1 \times 10^8$ M^{-1} s^{-1} as in the previous simulations. The two remaining rate constants k_{11} and k_{21} were instead allowed to vary on a wide range of values, i.e., $1 \times 10^8 \leq k_{11} \leq 1 \times 10^{10}$ M^{-1} s^{-1} and $1 \times 10^5 \leq k_{21} \leq 5 \times 10^9$ M^{-1} s^{-1}.

For any value of k_{21}, the simulations were found totally independent of the assumed rate constant k_{11} up to 1×10^{10} M^{-1} s^{-1}; we therefore chose for it the reasonable value of 1×10^8 M^{-1} s^{-1}. On the other hand, to get good agreement with the experimental data concerning the consumption of BC, α-TOH, and oxygen, the rate constant k_{21} should be at least 5×10^7 M^{-1} s^{-1}. Such a high value seems to be quite reasonable in view of the strong exothermicity of reaction 21 and also by comparison with the rate constants for the similar dismutation reactions of catechol (3.9×10^8 M^{-1} s^{-1})[33] and of 2,6-dimethylhydroquinone (3.0×10^8 M^{-1} s^{-1}).[34] It can be therefore concluded that reaction 21, being so fast to overwhelm reaction −20, i.e., the hydrogen transfer from α-TOH to the semiquinone radical, represents the driving force of the regeneration process that makes the recycling of α-tocopherol practically irreversible under typical autoxidation conditions.[35] Since the regeneration of α-TOH is likely to be essentially irreversible with any catechol derivative, with this class of coantioxidants the regeneration factor α should depend only on the ratio k_r/k_{10}; in other words, if the regeneration of α-tocopherol by a substituted catechol is not complete, this should only depend on the low value of the rate constant k_r for hydrogen atom transfer.

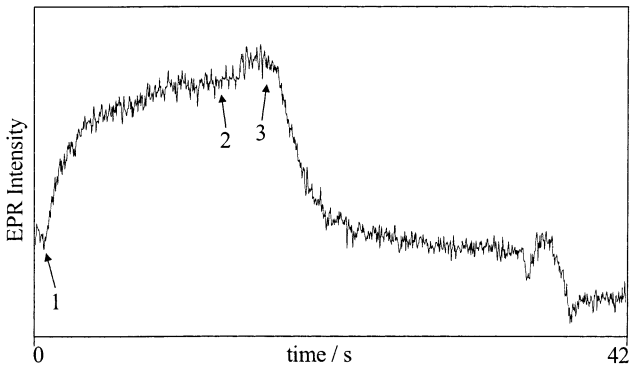

FIGURE 6. Time evolution of the EPR signal due to the aminyl radical generated at 298 K by UV irradiation of a benzene solution of 1,9-dimethylphenothiazine (0.001 M) containing ^{t}BuOO^{t}Bu (0.1 M). Irradiation was started at time 1 and stopped at time 2; α-tocopherol was injected at time 3. No radical decay was observed, in the absence of α-TOH, during the time of the experiment.

Rate Constants for the Regeneration of α-Tocopherol. To assess the feasibility of the regeneration reaction of α-tocopherol by coantioxidants (eq 9), the knowledge of the rate constants for hydrogen atom transfer from the coantioxidant to α-TO$^\bullet$ (k_r) is essential. We therefore carried out the determination of k_r in benzene solution at room temperature, by the kinetic EPR method, for several coantioxidants.

In the case of 1,9-dimethylphenothiazine, the reverse rate constant k_{-r} was measured for convenience, i.e., the rate for hydrogen atom transfer from α-tocopherol to the aminyl radical of MPTZ; then, k_r was calculated from the equilibrium constant K_r obtained from the known ΔBDE difference. The method consisted of monitoring the decay of a spectral line of the very persistent 1,9-dimethylphenothiazinyl radical, not superimposed onto that of α-TO$^\bullet$, after the instantaneous injection of a concentrated stock solution of α-tocopherol (final concentration of ca. 1 mM) in the sample tube, while continuously bubbling nitrogen (see Figure 6). Under the experimental conditions employed, the EPR signal completely decayed in approximately 5−10 s after the injection of α-tocopherol, following good pseudo-first-order kinetics. The measured rate constant k_{-r} was obtained as 6.5×10^2 M^{-1} s^{-1} from which the value $k_r = 1.5 \times 10^3$ M^{-1} s^{-1} in benzene at 298 K was calculated using the difference of 0.5 kcal/mol between the BDEs of α-TOH and MPTZ.[11,12]

Since the value for k_{-r} is close to the known rate constant for the bimolecular self-decay of α-tocopheroxyl radicals in benzene at the same temperature,[12b] the observed kinetics might be due to the occurrence of fast hydrogen transfer between α-tocopherol and the aminyl radical followed by the spontaneous decay of the resulting α-tocopheroxyl radical. To rule out this possibility, the rate constants of hydrogen transfer k_H (eq 22) to the aminyl radical from MPTZ from a series of substituted phenols having the same steric hindrance around the reaction center as α-TOH (i.e., two methyl groups *ortho* to the OH) have been measured and correlated with the O−H BDE values of the phenols. Experiments were

(32) Benson, S. W. *Thermochemical Kinetics*; Wiley & Sons: New York, 1968.
(33) Khudiakov, I. V.; Kuzmin, V. A.; Emanuel, N. M. *Int. J. Chem. Kinet.* **1978**, *10*, 1005.
(34) Foster, T.; Elliot, A. J.; Adeleke, B. B.; Wan, J. K. S. *Can. J. Chem.* **1978**, *56*, 869.
(35) Simulations were also done by assuming that dismutation of the semiquinone radical from BC could take place. No effect was found on the simulated traces by varying the rate constant in the range $0 \leq k_{dis} \leq 5 \times 10^9$ M^{-1} s^{-1}.

TABLE 1. Rate Constants k_H for the Hydrogen Transfer Reaction (eq 22) between *ortho*-Dimethyl Phenols and the 1,9-Dimethylphenothiazinyl Radical, in Benzene Solution at 298 K[a]

	k_H, (M^{-1} s^{-1})	BDE(O−H), (kcal/mol)	k_{-H} (M^{-1} s^{-1})
2,6-Me_2-C_6H_3OH	0.08	84.5[b]	6.4×10^3
2,4,6-Me_3-C_6H_2OH	0.70	82.7[b]	2.8×10^3
C_6Me_5OH	3.60	81.8[c]	3.2×10^3
α-TOH	650	78.2[b]	1.5×10^3
MPTZ	-	77.7[d]	

[a] O−H Bond Dissociation Enthalpies of the phenols and calculated rate constants k_{-H} of the reverse reactions are also reported. [b] From ref 12. [c] From the present work. [d] From ref 11.

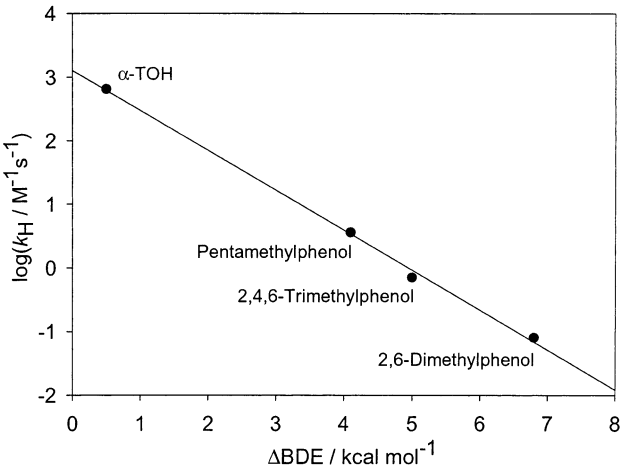

FIGURE 7. Relation between the logarithm of the rate constants k_H for the hydrogen transfer reaction from phenols to the 1,9-dimethylphenothiazinyl radical and the difference between the strengths of the O−H bond of phenols and the N−H bond of MPTZ (ΔBDE).

performed on solutions of both antioxidants by using high phenol concentrations because of the large BDE difference with MPTZ. The measured rate constants are reported in Table 1 together with the pertinent BDE values.

$$ArOH + Ar_2N^{\bullet} \underset{k_{-H}}{\overset{k_H}{\rightleftharpoons}} ArO^{\bullet} + Ar_2NH \qquad (22)$$

The O−H bond dissociation enthalpy for pentamethylphenol, previously unknown, was measured here from equilibration experiments as 81.8 ± 0.4 kcal/mol.[36]

As shown in Figure 7, for all the investigated phenols (*including* α-tocopherol), the correlation between log k_r and the difference between the BDE values of phenols and that of 1,9-dimethylphenothiazine (BDEOH − BDENH) is excellent. This guarantees that the measured rate constants actually pertain to the same chemical reaction, i.e., to the hydrogen atom transfer of eq 9.

The rate constant for the hydrogen transfer between 4-*tert*-butylcatechol and the α-tocopheroxyl radical (eq 20, k_r) was similarly measured in benzene by kinetic EPR using identical procedures and directly monitoring the decay of the EPR signal of the photolytically generated α-TO$^{\bullet}$ after the injection of a concentrated stock solution of BC (0.25−1 mM). Since, unlike the aminyl radicals from MPTZ, α-tocopheroxyl radicals undergo bimolecular self-decay in the time scale of the reaction with BC, care had to be taken to set the initial concentration of α-TO$^{\bullet}$ such that the bimolecular self-decay was negligible under our experimental conditions (less than 5% of the total apparent decay rate). Decay traces followed good pseudo-first-order kinetics from which the value for $2k_r$ was obtained as $(5.6 \pm 2.4) \times 10^3$ M^{-1} s^{-1} at 298 K.

Conclusions

It has been shown that the recycling of α-tocopherol by coantioxidants in homogeneous solutions may be completely described by the proposed kinetic model. This model has been applied to three limiting cases: in the first one, where the coantioxidant is vitamin C, the regeneration step is irreversible or, at least, is so fast with respect to the reverse reaction that it can be considered irreversible for practical purposes. Thus, the effectiveness of the recycling process depends on the magnitude of the rate constant k_r that, under normal experimental conditions, must be at least 10^3 M^{-1} s^{-1} in order for regeneration of α-TOH to be observed.

The second case is that of 1,3-dimethylphenothiazine (a model for sterically hindered coantioxidants characterized by low rate constants of inhibition) where the recycling is reversible and its effectiveness depends on the ratio $K_r k_{11}/k_{10}$, i.e., essentially on the equilibrium constant for the hydrogen atom transfer reaction between the two antioxidants and the corresponding radicals, being k_{11}/k_{10} normally close to 1. Since the magnitude of K_r is determined by the BDE difference of the two antioxidants, regeneration will only be observed when the BDE value of the coantioxidant is lower or at least close to that of α-TOH (78.2 kcal/mol).

The third case is that of 4-*tert*-butylcatechol (a model compound for polyphenolic antioxidants) where recycling of α-TOH is feasible even though the BDE value is significantly higher than that of vitamin E. In this case, the driving force for the recycling process is the removal of the semiquinone radical from the catechol derivative by the α-tocopheroxyl radical, which makes the regeneration of α-TOH practically irreversible. This may explain the strong antioxidant properties of natural products containing flavonoids or other polyphenolic antioxidants together with some amount of α-tocopherol.

Experimental Section

Materials. Solvents were of the highest grade commercially available and used as received. 2,2′-Azobis(2,4-dimethylvaleronitrile), AMVN, and α,α′-azobis(isobutyronitrile), AIBN, were stored at −20 °C. *RRR*-α-Tocopherol was purified by column chromatography on silica gel eluting with 92:8 hexane/ethyl acetate as previously described.[37] 4-*tert*-Butylcatechol, 2,4,6-trimethylphenol, and 2,6-dimethylphenol were commercially available and used without further purification. 1,9-Dimethylphenothiazine was available from previous studies.[11]

Pentamethylphenol was prepared according to a literature procedure[38] from 2,4,6-trimethylphenol, paraformaldehyde, and HBr in acetic acid. The obtained 3,5-bis(bromomethyl)-

(36) Experimental data will be reported on a forthcoming paper.

(37) Valgimigli, L.; Banks, J. T.; Ingold, K. U.; Lusztyk, J. *J. Am. Chem. Soc.* **1995**, *117*, 9966−9971.

(38) Nagel, M.; Hansen, H. J. *Helv. Chim. Acta* **2000**, *83*, 1022−1048.

2,4,6-trimethylphenol was reduced with $LiAlH_4$ in dry THF to afford the title compound.

Autoxidation Experiments. Autoxidation experiments were performed in a two-channel oxygen uptake apparatus, based on a Validyne DP 15 differential pressure transducer, that has already been described elsewere.[39] The entire apparatus was immersed in a thermostated bath that ensured a constant temperature within ±0.1 °C.

In a typical experiment, an air-saturated chlorobenzene solution of styrene containing the antioxidant mixture (from 2.5×10^{-5} to 1.5×10^{-4} M) was equilibrated with the reference solution containing only an excess of α-tocopherol (from 1×10^{-3} to 1×10^{-2} M) in the same solvent at 30 or 60 °C. After equilibration, a concentrated chlorobenzene solution of AMVN or AIBN (final concentration from 5×10^{-2} to 5×10^{-3} M) was injected in both the reference and sample flasks and the oxygen consumption in the sample was measured, after calibration of the apparatus, from the differential pressure recorded with time between the two channels. This instrumental setting allowed us to have the N_2 production and the oxygen consumption derived from the azo-initiator decomposition already corrected from the measured reaction rates. Initiation rates, R_i, were determined for each condition in preliminary experiments by the inhibitor method using α-tocopherol as areference antioxidant: $R_i = 2[\alpha\text{-tocopherol}]/\tau$.

Quantitative Determinations of Antioxidants. α-Tocopherol ($(0.5-5) \times 10^{-4}$ M), the coantioxidant (1×10^{-4} M), and the thermal initiator AIBN (2−5 mM) in chlorobenzene were allowed to react at 60 °C under air while stirring. Aliquots of the reaction mixtures were sampled at time intervals, cooled to 20 °C, diluted 1:10 with methanol, and analyzed by HPLC-MS using electrospray ionization (ESI) in a Waters 2695 separation module (equipped with an autosampler) coupled to a Micromass ZMD ESI-MS spectrometer. The most appropriate instrumental settings were determined in a preliminary set of experiments: injection volume, 20 μL; column, C18 (Waters X-Terra-MS, 3 × 150 mm, 3.5 μm); eluent, 97:3 methanol/water; flow rate (in column), 0.5 mL/min; splitting ratio, 5:1; ESI type, negative ions; desolvation gas (N_2), 750 L/h; cone gas (skimmer), 76 L/h; desolvation temp, 380 °C; capillary voltage, −2.8 kV; cone voltage: −30 V; hexapole extractor, −3 V. Calibration curves were obtained for each analyte using the same instrumental settings and authentic samples dissolved in 1:10 chlorobenzene/methanol.

Kinetic Measurements by EPR. The rate of hydrogen exchange between α-tocopherol and the aminyl radical from 1,9-dimethylphenothiazine was measured at 298 K by a previously described kinetic−EPR method.[40] Briefly, an open EPR suprasyl quartz tube containing a benzene solution of 1,9-dimethylphenothiazine ($(1-5) \times 10^{-5}$ M) and di-*tert*-butylperoxide (0.1 M) was placed in the thermostated cavity of a Bruker ESP 300 spectrometer equipped with a Bruker ER033M Field-Frequency Lock accessory. For each experiment, a single time-sweep EPR scan was recorded with the following settings: microwave power = 5 mW, time constant = 4 ms, conversion time = 20−164 ms. The solution was continuously bubbled with a fine stream of nitrogen through a capillary glass tube, which ensured rapid mixing of the solutions and continuous removal of oxygen. The aminyl radical was generated by a short pulse of UV light from an unfiltered 500 W high-pressure Hg lamp. Briefly after the irradiation had been interrupted, a concentrated stock solution of α-tocopherol (final concentration = $(1-5) \times 10^{-3}$ M was rapidly (<1s) injected and the time-dependence of the EPR signal of a spectral line from the aminyl radical was monitored. The EPR line not superimposed onto those of the phenoxyl radical was previously chosen in a separate set of experiments. The reaction of the aminyl radical with 2,6-dimethylphenol, 2,4,6-trimethylphenol, and pentamethylphenol was similarly obtained, although in this case, the two antioxidants were both present in the system during photolysis: a deoxygenated benzene solution containing di-*tert*-butylperoxide (0.1 M), 1,9-dimethyl phenothiazine (1×10^{-4}-0.1 M), and one of the phenols (0.1−1 M) was sealed in a quartz tube sitting inside the cavity of the EPR spectrometer and shortly irradiated. The time evolution of the EPR signal from the aminyl radicals was monitored during and after UV irradiation using the instrumental setting described above. The actual concentrations of the two antioxidants (1,9-dimethylphenothiazine and phenol) had ratios ranging between 1:10 and 1:2000, which were chosen taking into account the BDE difference. The aminyl radical initially formed then decayed, following pseudo-first-order kinetics, by reacting with the excess phenol. For each phenol investigated, measures were repeated at 5 different phenol concentrations. Plots of the initial pseudo-first-order rate constant of EPR signal decay versus the concentration of the phenol gave excellent straight lines ($r > 0.9$) whose slopes provided the second-order rate constant for hydrogen transfer from the phenol to the aminyl radical.

The second-order rate constant for the hydrogen abstraction by α-tocopheroxyl radical from 4-*tert*-butylcatechol was measured at 298 K in benzene containing di-*tert*-butyl peroxide (0.1 M) by kinetic EPR by monitoring the decay of the EPR trace of photolytically generated α-TO$^\bullet$ radical with and without the injection of a concentrated stock solution of 4-*tert*-butylcatechol in benzene (final concentration of 0.25−1 mM). The procedure and experimental settings were otherwise identical to those previously described for the reaction of α-TOH with the aminyl radical from MPTZ. The initial concentrations of the reactants and the amount of tocopheroxyl radical produced in solution by short UV photolysis were accurately chosen to ensure clean decay traces under pseudo-first-order kinetics and to minimize other reactions, including the bimolecular self-decay of the α-tocopheroxyl radical. This last reaction was determined for each experiment and set to account for less than 5% the total apparent decay rate, therefore being negligible under the employed conditions. Five measurements were performed with different amounts of injected BC, and from the resulting pseudo-first-order rate constants, the bimolecular rate constant k_r was obtained.

Simulation of Reaction Kinetics. Simulation of the experimental traces of the consumption of the antioxidants and oxygen were carried out by means of a stochastic simulation program (CKS, developed at IBM, Almaden, San Jose, CA) that calculates concentrations of all reactants and products in chemical systems as function of time (for example, see ref 41). This is done by representing the reaction system with a volume containing an adequate number of particles. The particles are distributed among reactants present at the beginning of the simulation according to their initial concentrations, and then the system is allowed to evolve following the scheme and kinetic constants defined by the user, with time steps inversely proportional to the reaction rate. The simulations were carried out using the higher number of molecules available (2×10^9) and the kinetic equations and rate constants described above.

Acknowledgment. Financial support from the University of Bologna and MURST (Research project "Free Radical Processes in Chemistry and Biology: Syntheses, Mechanisms, Applications") is gratefully acknowledged.

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(39) Amorati, R.; Pedulli, G. F.; Valgimigli, L.; Attanasi, O. A.; Filippone, P.; Fiorucci, C.; Saladino, R. *J. Chem. Soc., Perkin Trans. 2* **2001**, 2142−2146.

(40) Valgimigli, L.; Lucarini, M.; Pedulli, G. F.; Ingold, K. U. *J. Am. Chem. Soc.* **1997**, *119*, 8095−8096.

(41) Chen, J.; Korner, S.; Craig, S. L.; Lin, S.; Rudkevich, D. M.; Rebek, J. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 2593−2596.