REVIEW

The Chemistry and Antioxidant Properties of Tocopherols and Tocotrienols

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ABSTRACT: This article is a review of the fundamental chemistry of the tocopherols and tocotrienols relevant to their antioxidant action. Despite the general agreement that α-tocopherol is the most efficient antioxidant and vitamin E homologue *in vivo*, there was always a considerable discrepancy in its "absolute" and "relative" antioxidant effectiveness *in vitro*, especially when compared to γ-tocopherol. Many chemical, physical, biochemical, physicochemical, and other factors seem responsible for the observed discrepancy between the relative antioxidant potencies of the tocopherols *in vivo* and *in vitro*. This paper aims at highlighting some possible reasons for the observed differences between the tocopherols (α-, β-, γ-, and δ-) in relation to their interactions with the important chemical species involved in lipid peroxidation, specifically trace metal ions, singlet oxygen, nitrogen oxides, and antioxidant synergists. Although literature reports related to the chemistry of the tocotrienols are quite meager, they also were included in the discussion in virtue of their structural and functional resemblance to the tocopherols.

Lipids 31, 671–701 (1996).

Lipid oxidation is a degradative, free radical mediated process responsible for the development of objectionable odors and flavors in oils, fats, and foods containing them (1–7). Moreover, oxidation of the polyunsaturated fatty acids (PUFA) of the biomembranes causes functional abnormalities and pathological changes (8–10). Although the mechanisms responsible for lipid oxidation have been extensively studied and documented in many books and excellent reviews (1,3,10–15), they are still not fully understood. Both the rates and pathways of lipid peroxidation are dramatically affected by other chemical species in the reaction medium as well as by the physical conditions of the reaction.

Vitamin E compounds (tocopherols and tocotrienols) are well recognized for their effective inhibition of lipid oxidation in foods and biological systems (16–29). Since vitamin E is only synthesized by plants, it is a very important dietary nutrient for humans and animals (7,30,31). Tocopherols are present in oil seeds, leaves, and other green parts of higher plants. α-Tocopherol is present mainly in the chloroplasts of plant cells, while β-, γ-, and δ-homologues are usually found outside these organelles (31). In contrast, the tocotrienols are not found in the green parts of the plants but, rather, in the bran and germ fractions of certain seeds and cereals (32). The tocopherol content of foods (33–35) is also important to protect food lipids against autoxidation and, thereby, to increase their storage life and their value as wholesome foods.

The antioxidant activity of the tocopherols and tocotrienols (grouped as chromanols) is mainly due to their ability to donate their phenolic hydrogens to lipid free-radicals (36–40). Although it is generally agreed that the relative antioxidant activity of the tocopherols *in vivo* is in the order $\alpha > \beta > \gamma > \delta$ (25,37,41,42), there is a widespread confusion concerning their relative potency *in vitro* (36). The chemical structures of the tocopherols and tocotrienols support a hydrogen-donating power in the order $\alpha > \beta > \gamma > \delta$ (40). This order also was obtained when the activity of the four tocopherols was compared in a homogeneous solution in dichlorobenzene (36), but a reversed order ($\delta > \gamma \approx \beta > \alpha$) was obtained when relative antioxidant potencies were compared in fats, oils, and lipoproteins *in vitro* (43–50). The reasons behind this reversed order is not yet clearly understood. However, it is now known that the "absolute" and "relative" *in vitro* activities of the tocopherols are not only dependent on their absolute chemical reactivities toward hydroperoxy and other free radicals, but also on many other possible side reactions. These side reactions, which are dramatically affected by tocopherol concentrations (46,51–53), by temperature and light (54,55), type of substrate (46,54,56,57) and solvent (58,59), and by other chemical species acting as prooxidants and synergists in the system, may be highly propagative. Thus, the mode in which the chromanols react is significantly affected by the interplay of all the chemical and physical parameters of the system.

The different roles that tocopherols can play in polyunsaturated lipid peroxidation are sufficiently described in a number of literature reports. In contrast to the tocopherols, there are very few articles on the antioxidative effects of the tocotrienols (60–69). It is unfortunate that the results from published studies on the *in vitro* antioxidant activities of the four tocopherols are not appropriate for critical intercomparison because these studies were made by different investigators on different lipid systems, generally of an unknown chemical

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Abbreviations: ESR, electron spin resonance; LDL, low density lipoprotein; NO, nitric oxide; PUFA, polyunsaturated fatty acids.

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composition and not fully specified physical parameters. By bringing together available knowledge in a form of a review, we hope that understanding the basic chemical mechanisms behind the tocopherol interference with lipid oxidation will increase. In addition, this review provides some reasoning for the controversial experimental results on the comparative antioxidant potency of the tocopherols *in vivo* and *in vitro*.

TOCOPHEROLS AND TOCOTRIENOLS: STRUCTURES, STEREOCHEMISTRY, AND NOMENCLATURE.

While studying the influence of nutrition on rat reproduction, Evans and Bishop (70) discovered vitamin E (at that time α-tocopherol) as a factor essential for reproduction. The name tocopherol, then, came from "tokos" (childbirth) and "phorein" (to bring forth) and the suffix "-ol" was added to indicate the phenolic nature. Now the term "vitamin E" is a generic name for all tocol and tocotrienol derivatives qualitatively exhibiting the biological activity of α-tocopherol. Structurally, the tocopherols and tocotrienols can be viewed as consisting of a chroman head (with two rings: one phenolic and one heterocyclic) and a phytyl tail (Table 1). The four tocopherols have saturated tails and vary only in the number of methyl substituents and the patterns of substitution in the phenolic ring. The four tocotrienols have chroman heads similar to those in their corresponding tocopherols but contain three isolated double bonds in their phytyl tails. While the tocopherols exist only as free phenols, the tocotrienols can occur naturally in esterified forms (32).

The tocopherol molecule has three chiral centers in its phytyl tail (2, 4′, and 8′), making a total of eight (2^3) stereoisomeric forms possible. Naming the different tocopherol stereoisomers is now done by following rules set by the International Union of Nutritional Sciences (71) and an International Union of Pure and Applied Chemistry–International Union of Biochemistry commission (72). Tocopherols of unspecified configuration should be named as methyl-substituted tocols, e.g., 5,7,8-trimethyl tocol, 5,8-dimethyl tocol, etc. All naturally occurring tocopherols (α-, β-, γ-, and δ-) have the same molecular configuration [*RRR*, 2D,4′D,8′D, *d*-tocopherols or (+)-tocopherols] in their phytyl groups. The condensation products of methyl-substituted hydroquinones and natural phytol [previously known as *l*-tocopherols, (-)-tocopherols or 2L,4′D,8′D-tocopherols] are epimeric only at position 2 with the recommended nomenclature of *SRR*-tocopherols or 2-*epi*-tocopherols. An equimolar synthetic mixture of a tocopherol with the natural configuration (*RRR)* and its 2-epimer [previously known as *dl*-tocopherol, (±)-tocopherol or 2DL,4′D,8′D-tocopherol] was recommended to be named 2-*ambo*-tocopherol. On the other hand, the condensation of methyl-substituted hydroquinones and racemic isophytol gives four possible enantiomeric pairs of diastereoisomers. α-Tocopherol, synthesized by the above reaction, is a mixture of approximately equal amounts of the eight possible stereoisomers: [2D,4′D,8′D (*RRR*), 2L,4′D,8′D (*SRR*), 2D,4′D,8′L (*RRS*), 2L,4′D,8′L (*SRS*), 2D,4′L,8′D (*RSR*), 2L,4′L,8′D (*SSR*), 2D,4′L,8′L (*RSS*), and 2L,4′L,8′L (*SSS*)] (25). This mixture is characterized as 2DL,4′DL,8′DL-α-tocopherol (should not be confused with 2DL-tocopherols) with the recommended name *all-rac*-α-tocopherol or [*dl*]-α-tocopherol. The distinction between the different tocopherol stereoisomers is of great importance for biological activity since the different α-tocopherol stereoisomers have different biopotencies *in vivo*.

The tocotrienols only have one chiral center at position 2, so they can only have 2D and 2L stereoisomers. However, the presence of the double bonds at positions 3′ and 7′ of the phytyl tail allows for the existence of four *cis/trans* geometrical isomers per tocotrienol. Thus, a total of eight isomers [2D, 3′*cis*, 7′*cis* (*R*, *cis-cis*); 2D, 3′*cis*, 7′*trans* (*R*, *cis-trans*); 2D, 3′*trans*, 7′*cis* (*R*, *trans-cis*); 2D, 3′*trans*, 7′*trans* (*R*, *trans-trans*); 2L, 3′*cis*, 7′*cis* (*S*, *cis-cis*); 2L, 3′*cis*, 7′*trans* (*S*, *cis-trans*); 2L, 3′*trans*, 7′*cis* (*S*, *trans-cis*); 2L, 3′*trans*, 7′*trans* (*S*, *trans-trans*)] can, at least theoretically, be present for each tocotrienol.

THE CHEMISTRY OF THE TOCOPHEROLS AND TOCOTRIENOLS: OXIDATION MECHANISMS AND PRODUCTS

The chemistry of oxidation of the chromanols is complicated, depending on the severity of the oxidation conditions and on the presence of other chemical compounds in the vicinity of the reacting tocols or tocotrienols. Oxidations of all chromanols by strong oxidizing agents (e.g., chromic acid, nitric acid, ferric chloride, etc.) generally give similar products, including lactones, quinones, and many degradation products. Different oxidation products were, on the other hand, obtained under milder conditions such as those involved in lipid oxidation (73–76).

Generally, the first step in the oxidation of a chromanol is the formation of resonance stabilized chromanoxyl (chroman-6-oxyl) radical, due to the donation of the phenolic hydrogen to a lipid peroxy radical (77–80). Evidence for the formation of tocopheroxyl radicals from the tocopherols and tocotrienols is available from electron spin resonance (ESR) and electron nuclear double resonance studies (64,81–86). The delocalization of the unpaired electrons also induces radical sites on the *ortho*- and *para*-positions (Fig. 1).

The chromanoxyl radicals are very reactive toward alkyl and alkylperoxy radicals, as will be explained in detail in the next section. Carbon-centered alkyl radicals generally add to the phenoxyl oxygen while oxygen-centered radicals prefer to add to an *ortho*- or a *para*-position of phenoxyl radicals. Thus, substitution at the *ortho*- position 5 accounts for the fundamental differences between the α- and β-chromanols and their γ- and δ-homologues and for the different oxidation pathways for the different tocols. The *ortho*-position (position 7), even if free, is sterically hindered, making the other *ortho*-position (position 5) to be the *ortho* site for radical-radical coupling reactions. Thus, γ- and δ-chromanoxyl radicals can rearrange to radicals capable of capturing lipid peroxy radicals at position 5; α-, and β-chromanoxyl radicals can re-

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TABLE 1
The Structures and Chemical Names of the Tocopherols and Tocotrienols

E-vitamer	R_1	R_2	Trivial names	Chemical abstract names
Tocopherols				
α-Tocopherol	Me	Me	5,7,8-Trimethyl tocol[a]	3,4-Dihydro-2,5,7,8-tetramethyl-2-(4′,8′,12′-trimethyltridecyl)-2H-1-benzopyran-6-ol; 2,5,7,8-tetramethyl-2-(4′,8′,12′-trimethyltridecyl-6-chromanol.
β-Tocopherol	Me	H	5,8-Dimethyl tocol	3,4-Dihydro-2,5,8-trimethyl-2-(4′,8′,12′-trimethyltridecyl)-2H-1-benzopyran-6-ol; 2,5,8-trimethyl-2-(4′,8′,12′-trimethyltridecyl)-6-chromanol.
γ-Tocopherol	H	Me	7,8-Dimethyl tocol	3,4-Dihydro-2,7,8-trimethyl-2-(4′,8′,12′-trimethyltridecyl)-2H-1-benzopyran-6-ol; 2,7,8-trimethyl-2-(4′,8′,12′-trimethyltridecyl)-6-chromanol.
δ-Tocopherol	H	H	8-Monomethyl tocol	3,4-Dihydro-2,8-dimethyl-2-(4′,8′,12′-trimethyltridecyl)-2H-1-benzopyran-6-ol; 2,8-dimethyl-2-(4′,8′,12′-trimethyltridecyl)-6-chromanol.
Tocotrienols				
α-Tocotrienol (formerly ε-tocopherol)	Me	Me	5,7,8-Trimethyl toctrienol	3,4-Dihydro-2,5,7,8-tetramethyl-2-(4′,8′,12′-trimethyl-3′,7′,11′-tridecatrienyl)-2H-1-benzopyran-6-ol; 2,5,7,8-tetramethyl-2-(4′,8′,12′-trimethyl-3′,7′,11′-tridecatrienyl)-6-chromanol.
β-Tocotrienol (formerly ζ_1-, ζ_2-tocopherol)	Me	H	5,8-Dimethyl toctrienol	3,4-Dihydro-2,5,8-trimethyl-2-(4′,8′,12′-trimethyl-3′,7′,11′-tridecatrienyl)-2H-1-benzopyran-6-ol; 2,5,8-trimethyl-2-(4′,8′,12′-trimethyl-3′,7′,11′-tridecatrienyl)-6-chromanol.
γ-Tocotrienol	H	Me	7,8-Dimethyl toctrienol	3,4-Dihydro-2,7,8-trimethyl-2-(4′,8′,12′-trimethyl-3′,7′,11′-tridecatrienyl)-2H-1-benzopyran-6-ol; 2,7,8-trimethyl-2-(4′,8′,12′-trimethyl-3′,7′,11′-tridecatrienyl)-6-chromanol.
δ-Tocotrienol	H	H	8-Monomethyl toctrienol	3,4-Dihydro-2,8-dimethyl-2-(4′,8′,12′-trimethyl-3′,7′,11′-tridecatrienyl)-2H-1-benzopyran-6-ol; 2,8-dimethyl-2-(4′,8′,12′-trimethyl-3′,7′,11′-tridecatrienyl)-6-chromanol.

[a]The chemical abstract name for tocol is: 3,4-dihydro-2-methyl-2-(4′,8′,12′-trimethyltridecyl)-2H-1-benzopyran-6-ol and the registry numbers for the tocopherols are: α-T [59-02-9]; β-T [16698-35-4]; γ-T [54-28-4]; and δ-T [119-13-1] and for the tocotrienols are: α-T-3 [1721-51-3; 493-35-6]; β-T-3 [490-23-3]; and γ-T-3 [91-86-1].

arrange to chromanol methide radicals or chromanol radicals with the radical site at position 8a of the chroman ring.

Oxidations of the α-tocopherol **[I]** in polar protic solvents, e.g., ethanol and water-saturated solvents, usually involve different pathways and lead to different products than oxidations in inert, nonpolar, aprotic solvents like petroleum (87–91). In protic solvents, where electron mobility is enhanced, the α-tocopheroxyl radical **[II]** will either donate an electron to another radical and form the tocopherloxylium cation **[III]** or donate a hydrogen atom to form the quinone methide **[IV]**. The possible oxidation products of α-tocopherol in an alcoholic solution are shown in Figure 2.

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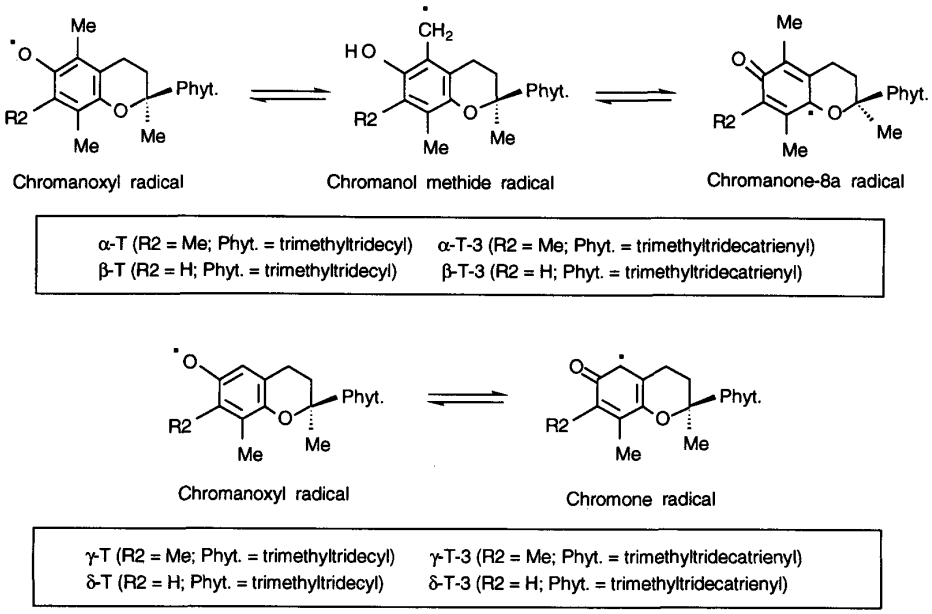

FIG. 1. The resonance forms of the chromanoxyl radicals; Phyt. = phytyl.

The tocopherloxylium cation **[III]** can react with alcohols and other protic species (Fig. 2), giving products with the structures **[V, VI]**. The tocopherol quinone methide **[IV]** can nucleophilically add water or alcohols (87,89,92) to form 5-hydroxymethyl-6-hydroxy **[VII]** or 5-alkoxymethyl-6-hydroxy **[VIII]** derivatives, respectively. The alkoxy group in **[VIII]** can be a cholesteryl function, suggesting a significant size contribution from the methyl group at position 5 of α-tocopherol (91). The 5-alkoxymethyl-6-hydroxy **[VIII]** also can add water, forming the hemi-acetal **[IX]** which can then lose the alcoholic residue to give the 5-formyl derivative **[XI]** *via* a 5-hydroxymethylene chromanol **[X]**. The dihydroxy derivatives **[VII]** also can be oxidized to the 5-hydroxymethylene chromanol **[X]**, 5-formyl derivative **[XI]**, **[XII]**, and **[XIII]** (88,89,91–93). No such studies were made for the other chromanols.

In aprotic (lipophilic) solvents, the chromanoxyl radicals tend to react mainly by radical-radical coupling reactions, forming dimers (94,95). In the presence of sufficient amounts of alkoxy, peroxy, hydroxy, or other radicals, adducts are formed with these radicals. In the absence of other potentially unstable radicals, the chromanoxyl radicals will undergo self-coupling. Phenoxy radicals having free *ortho* or *para* positions generally dimerize through these centers, forming two types of dimers: (i) diphenol dimers due to *ortho-ortho*, *ortho-para*, or *para-para* couplings and (ii) phenol-phenyl ether dimers, due to coupling of the phenoxyl oxygen of one phenol with an *ortho* or *para* position in the other phenol (96–98). The formation of the diphenyl dimers predominates in polar media while the dimerization to phenol-phenyl ethers predominates in nonpolar media (99).

Figure 3 shows the oxidation pathways for α-tocopherol in lipids and lipophilic solvents. The tocopheroxyl radical **[II]** can dimerize (*via* the tocopherol methide radical), resulting in the 1,2-*bis*-(α-tocopherol-5′-yl)-ethane dimer **[XVI]** (40). This dimer is a diphenol and can still act as an antioxidant. Upon further oxidation, this dimer can possibly end up as an α-tocopherol-α-tocopherlquinone spirodimer **[XXIII]**. Two α-tocopherol methide radicals **[II]** can easily disproportionate into a tocopherol molecule and a molecule of tocopherol quinone methide **[XV]**, which is a very versatile intermediate in lipophilic solvents (40,91,100). It undergoes a number of reactions and is a precursor of a wide range of compounds (92,93). It can add a tocopherol molecule by disproportion, forming the 1,2-*bis*-(α-tocopherol-5′-yl)-ethane dimer **[XVI]** (101), or by the Diels-Alder mechanism forming α-tocopherol-α-tocopheroxy dimers **[XVIII]** (92). It can undergo Diels-Alder polymerization to form a spirodimer **[XIX]** and a spirotrimer **[XX]** (101–106).

In the presence of peroxy radicals, α-tocopherol is primarily oxidized to 8a-peroxy-substituted tocopherones **[XIV]** (104–109) which will finally degrade to α-tocopherolquinone **[XVII]**, which is also the major metabolite of α-tocopherol *in vivo* (110), plus various tocopherone and quinone epoxides (107,108) and spirodimer and trimer (49,105). The epoxide formation did not proceed under anaerobic conditions (107).

Figure 4 shows the possible oxidation pathways for γ-tocopherol **[XXIV]** in lipids or lipophilic solvents. In the absence of peroxy radicals, the two atropic isomers of the diphenol dimer, 5-(tocopherol-5′-yl)-γ-tocopherol **[XXVI]**, were the predominant products in a similar way to many phenols (40,64,96,111–113). Upon further oxidation, 5-(tocopherol-

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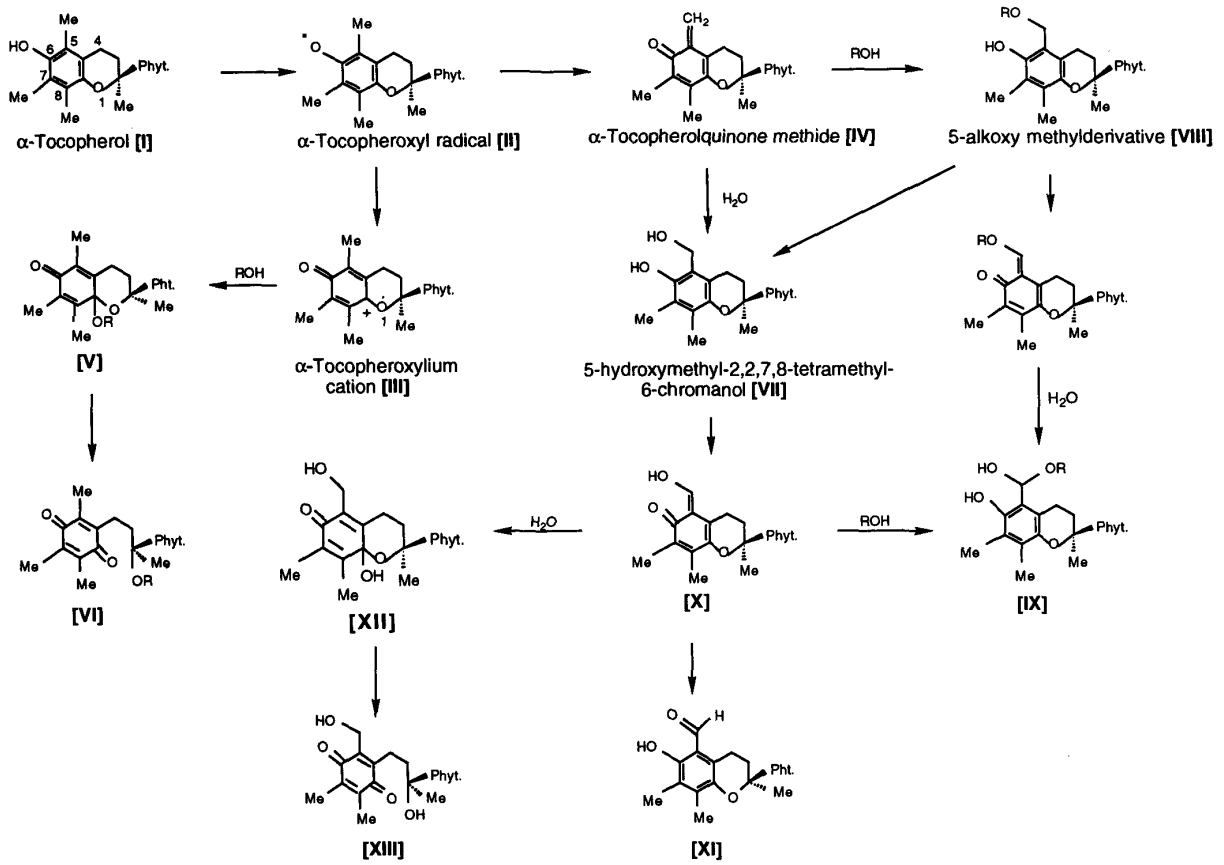

FIG. 2. The oxidation products of α-tocopherol in water and alcohols.

5′-yl)-γ-tocopherol **[XXVI]** may yield a γ-tocopherol dimer quinone **[XXVII]**. The phenol-phenyl ether dimer, 5-(γ-tocopheroxyl)-γ-tocopherol **[XXVIII]**, is also formed in autoxidizing lipids, though less frequently (49,64,114,115). Both dimers are still able to act as antioxidants by virtue of their free OH protons. In the presence of lipid hydroperoxides, the two atropic isomers of the diphenol dimer **[XXVI]**, 5-γ-tocopheroxyl-8a-peroxy-γ-tocopherone **[XXIX]**, the phenol-phenyl ether dimer **[XXVIII]**, the four isomeric 8a-peroxy-γ-tocopherones **[XXX]**, in addition to γ-tocored **[XXXII]**, were identified (112).

The apparently superior activity of γ-tocopherol compared to α-tocopherol in many *in vitro* systems was related by Gottstein and Grosch (49) to the fact that the former is dimerizable to compounds that can still be effective as antioxidants. However, this does not appear to be the full explanation, especially when α-tocopherol is present in high concentration. Further, α-tocopherol also was reported to dimerize to potent antioxidants, such as the α-tocopherol ethane dimer **[XVI]** and its intermediate degradation products **[XXI** and **XXII]** (93). Burton *et al.* (80) measured the rates of bimolecular coupling reactions of the four tocopherol isomers by kinetic ESR in benzene/di-*tert*-butyl peroxide (10:1, vol/vol) at 23°C for tocopherol concentrations of 1×10^{-3}, 5×10^{-3}, and 50×10^{-3} M. Rate constants of 3×10^3, 4×10^4, 4.5×10^4, and 1.5×10^5 $M^{-1}s^{-1}$ were obtained for the bimolecular couplings α/α, β/β, γ/γ, and δ/δ, respectively. In the presence of peroxides, the rate of tocopherol radical dimerization is too low compared with k_a (Eq. 5). Thus, when tocopherols are present with other radicals, the antioxidation reaction, i.e., coupling with peroxy and other radicals, will predominate but dimeric tocopherol products also will be formed (110,112, 116–119). However, if the autoxidation reaction is allowed to reach termination, the final products of tocopherol autoxidation are expected to be α-tocopherol quinone **[XVII]** and α-tocopherol-α-tocopherol quinone dimer **[XXIII]** in case of α-tocopherol, and γ-tocored **[XXXII]** in the case of γ-tocopherol. Kwi-Hyun and Igarashi (118) studied the oxidation products of two kinds of tocopherols [either (α- + γ-) or (α- + δ-)] co-existing during the autoxidation of methyl linoleate. Since no mixed dimers were detected, it was suggested that the oxidation of α-tocopherol proceeds and is then followed by the decomposition of γ-tocopherol and then δ-tocopherol after the approximate consumption of α-tocopherol.

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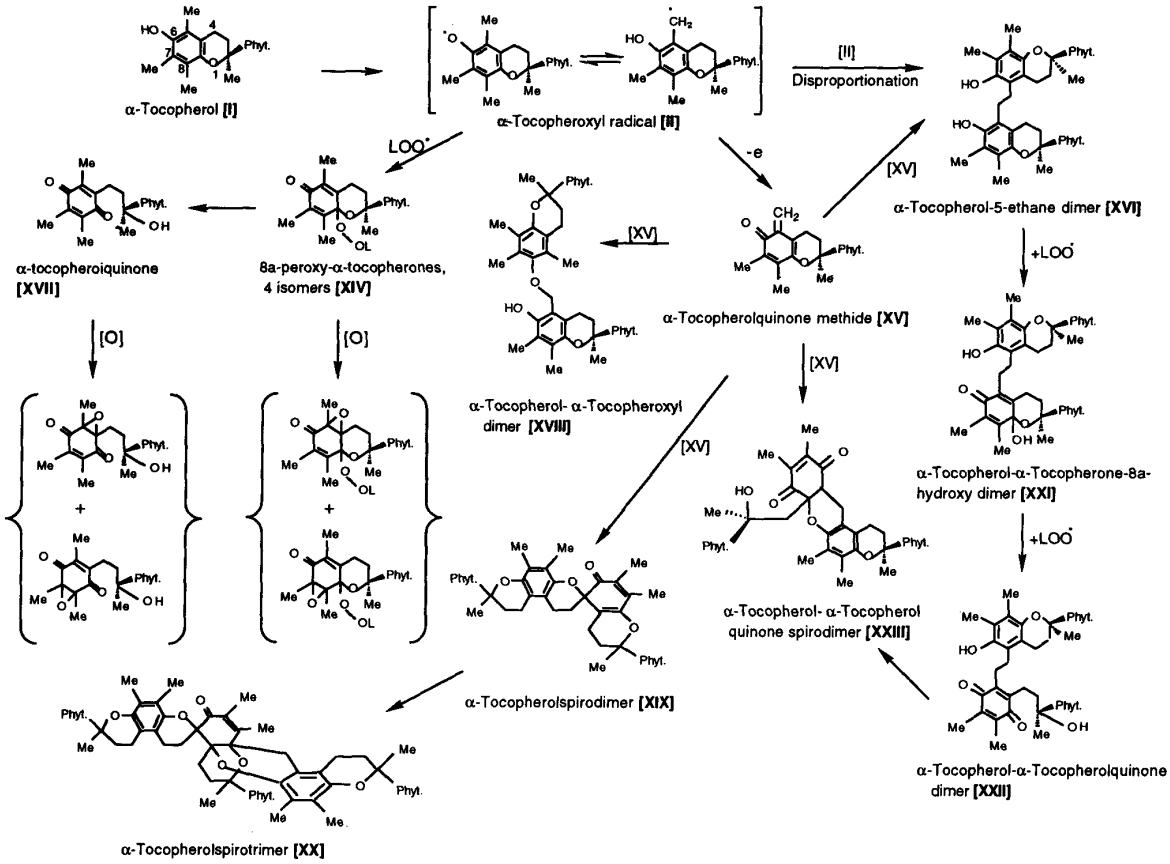

FIG. 3. The autoxidation products of α-tocopherol.

Products from, and thereby mechanisms for, the antioxidative reactions of the tocotrienols have not been studied yet, but are expected to be similar to those of the tocopherols. Goh *et al.* (64) generated some tocotrienoxyl radicals and studied them by ESR. The tocotrienols gave the same type of radicals and dimeric products as their corresponding tocopherols. In a mixture of α- and γ-tocotrienols, the radical of the latter was not seen until the signal for the α-tocotrienol radical disappeared in a similar way to the tocopherols. The presence of the three isolated double bonds in the phytyl tail seems to render the tocotrienols less efficient than the tocopherols as *in vivo* antioxidants (42). It is not yet known if the unsaturation of the phytyl tail will make any difference in the *in vitro* activity of the tocotrienols relative to their corresponding tocopherols.

EFFECTS OF STRUCTURE AND STEREOCHEMISTRY ON REACTIVITY AND BIOKINETICS

In general, unsubstituted phenols are inactive as hydrogen donors. Differences in the relative reactivities of substituted phenols can be explained by considering two main factors: (i) inductive effects related to the presence of electron-releasing substituents in positions *ortho-* and/or *para-* to the hydroxy function, and (ii) stereoelectronic effects concerned with the orientation of these substituents with repect to the aromatic plane (80).

The presence of electron-releasing substituents in positions *ortho-* and/or *para-* to the hydroxy function increases the electron density of the active centers facilitating the homolytic fission of the O-H bond, increasing the stability (lifetime) of the phenoxyl radical and improving the reactivity with peroxy radicals (40,120,121). Thus, butylated hydroxy anisole, butylated hydroxy toluene, and tertiarybutyl hydroquinone and other substituted phenols are active (121) while simple, unsubstituted phenol is not. Likewise, α-tocopherol is structurally expected to be more potent as a hydrogen donor than β-, γ-, and δ-tocopherols because these lack one or two *ortho* methyl groups. The oxidation reduction potentials for α-, β-, γ-, and δ-tocopherols were reported as +0.273, +0.343, +0.348, and +0.405 volts, respectively (122). Moreover, α-tocopherol was found to regenerate β-, γ-, and δ-tocopherols from their radicals in homogeneous solutions (123).

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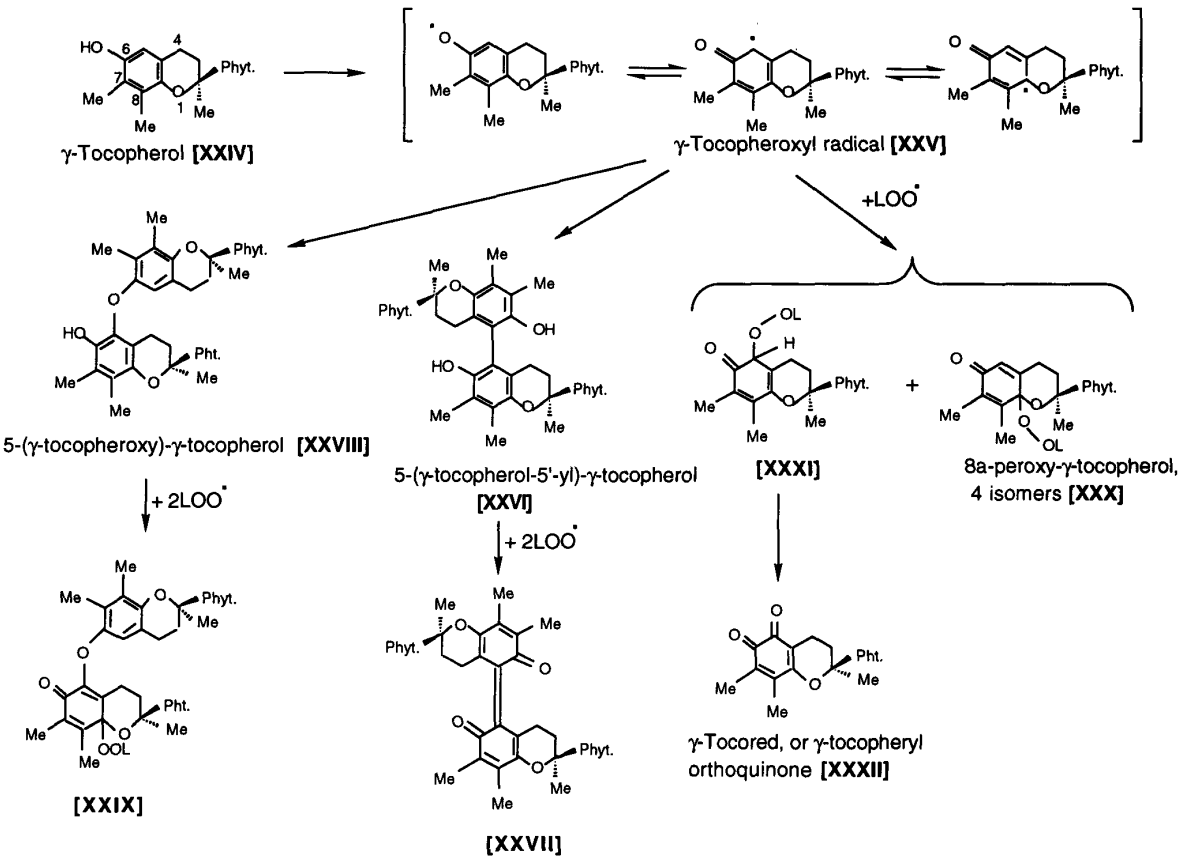

FIG. 4. The autoxidation products of γ-tocopherol.

The fact that γ-tocopherol was sometimes found to be a stronger antioxidant than α-tocopherol *in vitro* (43–50) cannot be directly explained by simple structural comparison. The decomposition of tocopherols was investigated in vegetable oils during accelerated storage test and α-tocopherol was found to be used up first, followed by β-, γ-, and then δ-tocopherol (118). In the following sections, we will try to give a hypothetical explanation for the experimental findings that α-tocopherol was found to be a less potent antioxidant than γ-tocopherol in many *in vitro* cases.

Compared with other phenolic analogues, the tocopherols owe their excellent antioxidant properties (*ca.* 250 times that of butylated hydroxytoluene) mainly to the heterocyclic ring in the chroman moiety (39). Phenols with an oxy substituent in the *para* position of a phenol are known to give more stable phenoxyl radicals and therefore to have a higher antioxidant activity compared to phenols lacking this function, e.g., 2,3,5,6-tetramethyl-4-methoxy phenol was more active than pentamethyl phenol in the inhibition of styrene autoxidation (Table 2, Ref. 80). A stereoelectronic theory for the contribution of the heterocyclic ring, with an oxygen atom *para* to the phenolic function, to the antioxidant activity of the tocopherols was proposed by Burton and Ingold (37) and Burton *et al.* (80,124). According to this theory, the excellent antioxidant properties of the tocopherols are due to the fused heterocyclic ring. In this ring, the p-type lone electron pair of oxygen is kept almost perpendicular to the aromatic plane. This p-type lone pair orbital overlaps with the semi-occupied molecular orbitals of the radical, the effect of which stabilizes the phenoxyl radical by conjugative electron delocalization. Thus, the larger the orbital overlap, the higher the antioxidant activity. The extent of the orbital overlap is dependent on the dihedral angle, θ, between the oxygen p orbital and a plane perpendicular to the aromatic plane. This angle, θ, should be equal to the dihedral angle, θ′, between the O-C_2 bond and the aromatic plane. With decreasing θ, the spin density on the aromatic ring and the strength of the O-H bond decrease. Provided that an oxy group is present at the *para* position, stabilization is at maximum when θ and θ′ approach 0° and at minimum when they approach 90° as in 2,6-ditertiarybutyl-3,4-dimethyl phenol. The tocopherols, with the heterocylic ring, have θ =17–21.4° while *para*-methoxy derivatives of the phenolic ring will give θ = 89° with low antioxidant activity (37). Comparison of α-tocopherol with an analogue having another

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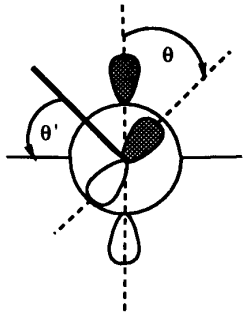
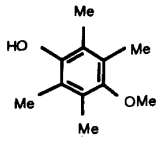
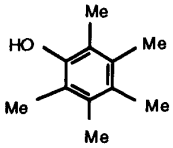
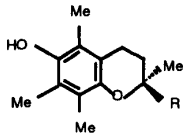
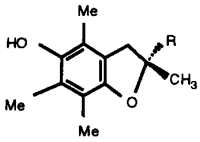
TABLE 2
The Effect of Structure on the Antioxidant Properties of Some Tocopherol-Related Compounds as Measured by the Inhibition of Styrene Autoxidation at 30°C[a]



Structure		Relative reactivity	θ
	4-Methoxy-2,3,5,6-tetramethyl phenol	12	89°
	Pentamethyl phenol	11	—
	*R* = Phytyl (α-tocopherol) *R* = Me	100 119	17°
	*R* = H *R* = Me *R* = Phytyl	169 178 147	6°

[a]Values for θ were taken from References 37 and 80, and values for relative reactivities of the phenols were recalculated from the same references.

methyl group at carbon 2 instead of the phytyl tail confirmed the assumption that it is the chroman moeity that is responsible for the antioxidant activity of the tocopherols, and that the phytyl tail had no or only a very minor role (Table 2).

Applying this theory to the four tocopherol homologues, Mukai *et al.* (125) studied the molecular structures of cation radicals of model compounds of the four tocopherol homologues using electron nuclear double resonance spectroscopy. Although γ- and δ-tocopherols had slightly smaller θ values than α- and β-tocopherols, the antioxidant potency of the four tocopherols was in a decreasing order α > β = γ > δ (125). Thus, it was concluded that the reactivity of the tocopherols is not only dependent on the dihedral angle (θ), but also on the facility of H-transfer. The same conclusion was forwarded by Nagaoka *et al.* (126,127) who used photoelectron spectroscopy and *ab initio* calculation to study the antioxidant action of vitamin E. The following values of θ were reported: α- (21°), β- (21.4°), γ- (18.9°), and δ-tocopherol (20.2°). Ac-

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cordingly, the extent of the orbital overlap in α- and β-tocopherols is smaller than in γ- and δ-tocopherols (128). Thus, the substitution pattern in the phenolic ring of the chroman moiety of the tocopherols seems to be the main factor determining the relative antioxidant effectiveness of, at least, these four homologues. The presence of more methyl substituents in the phenolic ring of the tocopherol does not only enhance its antioxidant activity, but also increases its lipophilic properties, making the α-homologue the most soluble tocopherol in lipid substrates (129). Substitution at *ortho*- and *para*-positions is known to hinder the phenoxy radicals from further reactions (99) and to decrease tendency toward oxidation by atmospheric oxygen (40). Thus, α-tocopherol (with two *ortho*-methyl substituents) is expected to be a more potent antioxidant (hydrogen donor) than either β- or γ-tocopherols (with only one *ortho*-methyl substituent) which, in turn, are more potent than δ-tocopherol (with no *ortho*- methyl substituent) (130–132).

When considering the relative effects of the tocopherols and tocotrienols *in vivo*, the situation is more complicated. The utilization of the tocopherols and tocotrienols in biological tissues is not only governed by their chemical reactivities but also by the biokinetics of their distribution and transport, or bioavailability (133). Ingold *et al.* (134–137) summarized the following structural requirements for a good chain-breaking tocopherol analogue for *in vivo* antioxidation: (i) the aromatic ring should be fully methylated, (ii) the size of the heterocyclic ring is important since the reduction of the ring size from six atoms in α-tocopherol to five atoms in the dihydrofuran analogue increased the activity (138,139), (iii) the stereochemistry at position 2 of both α-tocopherol and its dihydrofuran analogue should have the 2*R*-configuration for maximum *in vivo* activity (140–145), and (iv) methyl branching in the lipophilic "tail" is unimportant, but the length of the tail (optimum chain length being 11–13 carbons) is important.

The lipophilicity of the molecule (as determined by the number of methyl substituents in the chroman ring and the structure and stereochemistry of the phytyl tail) is an important feature for the biological activity of the tocopherols since it determines the kinetics of their transport and retention within the membranes (25). The chroman group is, thus, not only responsible for the antioxidant potential of the tocopherols and tocotrienols but also for their lipophilic properties. The phytyl tail, on the other hand, has no effect on the chemical reactivity of vitamin E antioxidants but is responsible for the very high lipophilic properties of these compounds (25,36,37,39,80,146) important for proper positioning in the biomembranes (147–150).

Table 3 shows the biological activities of some tocopherols and tocotrienols relative to natural (*RRR*) α-tocopherol as assayed by the rat resorption–gestation test (143). The fact that natural α-tocopherol was biologically more potent than the synthetic (*all-rac*) α-tocopherol mixture (42,140,141,151) indicates that the natural *RRR* configuration of the phytyl tail is optimum for maximum biopotency. The biological activity of the different tocopherols is also low for the less lipophilic δ- and γ-tocopherols compared with α- and β-tocopherols. The unsaturation of the phytyl tail seems to reduce the biological activities of the tocotrienols relative to the tocopherols since α-tocotrienol only has 30% of the vitamin E activity of α-tocopherol and β-tocotrienol only has 10% of the biological activity of β-tocopherol (152).

TABLE 3
The Biological Activity of Some Tocols and Tocotrienols[a]

Name/configuration	Relative biological activity (%)
d-α-Tocopheryl acetate (2R,4′R,8′R)	100
2*R*,4′*R*,8′*S*-α-tocopheryl acetate	90
2*R*,4′*S*,8′*S*-α-tocopheryl acetate	73
2*R*,4′*S*,8′*R*-α-tocopheryl acetate	57
1-α-Tocopheryl acetate (2S,4′R,8′R)	31
2*S*,4′*R*,8′*S*,-α-tocopheryl acetate	37
2S,4′*S*,8′*R*-α-tocopheryl acetate	21
2*S*,4′*S*,8′*S*-α-tocopheryl acetate	60
d-α-Tocopherol	100
d-β-Tocopherol	50
d-γ-Tocopherol	10
d-δ-Tocopherol	3
d-α-Tocotrienol	30
d-β-Tocotrienol	5
d-γ-Tocotrienol	Not known
d-δ-Tocotrienol	Not known

[a]Data from VERIS (Ref. 42).

The very large differences in biopotency among the tocopherols and tocotrienols are mainly due to differences in retention in tissues and membranes. Comparative studies in humans using deuterium-labeled tocopherols showed that there is no discrimination between α- and γ-tocopherol during absorption, secretion in the chylomicrons, or transport to the liver, but subsequently there is a preferential enrichment of the very low density lipoprotein with α-tocopherol (153–155). Other studies showed that a similar biodiscrimination occurs between the eight α-tocopherol stereoisomers in favor of the *RRR* form (156–159). This discrimination between the tocopherol isomers and homologous is related to a specific tocopherol-binding protein which recognizes the following structural features (137,160–163): (i) a fully methylated aromatic ring, (ii) a saturated phytyl side chain, and (iii) a stereochemical *RRR*-configuration of the methyl groups branching of the side chain. The importance of this tocopherol-binding protein in tocopherol bioavailability was recently evidenced by the finding that patients with isolated vitamin E deficiency have an impaired ability to incorporate α-tocopherol into lipoproteins, due to abnormality caused by a mutation in the gene for this protein (164).

Although α-tocotrienol is known to possess a lower biological vitamin E activity than α-tocopherol (152,165), recent research suggested that α-tocotrienol is a better antioxidant (67,69). α-Tocotrienol possessed remarkably higher antioxidant activity *in vitro* in rat liver microsomes against (Fe^{2+}-ascorbate)- and (Fe^{2+}-NADPH)-induced lipid peroxidation and better protection of the intrinsic membrane protein (Cy-

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tochrome P-450) against oxidative damage than α-tocopherol. The higher antioxidant potency of α-tocotrienol over α-tocopherol was hypothesized to be due to the combined effects of three properties (67): (i) α-tocotrienol was mentioned to have a higher recycling efficiency from its chromanoxyl radicals than α-tocopherol, (ii) α-tocotrienol is significantly less associated in clusters and is more uniformly distributed in membrane bilayers than α-tocopherol, (iii) α-tocotrienol has a strong disordering effect on membrane lipids which makes interaction of the chromanols with lipid radicals more efficient.

Suzuki *et al.* (69) presented evidence that α-tocotrienol exhibited a greater potency than α-tocopherol in eliminating chemiluminescence and fluorescence characteristics resulting from 2,2′-azo-*bis*(2,4-dimethylvaleronitrile)-generated peroxy radicals in the liposomes. However, the free radical scavenging effects of α-tocotrienol and α-tocopherol were similar in solution. Suarna *et al.* (68) found that the antioxidant activity of low density lipoprotein (LDL)-associated α-tocotrienol is the same as that of α-tocopherol in contrast to the reports of Serbinova *et al.* (67) and Suzuki *et al.* (69).

THE EFFECTS OF TOCOPHEROLS ON LIPID PEROXIDATION: TOCOPHEROLS AS FREE RADICAL SCAVENGERS

PUFA autoxidation reactions generally can be divided into three major reactions: initiation, propagation (which includes chain transfer and chain branching), and termination (15). The initiation reaction (Eq. 1) involves the generation of a radical, generally an alkyl radical ($L^\bullet$) from an unsaturated fatty acid (LH). The initiation reaction is very slow (thus rate-limiting) and is dependent on the initiator (*I*) employed. The reaction can be catalyzed by heat, light, trace metals, and/or certain enzymes.

$$\text{initiation} \qquad I + LH \xrightarrow{R_i} L^\bullet + IH \qquad [1]$$

The alkyl radicals ($L^\bullet$) formed in the initiation step are very reactive, and they generally combine with available oxygen at a very high rate ($k = 10^9\ M^{-1}s^{-1}$), giving peroxy radicals ($LOO^\bullet$) (Eq. 2) (169). The peroxy radicals disappear *via* a much slower reaction (Eq. 3) ($k_p = 10–60\ M^{-1}s^{-1}$) (22, 36, 40, 166–168), giving rise to the lipid hydroperoxides (LOOH) and new alkyl radicals which will propagate the reaction chain (Eq. 3) (170):

$$\text{propagation} \qquad L^\bullet + O_2 \xrightarrow{\text{fast}} LOO^\bullet \qquad [2]$$

$$LOO^\bullet + LH \xrightarrow{k_p} LOOH + L^\bullet \qquad [3]$$

When almost all the unperoxidized lipids (LH) are consumed, the radicals will tend to dimerize and terminate the chain reaction:

$$\text{termination} \qquad LOO^\bullet \xrightarrow{k_{t1}} \text{nonradical products} \qquad [4a]$$

$$LO^\bullet \xrightarrow{k_{t2}} \text{nonradical products} \qquad [4b]$$

Nagaoka *et al.* (126) offered a probable explanation for the mechanism of antioxidant action of the tocopherols (TOH). Initially, the tocopherol molecule and the peroxy radical (susceptible to donating and accepting an electron, respectively) approach each other and their electron clouds begin to overlap. Thus, a transition state which has the property of the charge transfer species (LOO^----TOH^+) will be reached. When the tocopherol molecule and the peroxy radical approach each other to an appreciable extent ($LOO^{\delta-}$---$TOH^{\delta+}$), proton tunneling will take place where the chromanol molecule will lose a hydrogen atom to a lipid peroxy radical forming the chromanoxyl radical (Eq. 5).

$$LOO^\bullet + TOH \xrightarrow{k_a} LOOH + TO^\bullet \qquad [5]$$

The chromanoxyl radical ($TO^\bullet$) may undergo radical-radical coupling with other radicals forming adducts (Eq. 6) (36,171,172). As mentioned before, the chromanoxyl radicals react differently with carbon-centered and oxygen-centered oxidized lipid radicals. Carbon-centered radicals (either pentadienyl or epoxyallylic), formed under anaerobic conditions, tend to add to the chromanoxyl oxygen forming 6-*O*-lipid alkyl-chromanol adducts (173–175). On the other hand, oxygen-centered peroxyl radicals (either diene peroxyl or epoxyene peroxyl) tend to add at the 8a postion, forming 8a-alkyldioxy-tocopherones (174–176).

$$(n-1)(LOO^\bullet) + TO^\bullet \longrightarrow \text{adducts} \qquad [6]$$

In the absence of antioxidants, the rate of the lipid oxidation reaction is given by:

$$\frac{-d[O_2]}{dt} = Ri \bullet K_p[RH] \qquad [7]$$

and the inhibitory effect of an antioxidant on lipid oxidation reactions can be represented as follows:

$$\frac{-d[O_2]}{dt} = \frac{Ri \bullet Kp\,[RH]}{n \bullet Ka\,[TOH]} \qquad [8]$$

where: *Ri*, *Kp*, and *Ka* are the rates for the initiation, propagation, and antioxidation reactions, respectively (36). Thus, the antioxidant effect of the tocopherols and tocotrienols is dependent on their stoichiometric factors (*n*), the rates of their reactions with peroxy radicals (*Ka*), and their concentrations ([TOH]).

Since each tocopherol molecule can neutralize two peroxy radicals by reactions (Eqs. 5 and 6), the stoichiometric factor (*n*) for the four tocopherol isomers is theoretically considered to be equal to 2.0 (36). Values of $n = 1.3–1.6$ were reported

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for α-tocopherol in the autoxidation of PUFA within the microsomal membranes (177). The stoichiometric factors (*n*) for β-, γ-, and δ-tocopherols were reported as 2.04, 1.89, and 1.78 relative to a standard value of 2.00 for α-tocopherol (178). Values of *ka* for the four tocopherol isomers were measured for the inhibition of styrene oxidation initiated by 2,2′-*bis*(isobutyronitrile) at tocopherol concentrations of 2×10^{-4}–7×10^{-2} M at 30°C. The *ka* values were 23.5, 16.6, 15.9, and 6.5×10^5 $M^{-1}s^{-1}$ for α-, β-, γ-, and δ-tocopherols, respectively, suggesting a relative antioxidant potency of the order α > β ≈ γ > δ (36). The magnitude of *ka* for α-tocopherol was also measured in a number of substrates and values reported ranged from as low as 2×10^5 $M^{-1}s^{-1}$ for $C_6H_5.CMe_2.OO^{\bullet}$ at 60°C (179) to as high as 5×10^8 $M^{-1}s^{-1}$ for $Cl_3OO^{\bullet}$ at room temperature (180). The value of *ka* is, thus, not only dependent on the tocopherol isomer but also on the substrate and reaction conditions.

The chromanols seem to be the most efficient lipid antioxidants provided by nature. This antioxidant activity is apparently due to the following facts. Firstly, these phenolic compounds have special structural properties (long phytyl tails) that render them lipid-soluble. Since lipid peroxy radicals react with the tocopherols several orders of magnitude faster ($ka = 10^4$–10^9 M^{-1} s^{-1}) than their reactions with acyl lipids ($k = 10$–60 $M^{-1}s^{-1}$) (22,36,40,165–167), one tocopherol molecule can protect about 10^3–10^8 PUFA molecules at low peroxide levels (181). This may explain why the small α-tocopherol/PUFA ratio in biomemebranes (e.g., 1:500 α-tocopherol/arachidonic acid molecules in erythrocyte membrane) is enough to interrupt the free radical chain reactions (147). Alternatively, the presence of catalase, glutathione peroxidase, and superoxide dismutase in biological systems may diminish the flux of radicals sufficiently so that the small amount of vitamin E available only operates as a second line of defense dealing with unusual "overflow" of radicals (182).

The tocopherols also can react with alkoxy radicals ($LO^{\bullet}$), formed in the propagation step (Eq. 9), or undergo self-coupling to form dimers and/or trimers (Eq. 10) (80).

$$LO^{\bullet} + TOH \longrightarrow TO^{\bullet} + LOH \quad [9]$$

$$TO^{\bullet} + TO^{\bullet} \longrightarrow TO\text{–}TO \quad [10]$$

In very special cases, when oxygen is present in trace amounts and hydroperoxides are present in negligible concentrations, the tocopherols can react directly with alkyl radicals ($L^{\bullet}$):

$$TOH + L^{\bullet} \longrightarrow TO^{\bullet} + LH \quad [11]$$

The oxidation of α-tocopherol by the reactive oxygen species, hydroxyl ($^{\bullet}OH$), perhydroxy ($^{\bullet}OOH$), and superoxide ($O_2^{\bullet-}$) radicals, was studied in miscelles and liposomes (183). The hydroxy radicals were efficient oxidants of α-tocopherol through an indirect reaction, due to their low diffusibility. The $HO^{\bullet}$ first react with the solvent (ethanol, membrane lipids, etc.) molecules in their vicinity compared to the tocopherol molecules (Eq. 12) and the solvent radicals will afterward oxidize the tocopherol molecules (Eq. 13):

$$OH^{\bullet} + SH \longrightarrow H_2O + S^{\bullet} \quad [12]$$

$$S^{\bullet} + TOH \longrightarrow SH + TO^{\bullet} \quad [13]$$

The $HO_2^{\bullet}$ and $O_2^{\bullet-}$ radicals are always present in equilibrium (Lewis acid and base) in protonated solvents:

$$O_2^{\bullet-} + H^+ \rightleftharpoons HO_2^{\bullet} \quad [14]$$

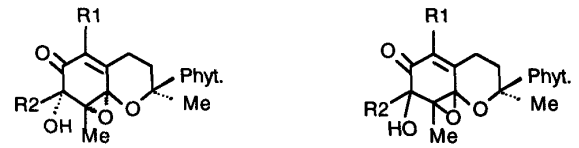
The oxidizing ability of the three radicals was in the order $OH^{\bullet} > HO_2^{\bullet} > O_2^{\bullet-}$. Although $HO_2^{\bullet}$ is reactive enough to attack lipids, the concentration ratio $O_2^{\bullet-}/HO_2^{\bullet}$ in protonated solvents and at neutral pH is 400. Under these conditions, the oxidation is very slow and it has also to compete with the dismutation (183).

$$2HO_2^{\bullet} \xrightarrow{H^+} H_2O_2 + O_2 \quad [15]$$

The reaction of α-tocopherol with the superoxide anion ($O_2^{\bullet-}$) was studied in the absence of other lipids where different products were obtained in protic and aprotic solvents (184,185). In aprotic solvents *cis*-7-hydroxy-*cis*-8,8a-epoxy-α-tocopherone **[XXXIII]** and *trans*-7-hydroxy-*trans*-8,8a-epoxy-α-tocopherone **[XXXIV]** were the major products of α-tocopherol oxidation by $O_2^{\bullet-}$ (184,185).

Under protic conditions, the reaction of α-tocopherol with $O_2^{\bullet-}$ produced α-tocopherol ethane dimer **[XVI]**, α-tocopherol-α-tocopheroxy dimer **[XVIII]**, α-tocopherol quinone **[XVII]** (Fig. 4), and α-tocopherylquinone-4a,5-epoxide **[XXXV]**.

Nishikimi *et al.* (186) studied the oxidation of tocopherols, in a water-based model at physiological pH, by $O_2^{\bullet-}$ generated by a xanthine-xanthine oxidase system. All tocopherols (α-, β-, γ-, and δ-) were oxidized to the corresponding tocopherol quinones. In case of α- and β-tocopherols the oxidation proceeded through an intermediate 8a-hydroxy-tocopherone (187).



cis-7-hydroxy-*cis*-8,8a-epoxy-α-tocopherone **[XXXIII]**

trans-7-hydroxy-*trans*-8,8a-epoxy-α-tocopherone **[XXXIV]**

SCHEME 1

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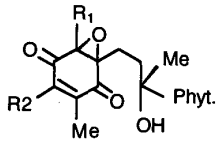

α-tocopheryl quinone-4a,5-epoxide [XXXV]

SCHEME 2

TOCOPHEROLS AS SINGLET OXYGEN QUENCHERS

The tocopherols not only inhibit free radical-induced lipid autoxidation, but they also inhibit the oxidations induced by the electronically excited singlet oxygen ($O_2{}^1\Delta_g$) (188–202) both *in vivo* and *in vitro*. Tocopherols react with singlet oxygen either by physical quenching or by chemical reactions. The balance of these processes depends on the properties of an initially formed exciplex to which three options were offered: reversion to reactants, collapse to ground state *via* intersystem crossing, and evolution to products (197). As a rule, physical quenching predominates but chemical quenching is by no means a negligible process, at least in polar solvents (202). In physical quenching, excited state singlet oxygen ($O_2{}^1\Delta_g$) is deactivated to ground state triplet oxygen ($O_2 \cdot {}^3\Sigma_g$) through a charge transfer mechanism. The charge transfer mechanism involves donation of an electron by the tocopherol to the electron-deficient singlet oxygen forming a charge transfer exciplex. The exciplex then undergoes intersystem crossing and then dissociates into a tocopherol molecule and a molecule of triplet oxygen (Eq. 16) (191,193,203)

$$TOH + {}^1O_2 \xrightarrow{K_q} [TOH^{\delta+} - {}^1O_2{}^{\delta-}] \rightarrow [TOH^{\delta+} - {}^3O_2{}^{\delta-}] \rightarrow TOH + {}^3O_2 \quad [16]$$

The rates of physical quenching (*Kq*) of singlet oxygen by the four tocopherols (α-, β-, γ-, and δ-) were reported as 4.2×10^7, 2.3×10^7, 1.1×10^7, and 0.5×10^7 $M^{-1}s^{-1}$, respectively (198). When the reactivity of different tocols toward singlet oxygen was compared, the relative effectiveness of α-, β, γ-, and δ-tocopherols as singlet oxygen quenchers was 100:55:26:10, respectively (188,191). The quenching efficiency of the four tocopherols was, thus, in the decreasing order $\alpha > \beta > \gamma > \delta$ (191,198,199). Recently Kough and Min (200) studied reactions of α-, γ-, and δ-tocopherols with singlet oxygen in methylene chloride containing 1.0×10^{-5} M chlorophyll under light at 25°C for 60 min. The three isomers were lost at the following rates 6.6×10^{-6}, 5.0×10^{-6}, and 2.9×10^{-6} M/min, at ratios of 21, 16, and 9%, respectively. Since the reactivity of the tocopherols with singlet oxygen was in the same order as their vitamin E activity, Grams and Eskins (188) concluded that singlet oxygen reactivity is the mechanism by which tocopherols inhibit lipid oxidation *in vivo*. The effect seems dependent on the free phenolic hydrogen since the presence of an ether or ester bond eliminated the activity (199). If reactions with singlet oxygen were run for long times, γ- and δ-tocopherols may prove more effective than α-tocopherol since the effects of γ- and δ-tocopherols can remain for longer times because of their higher stability to photosensitized oxidation (193).

Further, the tocopherols also react chemically with singlet oxygen and get destroyed after the reaction. The rate of physical quenching (*Kq*) of singlet oxygen by the tocopherols is several orders of magnitude higher than the rates of chemical reactions (191,197,204,205). Although tocopherols are poor quenchers (50-fold less) compared with the carotenoids (166,206,207), they can deactivate about 40–120 singlet oxygen molecules before they are destroyed (218,221). The chemical reaction proceeds through an intermediate hydroperoxide which will decompose to form the secondary products: the tocopherol quinone and the tocopherol quinone-4a,5-epoxide (193,196,198). Reaction products involving the "ene"-type mechanism were also isolated from the reactions of tocopherols with singlet oxygen (209,210). A scheme for the reported oxidation products of α-tocopherol by singlet oxygen is shown in Figure 5.

α-Tocopherol hydroperoxides formed in reactions with singlet oxygen were mentioned to generate free radicals and act as prooxidants in PUFA autoxidation (196,211,212). The effect of different reaction conditions and solvents on the type of oxidation products of the tocopherols by singlet oxygen needs further investigations. Fragata and Bellemare (205) found that the ratio *Kq/Kr* increases with decreasing lipophilicity of the medium. In a similar way, Gorman *et al.* (197) found the rate of physical quenching of ${}^1O_2$ by α-tocopherol to increase with increasing solvent polarity.

TOCOPHEROLS AS PROOXIDANTS

Effective antioxidants should generally yield radicals that are unreactive toward stable molecules (mainly molecular oxygen, lipid molecules, and lipid hydroperoxides) and that are limiting their reactions only to donation of hydrogen(s) to radicals and to radical-radical coupling. Unfortunately, this goal is not ideally achieved since the antioxidants and/or their radicals often undergo other side reactions which may be classified as prooxidative. The degree of such reactions is determined by different factors, mainly the antioxidant structure, concentration, temperature, etc. (54,56). These factors, which will be discussed in more detail in the next section, affect the absolute and relative potency of antioxidants and may explain the inconsistency in the reported relative activities of the different tocopherols *in vivo* and *in vitro*.

The prooxidant effect of α-tocopherol was related to its tocopheroxyl radicals (α-TO•) (40). This was based on the assumption that when TO• is present in high concentration, there is more possibility for a number of undesirable side reactions, which may initiate a reaction chain (40) or enhance the rate of peroxidation. The following reaction mechanisms were suggested for the prooxidant effects of α-TO•: (i) α-TO• was mentioned to react reversibly with unperoxidized lipids (LH) and with lipid hydroperoxides (LOOH) by chain-transfer generating alkyl (Eq. 17) and peroxy (Eq. 18) radicals, respectively:

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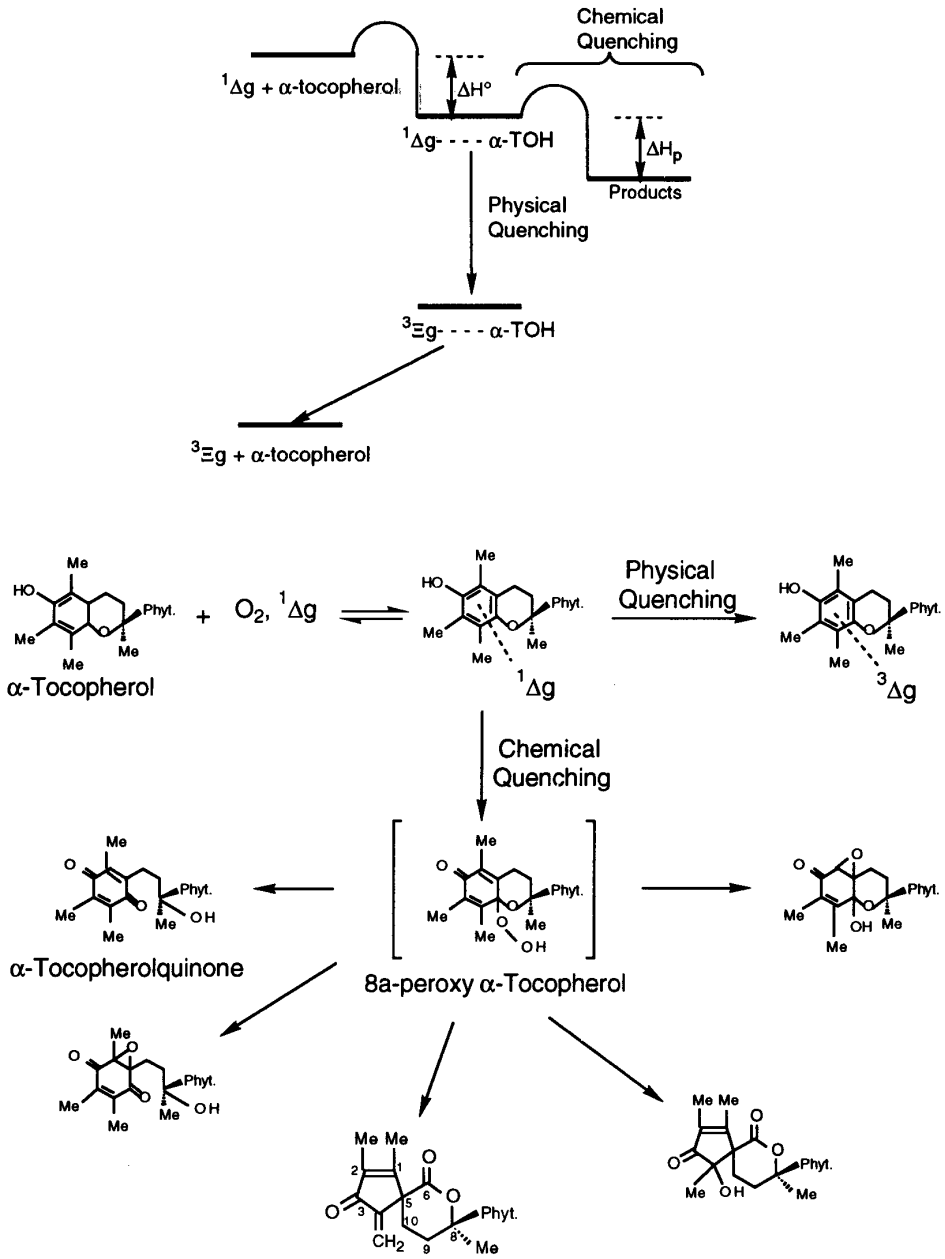

FIG. 5. The singlet oxygen quenching by α-tocopherol. The upper part shows a schematic enthalpy profile for the scavenging of singlet oxygen ($^1\Delta g$) by α-tocopherol. The physical quenching process involves a horizontal nonradiative step and a relaxation step. The lower part shows the different products resulting from chemical quenching.

$$\alpha\text{–TO}^{\bullet} + \text{LH} \longrightarrow \alpha\text{–TOH} + \text{L}^{\bullet} \quad [17]$$

$$\alpha\text{–TO}^{\bullet} + \text{LOOH} \longrightarrow \alpha\text{–TOH} + \text{LOO}^{\bullet} \quad [18]$$

The rate constants of the reverse reactions of related α-tocopheroxyl radicals with the ethyl esters of oleic, linoleic, linolenic, and arachidonic acids (Eq. 17) were reported as 1.04×10^{-5}, 1.82×10^{-2}, 3.84×10^{-2}, and 4.83×10^{-2} $M^{-1}s^{-1}$, respectively (213). On the other hand, the rate constants for the reactions of α-tocopheroxyl radicals with the hydroperoxides of oleic, linoleic, linolenic, and arachidonic acids (Eq. 18) were in the range of $1.3–3.6 \times 10^{-1}$ M^{-1} s^{-1} (214). More recent studies on the kinetics of the abstraction of hydrogen by the α-TO• from fatty acids (215) or from

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lipid hydroperoxides (216) showed that while the rate constant of the forward reaction of the α-TOH with the peroxy radical [5], *ka*, was $3.2 \times 10^6\ M^{-1}s^{-1}$, that of the backward reaction of the α-TO• with the lipid hydroperoxide, k_{-a}, was $5 \times 10^{-1}\ M^{-1}s^{-1}$ (Eq. 18). On the other hand, the rate of hydrogen abstraction from a PUFA by the α-TO•, K_3, was six times less than K_{-a} (Eq. 17). Moreover, the facts that these reactions cannot be significant in the presence of high concentrations of unoxidized tocopherol and that the rates of tocopheroxyl radicals bimolecular couplings were found to range from 3×10^3–$5 \times 10^5\ M^{-1}\ s^{-1}$ (80) suggest that the prooxidant effect of high concentrations of tocopherols in bulk oil phases is not due to the backward reactions (Eq. 17 and Eq. 18), which have very low rates compared to both antioxidation (Eq. 5) and tocopheroxyl coupling reactions (Eq. 10).

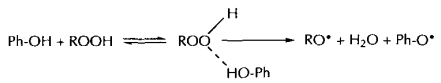
Some studies on LDL oxidations have, on the other hand, suggested that α-tocopherol actually accelerates the peroxidation of the LDL lipids under mild free radical conditions (217–219). A mechanism was proposed whereby LDL peroxidation is initiated by reactions of α-TOH, residing at or near the surface of membranes, with attacking aqueous radicals to give α-TO•. The inability of thus-formed α-TO• to escape from the LDL particle forces it to propagate the radical chain *via* its reaction with the PUFA within the particle. Since α-TO• radicals are localized far from each other and their mobility within the LDL particle is limited, tocopheroxyl coupling reactions (Eq. 10) cannot compete with reactions Equation 17 and Equation 18. This effect can, however, be eliminated if ascorbic acid is present in the aqueous phase outside the LDL particle.

(ii) The autoxidation rates and the prooxidant effect of the tocopherols also was mentioned to be enhanced by the presence of high initial levels of hydroperoxides (220). Hydroperoxide decomposition *via* unimolecular (Eq. 19) or bimolecular (Eq. 20) mechanisms seems responsible for the peroxide-catalyzed propagation of autoxidation reactions:

$$\mathrm{LOOH} \longrightarrow \mathrm{LO}^{\bullet} + \mathrm{OH}^{\bullet} \qquad [19]$$

$$2\mathrm{LOOH} \longrightarrow \mathrm{LO}^{\bullet} + \mathrm{LO}_2^{\bullet} + \mathrm{H_2O} \qquad [20]$$

Phenols were oxidized slowly by hydroperoxides (ROOH). The reaction apparently involves abstraction of a hydrogen atom from the phenol and scission of the O-O bond (99,221). The reaction is proceeded by H-bonding between the phenol and the peroxide:



[21]

In homogeneous solutions, the alkoxy radicals which are formed as a result of this reaction may propagate the autooxidation reaction after the complete consumption of the phenolic antioxidant. In the presence of lipid hydroperoxides, α-tocopherol is primarily oxidized to 8a-peroxy-α-tocopherones (α-TO-8a-OOL). These adducts are not stable in acid and are known to hydrolyze to α-tocopherol quinone **[XVII]** (Fig. 3). At low temperatures favoring hydrogen-bonding, tocopherol molecules can donate hydrogen atoms to lipid hydroperoxides (LOOH or LOOL) or to the α-TO-8a-OOL decomposing them to stable lipid alkoxides (LOH) plus alkoxy radicals (21) or to α-TO-O• radicals (Fig. 6), respectively. The 8a-oxy-α-tocopherone radical (α-TO-O•) is expected to be unstable and to abstract a hydrogen atom from any available α-TOH and to finally rearrange to α-tocopherolquinone **[XVII]**, which was reported to be formed during Fe(III)-catalyzed reactions between α-TOH and methyl linoleate hydroperoxides (222,223). These radicals (LO• and α-TO-O•) are not expected to play any significant prooxidative roles at the initial stage of autoxidation because they will be neutralized by α-TOH. However, we propose that this mechanism may be more significant than the first mechanism in bulk oils at low temperatures and at stages when the tocopherol/hydroperoxide ratio is equal to or less than one. If the conditions for such a reaction are fulfilled, α-tocopherol is expected to decompose LOOH at a faster rate compared to the other tocopherols due to its greater willingness to donate its phenolic hydrogen. The presence of the free, sterically unhindered position 5 in

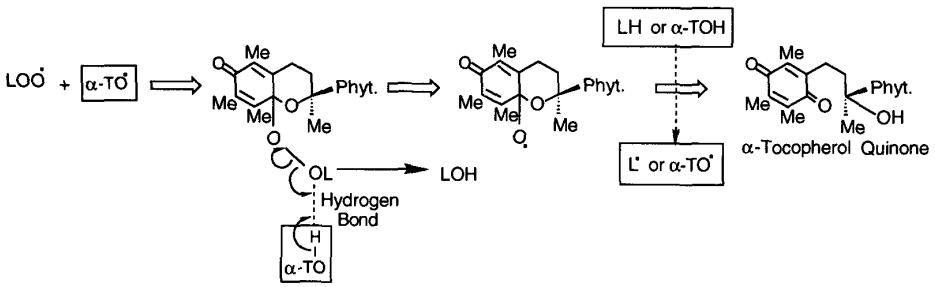


FIG. 6. A proposed mechanism for α-tocopherol-catalyzed decomposition of α-tocopheroxy-peroxy radical adducts at low temperatures.

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γ-tocopherol makes the pathway for the peroxy radical addition to this tocol different from that of α-tocopherol which may partially explain the observed higher antioxidant potency of γ-tocopherol in many *in vitro* systems (Fig. 4).

(iii) The tocopherols and the tocopheroxyl radicals also are known to reduce metal ions to lower state valences by donation of an electron (Eqs. 22, 23) (171,224,225):

$$\mathrm{TOH} + \mathrm{M}^{(n+1)+} \longrightarrow \mathrm{TO}^{\bullet} + \mathrm{H}^{+} + \mathrm{Mn}^{n+} \qquad [22]$$

$$\mathrm{TO}^{\bullet} + \mathrm{M}^{(n+1)+} \longrightarrow \mathrm{TO}^{+} + \mathrm{Mn}^{n+} \qquad [23]$$

While alcoholic solutions of α- and γ-tocopherols (at 1 mg/mL concentration) were stable for one year, no residual tocopherol was present after 30 d storage upon the addition of 50 ppm Cu^{2+} or Fe^{3+}. Metal chelators such as EDTA and citric acid gave some protection to the tocopherols, but only ascorbic acid prevented the destruction by acting as a reducing agent itself (226). The tocopherols, thereby, recycle transition metal ions, to act as prooxidants, by Fenton (Eq. 24) and Fenton-like (Eq. 25) reactions:

$$\mathrm{H_2O_2} + \mathrm{M}^{n+} \longrightarrow \mathrm{OH}^{\bullet} + \mathrm{OH}^{-} + \mathrm{M}^{(n+1)+} \qquad [24]$$

$$\mathrm{LOOH} + \mathrm{M}^{n+} \longrightarrow \mathrm{LO}^{\bullet} + \mathrm{OH}^{-} + \mathrm{M}^{(n+1)+} \qquad [25]$$

Although Fenton reactions are relatively slow ($k = 621\ \mathrm{M^{-1}s^{-1}}$) compared with chain propagation reactions (227), they are not slow for chain reinitiation, especially when lipid peroxides (or hydrogen peroxide), tocopherols, and trace metal ions are present in sufficient amounts.

(iv) Another chemical species that is always found in autoxidizing lipids is triplet molecular oxygen ($^3O_2$). As with most organic compounds, the direct reaction of the tocopherols with $^3O_2$ is spin-forbidden, and Denisov and Khudyakov (99) gave kinetic evidence that the reactions of the phenols with oxygen are less significant than their reactions with hydroperoxides. $^3O_2$ can, however, react with some radicals and Gottstein and Grosch (49) suggested that this reaction is the reason for the prooxidant effect of α-tocopherol (Eq. 26).

The formation of the α-tocopherol quinone **[XVII]** is expected to proceed *via* an 8a-hydroperoxy intermediate (187) for both α- and β-tocopherols. Again, the situation may not be the same for γ- and δ-tocopherols having a free *ortho*-position (C-5) for oxygen. At high oxygen pressures, the availability of a sterically unhindered *ortho*-position (position 5) in γ- and δ-tocopherols makes further oxidation of their tocopheroxyl radicals to γ-tocored **[XXXII]** possible.

Doba *et al.* (228), on the other hand, reported that oxygen lacks any effect on the α-tocopheroxyl radical decay and Burton *et al.* (80) also reported that this radical reacts very slowly with oxygen ($K_{25°C} = 6.5\ \mathrm{M^{-1}\ s^{-1}}$). However, in the presence of transition metal ions (which are recycled by α-tocopherol), the formation of lipid hydroperoxides and α-tocopherol-8a-peroxides from unsaturated lipids, α-TOH or α-TO• may be enhanced due to the generation of reactive $O_2^{\bullet -}$ or $HO_2^{\bullet}$ from $^3O_2$ (229). The following equations (27–29) summarize the metal-mediated autoxidation of lipid molecules (RH, including α-TOH) to lipid hydroperoxides:

$$^3\mathrm{O_2} + \mathrm{M}^{n+} \longrightarrow \mathrm{O_2^{\bullet -}} + \mathrm{M}^{(n+1)+} \qquad [27]$$

$$\mathrm{M}^{(n+1)+} + \mathrm{O_2^{\bullet -}} + \mathrm{RH} \longrightarrow \mathrm{ROOH} + \mathrm{M}^{n+} \qquad [28]$$

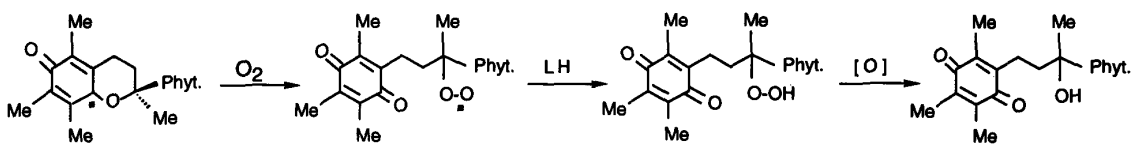
$$\text{sum} \quad {}^3\mathrm{O_2} + \mathrm{RH} \xrightarrow{\mathrm{M}^{n+}} \mathrm{ROOH} \qquad [29]$$

γ-Tocopherol is theoretically expected to show weaker prooxidant effects, if any, for its lower reduction effects on high-valent metal ions, lower relative reactivity with $O_2^{\bullet -}$, and the formation of 5-tocopheroxyperoxy adduct which will dissociate to γ-tocored **[XXXII]** and alkyl alcohol (LOH).

From the above discussion, although the α-tocopheroxyl radical can exert prooxidant effects in lipid micelles and membranes by direct reactions with unperoxidized lipids in the absence of a tocopherol-regenerating agent (e.g., ascorbic acid), a direct prooxidant effect of α-tocopherol in bulk oil phases seems unlikely. However, the α-tocopheroxyl radical can act as a prooxidant mainly in bulk oils by recycling metal ions to low-valent metals which can then act as real prooxidants by Fenton (or Fenton-like) reactions or by the generation of superoxide radical anions ($O_2^{\bullet -}$), which will again react with the tocopheroxyl radicals exerting a prooxidant action. In this case, the tocopherols cannot be considered as direct prooxidants but rather as cooxidants (or prooxidant synergists).

SOME MAJOR FACTORS AFFECTING TOCOPHEROL ANTIOXIDANT POTENCY: CONCENTRATION

Several investigators have demonstrated that the tocopherols (particularly α-tocopherol) act as prooxidants, when present in high concentrations in vegetable oils (47,58,59,230—233).



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The prooxidant effects of the tocopherols were attributed to the tocopheroxyl radical (TO•) (40). We tested the effects of α- and γ-tocopherols (at 0–2000 ppm concentrations) on the development of peroxides in purified sunflower and rapeseed oils (containing no tocopherols, no iron or copper, and no pigments) after incubation at 55°C for 1–3 d. Both tocopherols did not show any prooxidant effect after these incubations even at the very high concentrations used (Kamal-Eldin, A., and Appelqvist, L.-Å., unpublished results). This experiment confirmed the assumption that the tocopherols are not prooxidants in themselves but can act as prooxidant synergists (coprooxidants?) when present at high concentrations together with known prooxidants like transition metal ions, lipid peroxides, or other oxidizing agents.

The different tocopherols have different affinities toward these oxidation reactions in line with their oxidation–reduction potentials. Prooxidant effects of the order α → γ → δ-tocopherols were reported in soybean oil (52). Thus, each antioxidant/substrate combination has critical concentration ratios for the maximum stability. Below these critical concentration ratios, inhibition is below optimum and above which the antioxidant tocopherols may invert their effects and synergize the present prooxidants (40,233). This phenomena, called inversion of activity, seems to be related to redox potential where antioxidants with lower reduction potentials, e.g., ascorbic acid, show inversion of activity at lower concentrations than those with higher reduction potentials, e.g., δ-tocopherol (7,234,235).

The tocopherols were found to affect the rate of the initiation of PUFA autoxidation (*Ri*). γ- and δ-Tocopherols greatly decreased the value of *Ri* for methyl linoleate, independent of their concentrations. However, when α-, γ-, and δ-Tocopherols were added to ethyl eicosapentaenoate, or when α-tocopherol was added to methyl linoleate, *Ri* decreased up to a certain concentration and then started to increase with increasing tocopherol concentration. In such cases, the tocopherols showed only slight prooxidative effects (236).

If the tocopheroxyl radical is the real prooxidant species, the prooxidant activity of the tocopherols at high concentrations (46,51,52) and temperatures can be theoretically explained by applying equilibrium rules for the homolytic dissociation of the O-H bond:

$$\mathrm{TOH} \longrightarrow \mathrm{TO}^{\bullet} + \mathrm{H}^{\bullet} \qquad [30]$$

The equilibrium constant, K_e, for this reaction is given by Equation 31, and the concentration of the tocopheroxyl radical (TO•) by Equation 32:

$$K_e = \frac{[\mathrm{TO}^{\bullet}][\mathrm{H}^{\bullet}]}{[\mathrm{TOH}]} \qquad [31]$$

since [TO•] = [H•] and since [TO•] also decays by other mechanisms, then

$$[\mathrm{TO}^{\bullet}] \propto \sqrt{K_e[\mathrm{TOH}]} \qquad [32]$$

The magnitude of the equilibrium constant is linearly related to the temperature and the ease of O-H bond dissociation. The facility of O-H breakage is dependent on the structure as was explained previously, and the magnitude of k_e is in the order $k_\alpha > k_\beta > k_\gamma > k_\delta$. Thus, for the same concentration of tocopherol isomers under the same reaction conditions, the concentrations of the tocopheroxyl radicals will be α > β > γ > δ.

At 500 ppm in soybean oil, α-tocopherol had a net prooxidant effect, γ-tocopherol did not have noticeable effect, while δ-tocopherol was still having an antioxidant effect (52). α-Tocopherol also has been associated with enhanced tumor formation *in vivo* when taken in high doses (237). If tocopherols are added to lipids at the optimal concentrations required for maximum antioxidant potency, the order of activity will be in the order α > β > γ > δ (52,238). The optimum concentration varies from one substrate to another, and the natural amounts of tocopherols in natural systems are close to their optimal values (40). Molar antioxidant/substrate ratios under use in food industry are roughly in the range $1{:}10^3$ to $1{:}10^4$ (239).

TEMPERATURE

The antioxidant activity of the tocopherols was reported to be in the order α > β > γ > δ under low-to-mild temperatures and in the reverse order at higher temperatures, i.e., α < β < γ < δ (43,240,241). In contrast to these reports, Marinova and Yanishlien (242) recently found that the higher the temperature, the less the prooxidant effect of α-TOH, even at high concentrations. An explanation to this may be related to the fact that at high temperatures, oxygen has lower solubility in oils so that autoxidative peroxide formation proceeds at lower rates and becomes gradually substituted by polymerization reactions. Suppressing oxygen supply to autoxidizing lipids is known to have marked effects on the kinetics of the radical reactions between the antioxidant and the lipids (243). On the other hand, the higher the temperature, the higher the rate of hydroperoxide decomposition and the higher the reactivity of the transition metal ions and the greater the rates for redox reactions in general. The effect of temperature on the anti/prooxidant effect of the tocopherols seems to be highly related to the chemical composition and the other physical properties of the system.

LIGHT

In the presence of light and a suitable photosensitizer (e.g., chlorophyll), besides acting as free radical scavengers, tocopherols also react with singlet oxygen (188,193). Warner (244) studied the effects of adding α-, β-, γ-, and δ-tocopherols in various ratios on the photooxidative stability of stripped oils. Oils with higher levels of α-tocopherol had the best light stability but were the least stable after aging in the dark (60°C). This may be due to the previously mentioned differences in the actions of the tocopherols as free radical scavengers or singlet oxygen quenchers. In autoxidation re-

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actions (agitation at 60°C in the dark), the tocopherols may show prooxidant effect if trace metal ions are also present in the oil. The tocopherols were not mentioned to have prooxidative effects in their reactions with singlet oxygen. The efficiency of the tocopherols as singlet oxygen quenchers (in the light) was mentioned to have a direct correlation with their concentrations (189).

SUBSTRATE

Another factor that determines relative antioxidant potency is the nature of substrate or the medium in which activities were compared (54,56,245). The tocopherols were more effective in animal fats than vegetable oils (245). Thus, lipids with lower contents of tocopherols, such as animal fats and synthetic lipids used in the cosmetic industry, can be stabilized very well with the addition of tocopherols (35,50). Interestingly, Yuki and Ishikawa (246) found that among nine vegetable oils, those with higher levels of PUFA showed the least tocopherols loss during a frying test. This high stability of the tocopherols in highly unsaturated substrates may be explainable by the fact that the higher the degree of unsaturation the more competitive the fatty acids are toward oxidation.

Stronger antioxidants (e.g., α-tocopherol) were, however, mentioned to be more potent in highly oxidizable substrates (246) and in the absence of prooxidants. The presence of synergists (e.g., other phenols, vitamin C, carotenes, amines, amino acids, etc.) is expected to increase the antioxidant potency of the tocopherols by regeneration or metal chelation. The presence of other phenolic antioxidants may decrease the antioxidant potency of the tocopherols at high concentrations. When α-tocopherol was added to lard alone, maximum stability was achieved at 0.05% level. With the addition of 0.01% butylated hydroxyanisole, α-tocopherol showed decreased stabilization as its concentration increased from 0.01 to 0.05% (247). On the other hand, the presence of prooxidants in the substrate will cause the tocopherols to act as prooxidant synergists as shown before. The reason for the inconsistency in relative tocopherol potency in different substrates cannot be due to differences in relative rates of tocopheroxyl radical generation and reactions but is, most probably, due to differences in physical conditions and/or different chemical factors in the different substrates.

MEDIUM, POLARITY, VISCOSITY, AND pH

The structure of the whole oxidizing system largely influences the antioxidant/prooxidant behavior of hydrogen-donating antioxidants. Porter *et al.* (248) and Porter (249), in their "polar paradox" theory, suggested antioxidants that are relatively polar and hydrophilic are more effective in low surface-to-volume ratio displays of lipids (bulk oils and fats whether vegetable or animal or synthetic esters of fatty acids) while compounds that are relatively nonpolar are more effective in high surface-to-volume lipid matrices (emulsions, micelles, and membranes). The findings of Takahashi *et al.* (250) that α-tocopherol did scavenge radicals initiated in a lipid substrate by 2,2′-azobis(2,4-dimethylvaleronitrile) but less efficiently than radicals initiated in water by 2,2′-azobis(2-amidinopropane)dihydrochloride, is in support of the Porter theory (248,249). Recently, Frankel *et al.* (251) showed that the lipophilic antioxidants, α-tocopherol and ascorbyl palmitate, were more effective in an oil-in-water emulsion system than in bulk oil, while the opposite trend was found for the hydrophilic antioxidants trolox and ascorbic acid. This paradoxical behavior is related to the surface activity of antioxidants in biphasial system. Related to these are the previous findings that the antioxidant activity of α-tocopherol was found to be markedly reduced in micelle systems (252) and liposomal membranes (253) compared with homogeneous solutions. Castle and Perkins (254) proposed that the antioxidant activity of α-tocopherol in SDS micellar system was limited due to the fact that its intermicellar diffusion (i.e., the frequency with which it visits each micelle) may be slow compared to the lifetime of peroxy radicals which diffuse freely from one micelle to another. They found that trolox esters which are more hydrophobic than α-tocopherol were better antioxidants in this micellar system.

The polarity of the medium has a significant effect on the antioxidant/prooxidant behavior of the tocopherols. The prooxidant effect of α-tocopherol on linoleic acid was higher in aqueous media than in other polar protic (e.g., ethanol), polar aprotic (e.g., dimethyl sulfoxide, acetonitrile), and nonpolar solvents (e.g., hexane) (47,58,59,255). The prooxidant effect of α-tocopherol in aqueous systems (59) may be due to the high solubility of lipid peroxyl radicals and other prooxidants. Sumarno *et al.* (87) also showed that the oxidation of α-tocopherol is influenced by the nature of the solvent in which the reaction is carried out. Tertiary-butyl hydroperoxide in chloroform failed to oxidize *a*-tocopherol at 60°C for 3 h. Inclusion of a small amount of ethanol in the reaction mixture brought about immediate oxidation and formation of 5-ethoxymethyl-7,8-dimethyl tocol, 5-formyl-7,8-dimethyl tocol together with the spirodimer **[XIX]** and the spirotrimer **[XX]** of α-tocopherol. Ethanol, thus, acted as a solvating agent and as a participating reactant. The low antioxidant potency of α-tocopherol in protic solvents was related to the fact that hydrogen bonding occurs between the solvent and the phenolic group and thereby inhibits the antioxidant potency (256). The pH of the polar medium may be of interest since lipid peroxides are known to decompose at higher rates at high temperature and low pH in the presence of transition metals (257).

High water activity could enhance the metal catalysis by increasing the mobility of the metal catalyst and facilitating the reduction of metals by the tocopherols (Eq. 19). On the other hand, water-soluble antioxidants or synergists (SH) may be able to regenerate the tocopherols in the presence of low levels of water (Eq. 33) and the stability of the oil may be enhanced further.

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$$SH + TO^{\bullet} \longrightarrow TOH + S^{\bullet} \quad [33]$$

The presence of small amounts of water also has protective effects against hydroperoxide decomposition (12) by a mechanism involving both the solvation of the hydroperoxides and hydration of the metals catalyzing their decomposition (258).

The rates of the reaction of tocopherol with the peroxy radical (Eq. 5) also were reported to be dependent on the viscosity of the substrate (259) where highly viscous substrates/solvents seem to slow down this major antioxidation reaction. The rate constants for the antioxidation reaction of α-tocopherol with peroxy radicals (K_a, Eq. 5) were found to decrease when reactions were carried out in cyclohexane (υ = 0.32), *n*-dodecane (υ = 1.35), *n*-octanoic acid (υ = 7.5), and oleic acid (υ = 38), in respective order.

TOCOPHEROL INTERACTIONS WITH OTHER ANTIOXIDANTS AND SYNERGISTS

When more than a single component (antioxidant) is present in an oxidizing lipid system, their net antioxidant effect is frequently more than the sum of their individual effects (a phenomenon known as synergism). We can recognize at least three mechanisms by which synergism to the tocopherols can be explained.

The first mechanism, which involves some sort of tocopherol "sparing," occurs when the tocopherol is present with another real antioxidant working by the same or by a different mechanism (i.e., a radical scavenger or a singlet oxygen-quencher). In the case of another hydrogen donor, the effect may be the same as when having a higher tocopherol concentration. If the tocopherol is present alone, the relation between its concentration and its antioxidant effect is not expected to be linear but of a polynomial order (in the same way as the autoxidation reactions of PUFA). Thus, it may not be of great significance to have another antioxidant working by the same mechanism. It is important to note here that Parkhurst *et al.* (45) found that mixed tocopherols (α-, γ-, and δ-) gave better protection to lard than either alone and suggested some synergistic interaction. Phospholipids having a primary amine moiety (e.g., phosphatidylethanolamine and phosphatidylserine) and sulfur-containing amino acids (cysteine, cystine, and methionine) also were mentioned to function as peroxy radical scavengers (260–262) and, thereby, can have a sparing effect on the tocopherols. However, significant synergistic effects may be obtained when the other antioxidant is working by another mechanism, e.g., singlet oxygen quenching by β-carotene (263). In this case, the optimal conditions for the synergistic interaction (e.g., low oxygen pressure in case of β-carotene) have to be considered. β-Carotene is known to act as an antioxidant at low oxygen pressure but to have prooxidant effects at high oxygen pressure (264,265). Thus, α-tocopherol and β-carotene may play complementary roles or even show synergism in systems of low oxygen pressure (e.g., *in vivo*) while β-carotene can lead to a net prooxidant effect, even in the presence of tocopherol, in a high oxygen atmosphere.

The second mechanism operates when the tocopherol is present together with other substance(s) which is/are capable of "regenerating" it from its radicals or oxidation products (e.g., vitamin C or glutathione). Ascorbic acid was mentioned to regenerate α-tocopherol from its tocopheroxyl radical *in vivo* and *in vitro* and thereby restores its antioxidant activity (149,150,180,266–286). Glutathione is also capable of regenerating α-tocopherol from its radical (149,150,268). Phosphatidylethanolamine also was found to synergize α-tocopherol at high temperatures by regenerating it from α-tocopherylquinone (287). In this case, the "side effects" of these reducing regenerators have to be considered. Ascorbic acid, for instance, is also a strong reductant for trace metal ions and thereby shows prooxidant effects in many cases (288). The experimental evidences for the synergistic interaction between ascorbic acid and α-tocopherol were obtained from *in vivo* systems or from ESR studies of radicals of the two chemicals in solution. *In vivo*, the lipids are highly structured, and the interactions of α-tocopherol and other lipids with trace metals are minimal. Thus, it is of great importance to determine the exact conditions under which ascorbic acid can act as an antioxidant synergist to the tocopherols, if reference has to be made for conditions *in vitro*.

The third mechanism by which many synergists work is through "trace metal chelation." Tocopherols showed synergistic effects with phospholipids (289–296) which are mainly due to the metal-chelating properties of the latter (297). Phoshatidylcholine and phosphatidylethanolamine do not form inactive complexes with heavy metals, but phosphatidylinositol and other acidic phospholipids do (40). Amino acids and lower peptides also were mentioned to have synergistic interactions with the tocopherols because of their metal-chelating activity (298–300). Fifteen amino acids (in free and esterified forms) were tested for their antioxidant effects in lard with and without tocopherols (301). No antioxidant effect was observed in the absence of tocopherols. In the presence of tocopherols, all esters and free methionine, phenylalanine, proline, and tryptophan were active. Maillard reaction products (called melanoidins) produced by reactions of reducing sugars with amino acids were good inhibitors of linoleic acid oxidation, and acted as synergists with the tocopherols (302). The antioxidant properties of the melanoidins were partially related to their metal-chelating properties, but other mechanisms also may be involved (303). Citric acid is a useful chelating ingredient especially when copper ions are present (40).

Recently, a fourth mechanism was proposed by Kago and Terao (304) by which the phopholipids enhance the antioxidant potency of the tocopherols through a physical rather than a chemical action. These workers postulated that the phospholipids form reverse micelles (or microemulsions) when dissolved in organic solvents or bulk oils and that tocopherols are solubilized and positioned in these microemulsions with their active phenolic group near the polar region where peroxy radicals will be concentrated.

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THE EFFECT OF TOCOPHEROLS ON THE RELATIVE ABUNDANCE OF LIPID HYDROPEROXIDES

The tocopherols not only inhibit the peroxidation rates, but they also affect the relative abundance of the products, leading to an increase in the level of monohydroperoxides having conjugated structures (230,231). Further, they affect the thermal decomposition products of these hydroperoxides (305). In the absence of hydrogen donors, the hydroperoxy radicals are known to undergo rearrangement to isomers in which the hydroperoxy group is relocated and the stereochemistry of the double bond is changed (306,307). A mechanism for the autoxidation of LH explaining the effect of phenols on the stereochemistry (*cis-trans* /*trans-trans* ratio) of the peroxide isomers formed was proposed by Porter *et al.* (308). The effects of the tocopherols are explained by the fact that due to facile hydrogen donation, initially formed lipid peroxyl radicals are trapped before they can rearrange or undergo other radical reactions.

The autoxidation of methyl linoleate, in absence of tocopherols, usually gives a mixture of approximately equal proportions of four hydroperoxide isomers: 13-hydroperoxy-9-*cis*,11-*trans*-octadecadienoic (13-*cis-trans*, 23%), 13-hydroperoxy-9-*trans*,11-*trans*-octadecadienoic (13-*trans,trans*-, 28%), 9-hydroperoxy-10-*trans*,12-*cis*-octadecadienoic (9-*cis-trans*, 21%), and 9-hydroperoxy-10-*trans*,12-*trans*-octadecadienoic (9-*trans-trans*-, 28%). In the presence of 5% α-tocopherol, only 13-*cis-trans* (51%) and 9-*cis-trans* (49%) were obtained (231)

In the absence of tocopherols, methyl linolenate was found to oxidize to four inner hydroperoxides [12-*cis-trans* (6%), 13-*cis-trans* (7%), 12-*trans-trans* (2%), and 13-*trans-trans* (3%)] and four outer hydroperoxides [9-*cis-trans* (27%), 16-*cis-trans* (31%), 9-*trans-trans* (11%), and 16-*trans-trans* (13%)]. The addition of 5% α-tocopherol also led to an absolute formation of *cis-trans* isomers the four 9, 12, 13, and 16 hydroperoxides in approximately equal amounts (231). Thus, the proportion of the inner hydroperoxides increased from a total of 18 to 53% [12-*cis-trans* (29%) and 13-*cis-trans* (24%)] and the proportion of the outer hydroperoxides decreased from 82 to 47% [9-*cis-trans* (22%) and 16-*cis-trans* (25%)]. The differences in hydroperoxide formation in the absence and in the presence of 5% α-tocopherol are summarized in Figure 7. In the absence of hydrogen donors, the peroxy radicals of the inner 12- and 13-hydroperoxides were reported to undergo rapid 1,3-cyclization to form five-membered ring hydroperoxy epidioxides (231,309,310). This cyclization is a major pathway which accounts for the lower concentrations of the inner hydroperoxides compared to the outer hydroperoxides. At higher tocopherol concentrations and higher oxidation rates, dihydroperoxides will be formed at the expense of hydroperoxy epidioxides (309). The presence of 10% α-tocopherol led to selective formation of the 9,16-dihydroperoxide from methyl linolenate (311).

Autoxidation of methyl arachidonate in the absence of tocopherols produces twelve monohydroperoxides (six *cis-trans* and six *trans-trans* isomers) of the 15-, 12-, 11-, 9-, 8-, and 5-hydroperoxy positional isomers (230,312). The proportion of the outermost (15- and 5-) monohydroperoxide isomers is much higher than that of the innermost (9 and 11) isomers which is higher than that of the middle (8 and 12) isomers. In the presence of 1–5% α-tocopherol, the isomeric composition of the six arachidonate hydroperoxides was approximately homogeneous, and they were all in *cis-trans* configuration.

The qualitative and quantitative effects of α-tocopherol on the hydroperoxides will, of course, lead to consequent effects on their secondary decomposition products. Frankel and Gardner (305) found that the presence of α-tocopherol has affected both the total amount and the relative distribution of individual volatiles (pentane, hexanal, methyloctanoate, 2,4-decadienal, methyl-9-oxononanoate, and methyl-13-oxo-9,11-tridecadienoate) formed after thermal decomposition of methyl linoleate *cis-trans* and *trans-trans* 9- and 13-hydroperoxides.

TOCOPHEROLS AS ANTI-/PRO-NITROSATING AGENTS

Nitrous acid is another potent oxidizing agent that reacts with amines, forming the carcinogenic *N*-nitroso compounds. The rate of nitrosation in water is maximum at pH 3.0–3.4, close to the pK_a of nitrous acid (HONO; $pK_a = 3.14$ at 25°C). At higher pH, the concentration of nitrous acid decreases, and at lower pH the concentration of the unprotonated amine decreases (313). In moderately acidic aqueous solutions, the nitrosating agent is essentially nitrous anhydride, $N_2O_3^{\bullet}$, formed from 2 moles of nitrous acid.

Phenols generally react with nitrosating agents faster than amines. Phenols were reported to act as inhibitors for the nitrosation of amines at low pH (314), but may promote nitrosation of amines at higher pH (315). Both α- and γ-tocopherols were found to accelerate the disappearance on nitrite anion from acidic reaction mixtures (pH 2–4) with rapid disappearance at pH 2–3 (316). α-Tocopherol was more effective than γ-tocopherol in water miscible formulations at pH 1.3. The reaction products and inhibition mechanism are not yet fully understood, but α-tocopherol quinone **[XVII]** was at least one of the reaction products of α-tocopherol (317).

In the case of α-tocopherol, the reactions with nitrous acid had always yielded α-tocopherol quinone **[XVII]** and two moles of nitrogen monoxide gas:

$$\alpha\text{–TOH} + 2HNO_2 \longrightarrow \alpha\text{–TO.OH} + 2NO^{\bullet} + H_2O \qquad [34]$$

$$\alpha\text{–TOH} + N_2O_3^{\bullet} \longrightarrow \alpha\text{–TO.OH} + 2NO^{\bullet} \qquad [35]$$

The reaction is expected to proceed through the formation of *O*-nitroso derivatives at the phenoxy oxygen and/or at the oxygen atom in the *para*-position. These derivatives are highly unstable and can act as nitrosation catalysts for secondary amines.

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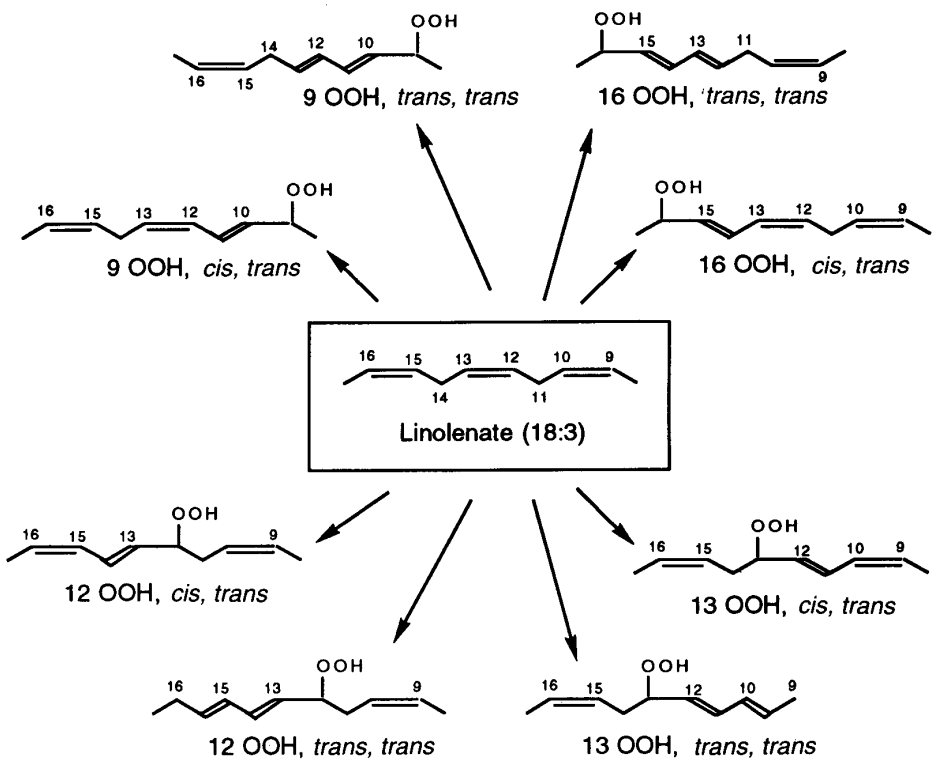

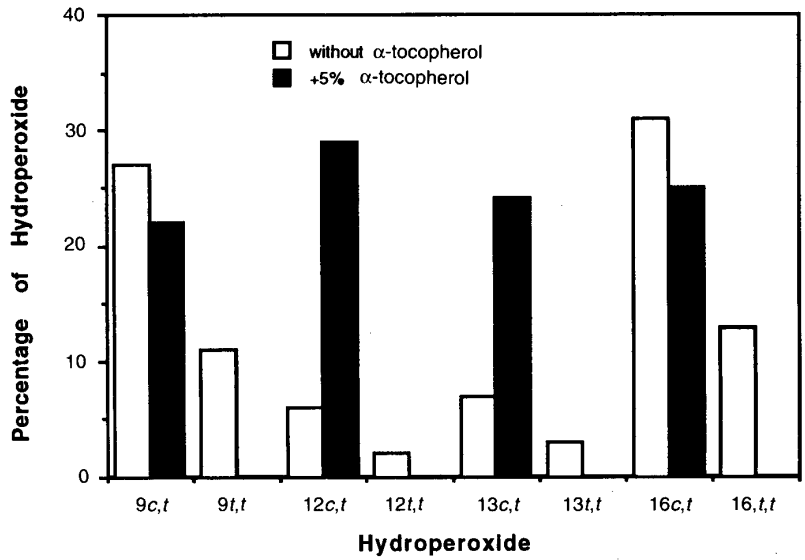

FIG. 7. The effect of α-tocopherol (α-TOH) on the relative abundance of linolenate hydroperoxides. The upper panel shows different hydroperoxides of linoleate oxidation in the absence of α-TOH. The lower panel shows the percentage distribution of the hydroperoxides in the absence and presence of 5% α-TOH. (The figure was adapted from data in Ref. 231.)

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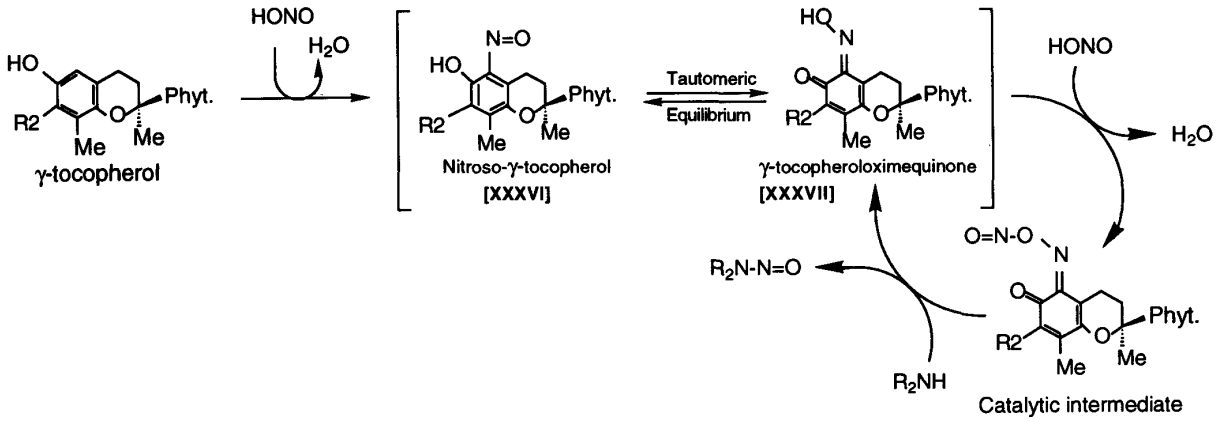

FIG. 8. The mechanism for the γ-tocopherol-catalyzed nitrosation of secondary amines by nitrous acid (HONO).

The full substitution of the aromatic ring of α-tocopherol is expected to be responsible for a fundamental difference in the reactivity of this homologue with nitrosating agents compared to the other tocopherols. The tocopherols with a free 5-position (γ- and δ-) are expected to react with nitrous acid and nitrites forming C-nitroso derivatives at this position (317). The nitrosophenols formed from γ-tocopherol are expected to exist in solution as a tautomeric mixture of nitroso-γ-tocopherol **[XXXVI]** and its γ-tocopherol oximequinone **[XXXVII]**. Phenolic oximequinones were reported to catalyze the formation of N-nitroso compounds (318–320). The reaction of γ- and δ-tocopherols with nitrous acid can be represented as shown in Figure 8.

Phenols generally inhibit nitrosamine formation, but they may catalyze the nitrosation under some conditions. The effect and its rate are dependent on the pH and the relative concentrations of phenol and nitrosating agent (315). It is of great importance, in this connection, to note that these types of C-nitrosophenols are relatively stable toward reactions with nitrosable substrates (e.g., amines) and seem to catalyze nitrosation only when present in low concentration compared to the nitrosating agent (315).

Since nitrous acid is water-soluble, reactions of the tocopherols with other, lipophilic, nitrosating agents (e.g., NO, N_2O_3, NO_2, N_2O_4) may be of greater importance. Such reactions may be of great importance in water–lipid interface systems (e.g., membranes, emulsions, etc.) where the lipophilic nitrosating agents can be generated in the water phase and then migrate to the lipid phase (321). The different nitrosating agents and their interactions in the aqueous and lipid phases are represented in Figure 9.

Nitric oxide (NO) is an important biochemical free radical in a variety of animal and human tissues (322). NO was reported to oxidize α-tocopherol to tocopheroxy radical which, subsequently, couples with excess NO forming an adduct. The stability of the tocopheroxy-NO adduct depends on the stability of tocopheroxy radical. It is expected that "more stable" radicals would form a weaker NO bond and dissociate reversibly, more rapidly and more completely in an equilibrium situation. Thus, α-tocopheroxy radicals could function as NO carriers in biological systems (322).

Mechanisms for the reactions of the tocopherols with NO_2 are shown in Figure 10. Reaction mechanisms are again expected to differ between α- and γ-tocopherols. In case of γ-tocopherol, nitrogen dioxide may add to the C_5 position as a nitrogen-centered radical ($^{\bullet}NO_2$), forming a nitro derivative which is rather stable, or as an oxygen-centered radical ($^{\bullet}ONO$), forming an *ortho O*-nitroso derivative which is relatively unstable (323). In case of α-, and probably β-tocopherol, the nitrosating agent only has the possibility to add to the *para*-position, forming a *para O*-nitroso derivative (324). These compounds are expected to be highly unstable and can serve as intermediates in the formation of toxic *N*-nitroso-derivatives from amines (324).

If tocopherols' relative antioxidant potency is to be related to the their ability of donating a hydrogen atom to the peroxy radi-

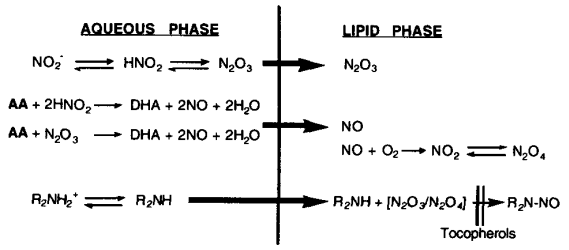

FIG. 9. A schematic representation of the nitrosation reactions in biological systems. NO, N_2O_3, NO_2, and N_2O_4 are different nitrogen oxides, AA = ascorbic acid, DHA = dihydroascorbic acid, R_2NH = a secondary amine, and R_2N-NO = *N*-nitrosoamine (modified from Tannenbaum and Mergens, Ref. 319).

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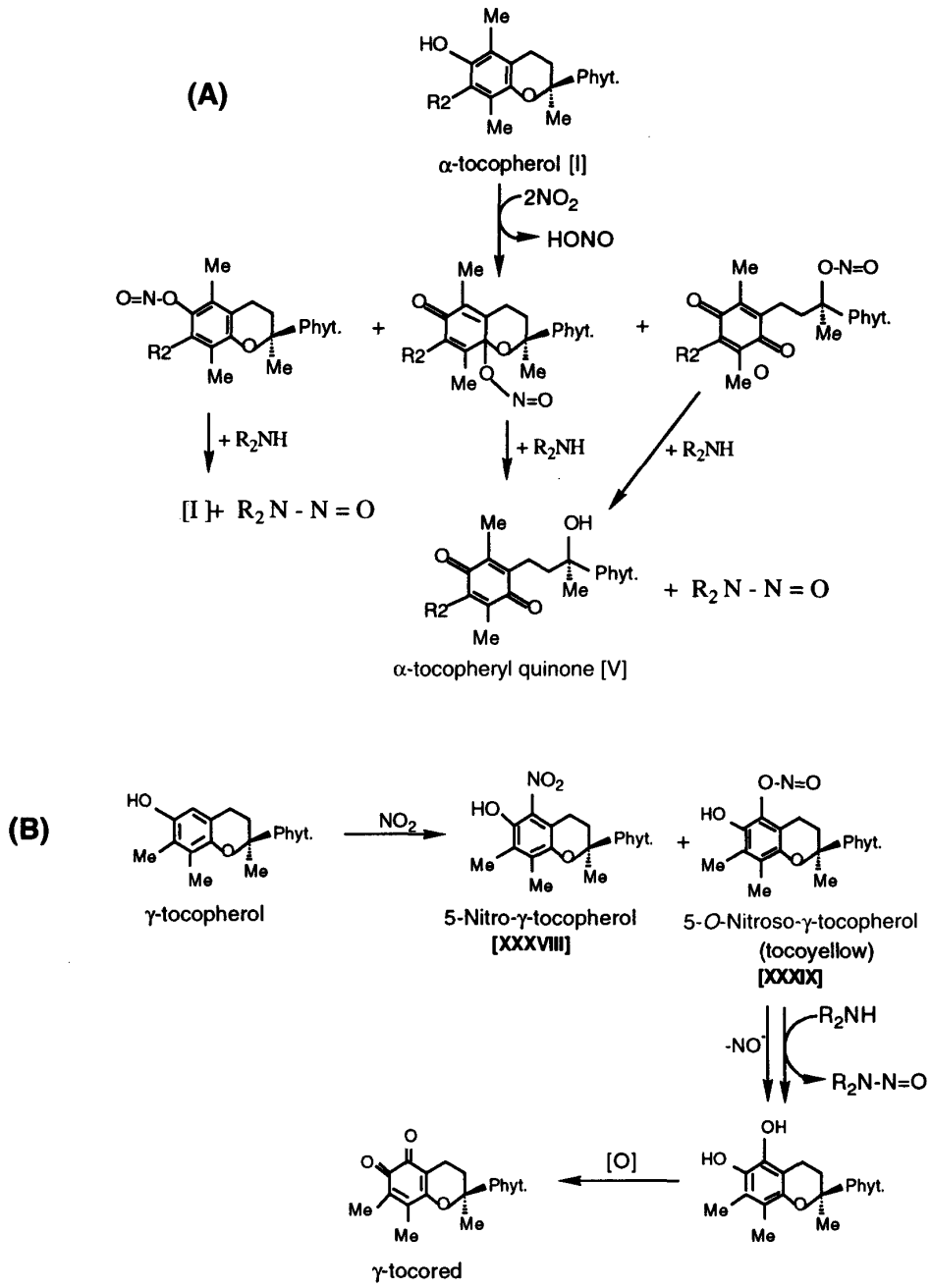

FIG. 10. Reactions of α-tocopherol (A) and γ-tocopherol (B) with NO_2.

cal, then α-tocopherol is absolutely the most potent homologue. However, a high hydrogen donation ability can cause many undesirable side reactions, e.g., reduction of trace metal ions and decomposition of hydroperoxides, that may lead to a net prooxidant effect. Moreover, the antioxidative potential of the tocopherols is also governed by all other chemical species in the lipid system, the physical parameters, and the structural build-up of the system (i.e., bulk phase, fat globule, membrane double-layer).

The differences in the antioxidant potency of the tocopherols *in vitro* and *in vivo* may be due to the major structural differences between the two systems (Table 4) or to differences in bioavailability which is due to differences in solubil-

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TABLE 4
Comparison of the Conditions *in vitro* Versus *in vivo*[a]

In vitro	*In vivo*
Temperature 45–65°C (?)	Temperature *ca.* 37°C
Higher oxygen pressures	Lower oxygen pressures
Tocopherols are present in contact with prooxidants	Tocopherols are buried in the membranes where they have structural protection.
Enzymes inactive	Antioxidant enzymes active

[a]A very important aspect to be considered is the differences between *in vito* and *in vitro* systems in regard to the main mechanism of tocopherol interaction (free radical scavengers or singlet oxygen quenchers). There is not enough literature to tabulate any difference in this respect.

ity and affinities to tissues and transporting proteins than to differences in chemical reactivity. While the *in vitro* antioxidant activity is highly dependent on the varying physical states and chemical compositions of the wide range of *in vitro* models (or systems), the *in vivo* vitamin E activity seems to be highly related to the lipophilicity of the vitamer. The number of methyl substituents on the phenolic ring and the structure of the phytyl tail (length, unsaturation, etc.) significantly affect the lipophilicity of the E-vitamers and thereby their transport within the body and retention in the different biocompartments. The observed differences between the *in vitro* and *in vivo* systems also may be due to differences in the relative importance of lipid oxidation mechanisms (radical-scavenging and singlet oxygen quenching). Of course, both reactions occur simultaneously and have a lot of interactions with each other, but the nature of the dominant reaction is expected to affect the tocopherol reactivity.

From a toxicological point-of-view, the best antioxidant should be the one that is active in as low a concentration as possible so that the concentration of the antioxidant radical will be at a minimum. If our hypothesis about the tocopherol-mediated catalysis of hydroperoxide formation and decomposition is correct, high concentrations of antioxidant radicals will certainly be problematic at some stage of the oxidation reaction. Nothing is known about the metabolism or the nutritional significance of oxidized tocopherols if foods containing them in high levels are eaten.

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The idea of writing this paper came after the question "Why is α-tocopherol a more potent antioxidant than γ-tocopherol *in vivo* and vice versa *in vitro*?" was posed by the faculty examiner, Dr. Michael H. Gordon (Department of Food Science and Technology, University of Reading, United Kingdom) during the defense of the Ph.D. thesis of the first author.

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