New Insights into the Oxidative Electrochemistry of Vitamin E

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ABSTRACT
A combination of electrochemical and spectroscopic experiments have proven that the α-, *β*-, *γ*-, and *δ*-forms (vitamers) of the tocopherols (vitamin E) undergo a series of chemically reversible proton- and electron-transfer steps in dry organic solvents, such as acetonitrile or dichloromethane, to form cationic compounds: the cation radical, the dication, and the phenoxonium cation. The cationic compounds are extremely unusual in their high persistence compared with what is presently known about the oxidative stability of other phenols, particularly the phenoxonium cation of α-tocopherol, which is stable for at least several hours in nonaqueous solvents and is formed quantitatively by oxidation of the starting material at an applied potential of approximately +0.5 V vs ferrocene$^{0/+}$ or with 2 mol equiv of NO^+.

1. Introduction

It is often argued that vitamin E's major role in mammalian tissues relates to its ability to act as an antioxidant, essentially preventing living cell membranes (lipids) from turning rancid and decomposing.[1−4] Vitamin E (TOH; Chart 1) has been proposed to inhibit (terminate) the autoxidation cycle by first reacting with an oxidized site in a cell membrane (LOO•) to yield a molecule of LOOH and the tocopheroxyl radical (TO•) (eq 1). Second, the TO• radical reacts with another LOO• radical (eq 2) so that overall one TOH molecule is able to inhibit two LOO• sites. The localization and mobility of TOH within the lipids is thought to be critical to its ability to function as well as its synergistic interactions with other species such as ascorbic acid, hydroquinones, and catechols.[3,4]

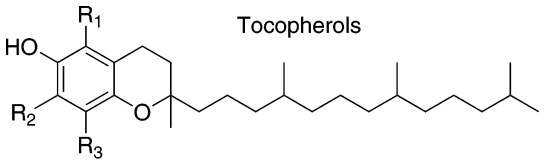
$$\mathrm{LOO^{\bullet} + TOH \rightarrow LOOH + TO^{\bullet}} \quad (1)$$

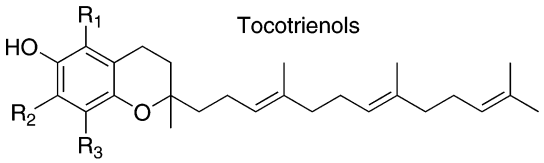
$$\mathrm{LOO^{\bullet} + TO^{\bullet} \rightarrow (TO)OOL} \quad (2)$$

While vitamin E is certainly capable of acting as a sacrificial compound to limit cell membrane deterioration, there is a growing body of evidence that it has other biological functions that involve specific interactions with other components of the cell, such as proteins and enzymes.[5−8] For example, protein kinases are a family of enzymes that regulate critical biological processes such as memory, hormone responses, and cell growth. Inhibition of protein kinase C (PKC) activity was found to be at the basis of vascular smooth muscle cell growth arrest induced by α-TOH.[7,8] *β*-TOH is ineffective at inhibition of PKC but prevents the inhibitory effect of α-TOH, whereas *γ*-TOH and *δ*-TOH have no effect on PKC.[6] The α-tocopherol transfer protein (α-TTP)[9] is a highly specialized protein in the liver that is responsible for delivering specifically α-TOH to very low density lipoprotein (VLDL), which is then transferred and delivered to peripheral cells.[5] There must exist an important mechanistic feature associated with α-TOH (that is not shared with the *β*-, *γ*-, and *δ*-vitamers) that is the reason for its preferential retention[10] and that causes it to react or interact in a special way with PKC. Other than the relatively small differences in rate constants for eq 1 measured in organic solvents[1] and the obvious difference in methyl substitution, there has been little discussion on chemical reasons why α-TOH differs from the other tocopherols.

In the following sections of this Account, the similarities and differences in the oxidative behavior of the α-, *β*-, *γ*-, and *δ*-tocopherols are discussed in relation to one another and in relation to other phenols. Most chemical studies on vitamin E have concentrated on its antioxidant properties and focused on the importance of the phenolic starting material and its associated phenoxyl radical.[1−4] The purpose of this Account is to illustrate that vitamin E undergoes a series of chemically reversible proton and electron transfers to form additional semistable oxidized compounds. It is reasoned that all of the oxidized forms of vitamin E should be taken into account when establishing chemical reasons for α-tocopherol's unique nonantioxidant biological function(s), because the *in vivo* behavior may not be entirely represented by eqs 1 and 2.[6−8,11]

Chart 1. Structures of Vitamin E





Tocopherol/ Tocotrienol	R_1	R_2	R_3
α-	CH_3	CH_3	CH_3
β-	CH_3	H	CH_3
γ-	H	CH_3	CH_3
δ-	H	H	CH_3

Richard Webster obtained his B.Sc. in Chemistry and Geology from the University of Auckland, New Zealand, and his Ph.D. in Chemistry in 1997 from La Trobe University, Australia, under the guidance of Alan Bond and Robert Cattrall. He received a Ramsay Memorial Fellowship to conduct postdoctoral research in the Physical and Theoretical Chemistry Laboratory at Oxford University working with Richard Compton. He held an Australian Research Council Queen Elizabeth II Fellowship at the Research School of Chemistry, Australian National University, between 2001 and 2006, before his present appointment at Nanyang Technological University in Singapore. He is interested in electron-transfer reactions in organic and inorganic systems and in developing spectroelectrochemical methods.

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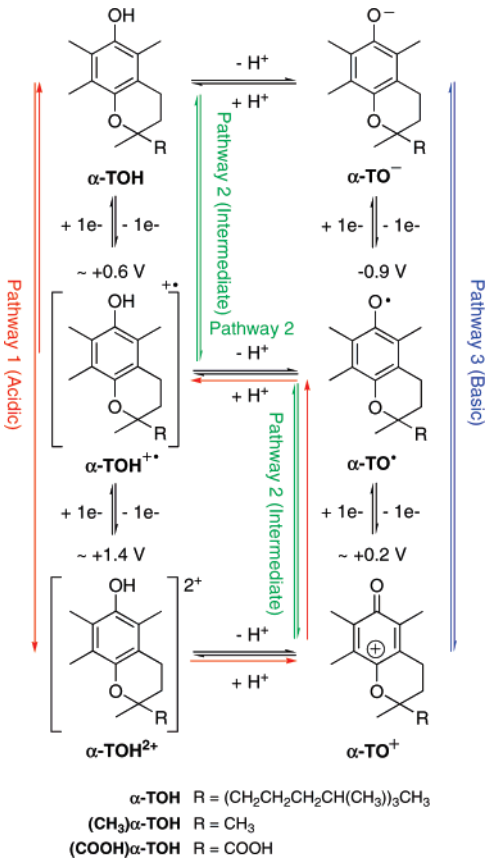
Scheme 1. Electrochemically Induced Transformations of α-TOH in Dry CH_3CN or CH_2Cl_2[15−17] *a*



a One resonance structure is displayed for each compound. The listed potentials (vs ferrocene$^{0/+}$) were obtained by voltammetry and are the approximate values necessary to bring about oxidation of the phenolic compounds but do not necessarily correspond to the formal potential. The counterions for the charged species are the supporting electrolyte cation [Bu_4N^+] and anion [PF_6^-], and the "H^+" ions exist coordinated to the organic solvent.

2. The Electrochemistry of Vitamin E

2.1. Electrochemistry of α-TOH. The electrochemical behavior of α-TOH has been established by detailed electrochemical and spectroscopic experiments in the aprotic solvents CH_3CN and CH_2Cl_2 and is represented through a series of proton- and electron-transfer steps (Scheme 1).[12−18] In this Account, only results that were obtained in pure organic solvents (with supporting electrolyte) and in organic solvents under dry acidic and basic conditions are discussed, since the absence of water drastically improves the stability of the oxidized species. Furthermore, organic solvents are likely to be closer to the natural environment of vitamin E, which exists *in vivo* in hydrophobic cell membranes, although cell membranes are themselves highly inhomogeneous media.[19]

Four heterogeneous one-electron-transfer steps can occur depending on the applied potential (eqs 3−6), as well as three homogeneous proton-transfer reactions involving the phenolic hydrogen ion (eqs 7−9). It is likely that the hydrogen ion released during the oxidation exists coordinated to the organic solvent molecules (i.e., [CH_3CNH]$^+$ or [CH_2Cl_2H]$^+$), with the supporting electrolyte acting as the counteranion.[15−18]

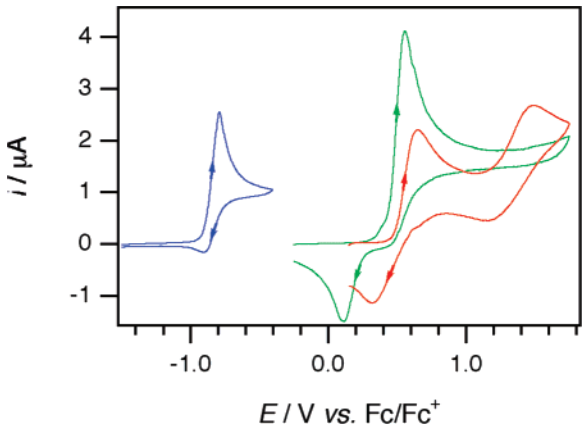


FIGURE 1. Cyclic voltammograms of 2 mM substrates at a scan rate of 100 mV s^{-1} at a 1 mm diameter planar Pt electrode in CH_3CN with 0.25 M Bu_4NPF_6: (green line) α-TOH; (red line) α-TOH with 0.1 M CF_3SO_3H; (blue line) α-TO^- prepared by reacting α-TOH with 2 mM Et_4NOH. Modified from data in ref 15.

$$\alpha\text{-TOH} - 1e^- \rightleftharpoons \alpha\text{-TOH}^{+\bullet} \quad (3)$$

$$\alpha\text{-TOH}^{+\bullet} - 1e^- \rightleftharpoons \alpha\text{-TOH}^{2+} \quad (4)$$

$$\alpha\text{-TO}^- - 1e^- \rightleftharpoons \alpha\text{-TO}^{\bullet} \quad (5)$$

$$\alpha\text{-TO}^{\bullet} - 1e^- \rightleftharpoons \alpha\text{-TO}^{+} \quad (6)$$

$$\alpha\text{-TOH} \rightleftharpoons \alpha\text{-TO}^- + H^+ \quad (7)$$

$$\alpha\text{-TOH}^{+\bullet} \rightleftharpoons \alpha\text{-TO}^{\bullet} + H^+ \quad (8)$$

$$\alpha\text{-TOH}^{2+} \rightleftharpoons \alpha\text{-TO}^{+} + H^+ \quad (9)$$

2.1.1. Pure CH_3CN and CH_2Cl_2 (Containing Bu_4NPF_6 as the Supporting Electrolyte). In acetonitrile in the absence of added acid or base, α-TOH can be voltammetrically oxidized at approximately +0.5 V vs Fc/Fc^+ (Fc = ferrocene) by one electron at solid electrodes to form the cation radical, α-$TOH^{+\bullet}$ (eq 3). α-$TOH^{+\bullet}$ quickly dissociates into α-$TO^{\bullet}$ (and H^+) (eq 8), which is immediately further oxidized at the electrode surface by one electron to form the brightly orange/red-colored phenoxonium cation, α-TO^+ (eq 6) (an ECE electrochemical mechanism, where E represents an electron transfer and C represents a chemical step; Scheme 1, pathway 2).[15−17] The reason α-$TO^{\bullet}$ is immediately further oxidized at the electrode surface is because it is approximately 0.4 V easier to oxidize than α-TOH; thus the cyclic voltammogram shows one process corresponding to a two-electron oxidation (Figure 1, green line). The true one-electron oxidation potential of α-TOH is close to +0.6 V vs Fc/Fc^+,[16] but because of the follow-up chemical and electrochemical reactions, the observed voltammetric wave is shifted to less positive potentials.

α-TO^+ is stable for at least several hours in the absence of water and can be reduced back to α-TOH on both the cyclic voltammetry (seconds or less) and controlled potential electrolysis (hours) time scales. α-TO^+ can also be

formed by homogeneous chemical oxidation of α-TOH with 2 mol equiv of NO^+ in CH_3CN.[16] There are only three other reports of phenols that form stable phenoxonium ions; two are organic compounds that have bulky groups in the 2- and 6-positions and an aromatic group in the 4-position,[20,21] and the third is a metal-stabilized phenoxonium complex, also with bulky groups in the 2- and 6-positions.[22]

The cyclic voltammogram displayed in Figure 1 in pure CH_3CN (green line) shows one anodic process and a cathodic process when the scan direction is reversed, albeit with a wide separation (~400 mV) between the forward and reverse peaks, indicating complicated electrochemical behavior. The presence of reverse peaks close to the main oxidation process is unusual for phenolic compounds, which normally display only chemically irreversible oxidation processes due to the oxidized compounds being very unstable and decomposing before they can be reduced back to the starting material.[15,23] In the case of α-TOH, the chemical reversibility in the voltammograms occurs because the phenoxonium cation is stable and because the equilibrium constant in eq 8 favors the protonation reaction. The K_{eq} value for the dissociation reaction in eq 8 has been estimated by digital simulations[24] of voltammetric data to be $\leq 1 \times 10^{-7}$ mol L^{-1} in CH_3CN at 243 K,[16] meaning that α-$TOH^{+\bullet}$ is very nonacidic (which is also highly unusual for phenolic compounds[23]). A further feature of the reaction in eq 8 is that the protonation must be fast in order to account for the complete chemical reversibility of pathway 2 in Scheme 1, because α-$TO^\bullet$ is not stable and reacts by a bimolecular self-reaction (eq 10).[25] Therefore, the protonation reaction in eq 8 must be much faster than the bimolecular reaction in eq 10; otherwise α-$TO^\bullet$ would dimerize before it was able to convert back to α-$TOH^{+\bullet}$ and subsequently be reduced back to α-TOH at the electrode surface. Digital simulation of voltammetric data indicated that the rate of the protonation reaction (k_b) in eq 8 is essentially diffusion controlled,[16] while the bimolecular reaction in eq 10 occurs at $\sim 1 \times 10^3$ L mol^{-1} s^{-1} [14] (which agrees with rate constants for eq 10 measured nonvoltametrically[25]). The rate constant for the protonation reaction in eq 8 is too fast to measure directly by electrochemical methods, and instead came from estimations of the ratio of the deprotonation rate constant (k_f) and equilibrium constant ($k_b = k_f/K_{eq}$).[16]

$$\alpha\text{-TO}^\bullet + \alpha\text{-TO}^\bullet \rightarrow \text{nonradical products} \qquad (10)$$

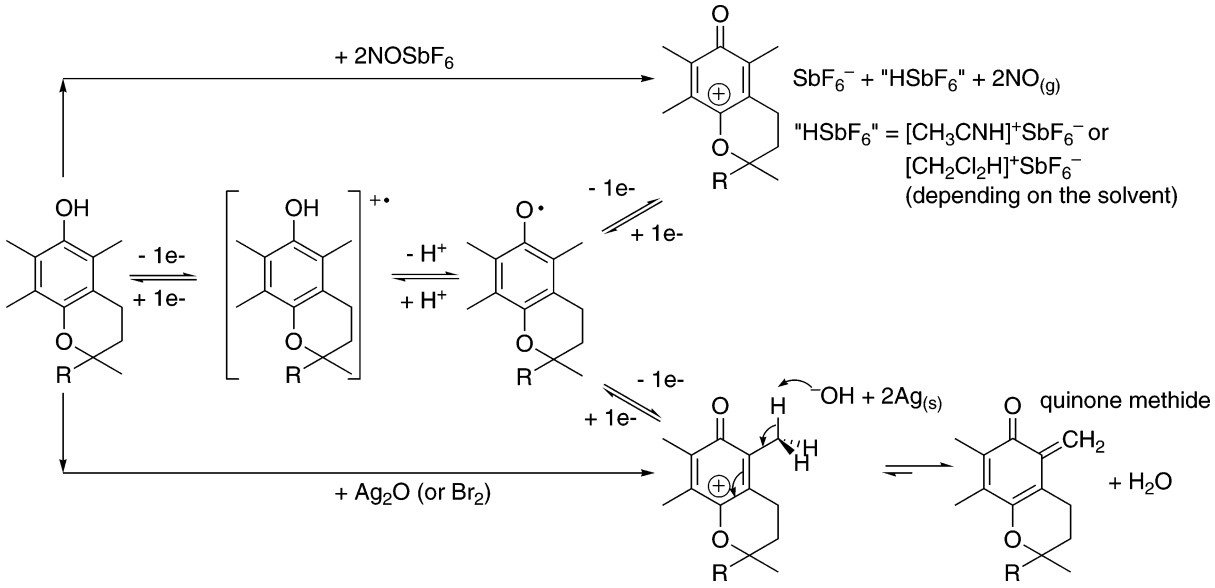
Because the potentials of the voltammetric waves are influenced by the kinetics of the homogeneous reaction (eq 8), the peak potentials (or half-wave potentials) of the cyclic voltammogram shown in Figure 1 (green line) do not correspond to the thermodynamic formal potentials ($E°$). The shapes of the cyclic voltammograms observed in pure CH_3CN or CH_2Cl_2 vary considerably as the solvent or temperature are changed because the rate and equilibrium constants in eq 8 also vary depending on the conditions. The voltammetric responses also change as the electrode surface is changed (Pt or GC) due to specific solute−electrode interactions and possibly due to changes in heterogeneous rate constants.[15−17]

2.1.2. Acidic Conditions. As acid (CF_3SO_3H or CF_3COOH) is added to solutions of α-TOH in CH_3CN or CH_2Cl_2, the equilibrium in eq 8 shifts toward the protonation reaction and α-$TOH^{+\bullet}$ becomes increasingly persistent.[12,15,17] At sufficiently high acid concentrations, the cyclic voltammetry behavior appears as a one-electron chemically reversible oxidation process at approximately +0.6 V vs Fc/Fc^+ (eq 3) due to complete inhibition of the deprotonation step (eq 8). It has been found that approximately 0.1 M CF_3SO_3H in CH_3CN or 1 M CF_3COOH in CH_2Cl_2 are needed to fully stabilize α-$TOH^{+\bullet}$, the difference in acid quantity relating to the degree of dissociation in the organic solvents. It is difficult to calculate the true concentration of "H^+" and hence the exact equilibrium constant in eq 8 because there will not be complete dissociation of the acids in organic media, especially in CH_2Cl_2 (although conductivity measurements performed on CF_3COOH in dichloroethane have indicated that significant dissociation does occur[26]). In strong acid conditions, the cation radical is able to be voltammetrically further oxidized by one electron to form the dication (α-TOH^{2+}) at approximately +1.4 V vs Fc/Fc^+ (eq 4), with a representative cyclic voltammogram displayed in Figure 1 (red line). The dication is not detectable in acid-free conditions (green line) because the intermediate monocation radical rapidly deprotonates. Therefore, the electrochemical behavior in strong acid conditions can be represented by pathway 1 in Scheme 1.

Electrolysis and spectroelectrochemical experiments have indicated that the dication is not stable for any more than a few seconds and immediately deprotonates to form α-TO^+ (eq 9), which is very stable in an acid environment and can be reduced back to the starting material in a two-electron, one-proton process.[12,17] However, in acid conditions the mechanism for the forward oxidation and reverse reduction reactions are different. The forward oxidation follows pathway 1 in Scheme 1, while the reverse reduction reaction follows the reverse of pathway 2, due to α-TOH^{2+} quickly deprotonating to form α-TO^+, which is then reduced to α-$TO^\bullet$ (which is immediately protonated to form α-$TOH^{+\bullet}$). The high stability of α-$TOH^{+\bullet}$ in mild acid conditions is unusual compared with most other phenols, which readily deprotonate even in strong acid conditions.[23]

2.1.3. Basic Conditions. The addition of an equimolar amount of organosoluble base (such as Et_4NOH) to CH_3CN solutions of α-TOH immediately induces the formation of the phenolate anion (α-TO^-),[13−15] which can be oxidized in two sequential one-electron steps to also form α-TO^+ (eqs 5 and 6; Scheme 1, pathway 3). Et_4NOH is commercially available as aqueous solutions, but small aliquots can be dried under vacuum to produce solid samples with a low water content.[14,15] α-TO^- is easier to oxidize than α-TOH by approximately −1.5 V, resulting in a substantial shift in potential of the voltammetric process and a decrease in anodic peak current due to a change from a two- to a one-electron oxidation process

Scheme 2. Intermediate Products Produced by Chemical Oxidation of α-TOH with $2NO^+$[16] or Ag_2O[31]



(Figure 1, blue line). Only a small cathodic peak is detected when the scan direction is reversed at slow scan rates during the oxidation of α-TO$^-$ to α-TO$^\bullet$ (Figure 1, blue line) because the phenoxyl radical is relatively unstable (eq 10). The voltammetric behavior observed for α-TOH in the presence of base is typical of most phenols, which are easily deprotonated to form the phenolate anions that are easier to oxidize than their corresponding phenols by *ca.* 1−2 V.[13−15,23,27,28]

2.1.4. Model Compounds. It was found that substituting the R group in Scheme 1 for a methyl group ((CH$_3$)α-TOH)[12,16−18] to form the simpler α-TOH model compound resulted in no change to the electrochemical behavior; thus the electrochemical responses shown in Scheme 1 are independent of the phytyl chain (and, therefore, not influenced by chirality effects). Some subtle differences were observed in the cyclic voltammetric behavior of the water-soluble analogue (COOH)α-TOH (Scheme 1), but electrolysis experiments (in organic solvents) indicated that the carboxylic acid derivative also underwent the same transformations as α-TOH.[16,17] Chirality effects are known to be very important in the biological function of vitamin E (since (*R*,*R*,*R*)-α-tocopherol is the most active), possibly by controlling a specific orientation adopted by α-TOH within the phospholipids.[3]

2.1.5. Long-Term Oxidation Products. Most preparative scale oxidative experiments conducted on vitamin E have been performed under conditions where none of the species in Scheme 1 were likely to be stable and resulted in a variety of products whose identities depended on the exact experimental conditions.[29] Furthermore, most long-term oxidation products are not readily converted back to vitamin E simply by reversing the applied potential, as occurs readily for the species in Scheme 1. One exception to this is a study that identified an *o*-quinone methide intermediate *via* oxidation of α-TOH in CH_2Cl_2 at −78 °C with Ag_2O.[30,31] The *o*-quinone methide was only stable for <10 s but could reportedly be converted back to α-TOH under reducing conditions.[31]

It is possible that the oxidative mechanism to account for the formation of the *o*-quinone methide also occurs *via* the phenoxonium cation (Scheme 2) although other nonradical reactions are also feasible. It is surprising that oxidation with Ag_2O (or Br_2) results in the formation of the *o*-quinone methide,[30,31] while oxidation with $2NO^+$ in CH_3CN or CH_2Cl_2 results in the formation of the phenoxonium cation[16] (which is stable in the absence of water and much more stable than the *o*-quinone methide). The reason for the immediate transformation of the phenoxonium cation to the *o*-quinone methide in the presence of Ag_2O or Br_2 may relate to the byproducts of the oxidation reaction (presumably HO^- or Br^-) reacting immediately with a methyl proton, which would need to be at least slightly acidic (Scheme 2).

2.2. Electrochemistry of *β*-, *γ*-, and *δ*-TOH. Voltammetric experiments have recently been performed on all the tocopherols under the same conditions as α-TOH.[17] It was found that *β*-TOH, *γ*-TOH, and *δ*-TOH undergo exactly the same mechanism as α-TOH (Scheme 1) with the most significant difference relating to the stability of the phenoxonium cations. Where α-TO$^+$ is stable in dry CH_3CN for at least several hours (longer at low temperatures), *β*-TO$^+$ is stable for several minutes, while *γ*-TO$^+$ and *δ*-TO$^+$ are stable for <1 s. Therefore, there is *at least* a 10^4 difference in stability between the different phenoxonium cations, demonstrating a significant difference in the oxidative behavior of the tocopherols beyond their proposed antioxidant functions. The 10^4 difference in stability of the phenoxonium cations can be compared to a <10-fold difference in the rates of reaction in eq 1 among all the tocopherols, which were measured in organic solvents.[1]

It is presently not clear why α-TO$^+$ and *β*-TO$^+$ have a much longer lifetime in solution than *γ*-TO$^+$ and *δ*-TO$^+$. Both α-TO$^+$ and *β*-TO$^+$ contain a methyl group in position R_1 (Chart 1), which interestingly is the group that reacts to form the *o*-quinone methide. It is also uncertain whether the phenolic methyl groups increase the stability

of the phenoxonium ions by a steric (by inhibiting reactions with potential nucleophiles) or electronic mechanism, since other phenols that contain bulky or electron-donating groups around the aromatic ring do not necessarily form stable phenoxonium cations when they are oxidized. It is thought that the kinetic stability of the individual phenoxonium cations (α, β, γ, and δ) is a property of the phenoxonium ions and not due to variations in the reactivity of the intermediate phenoxyl radicals. This conclusion is based on oxidative experiments performed in acid conditions (Scheme 1, pathway 1; eqs 3, 4, and 9), which still resulted in the β-, γ-, and δ-phenoxonium cations being unstable, even though their method of formation did not go through their intermediate phenoxyl radicals (Scheme 1, pathway 2; eqs 3, 8, and 6).[17]

2.3. Spectroscopic Characterization of Oxidized Compounds. A variety of spectroscopic data now exist for the compounds in Scheme 1, except for the dications, which have only been identified by cyclic voltammetry experiments.[12,15,17] UV−vis spectra of the cation radicals of all the tocopherols measured in CH_2Cl_2 with 1 M CF_3COOH[12,17] or CH_3CN with 0.1 M CF_3SO_3H[15] display two strong bands at ~22 000 cm^{-1} (with a shoulder) and 33 000 cm^{-1}. The UV−vis spectrum of α-$TOH^{+\bullet}$ was also obtained by pulse radiolysis experiments in hexane.[32] The UV−vis spectra of α-TO^+ (and model compounds) and β-TO^+ were similar to the cation radicals with absorbancies around 22 000 and 33 000 cm^{-1}, but the intensities and shapes of the bands were different.[12,15,17] Solutions of the phenoxonium cations were a vibrant orange/red color, while the cation radicals appeared dark green at high concentrations (millimolar) and pale yellow at very low concentrations (micromolar). α-$TO^{\bullet}$ shows characteristic bands in the UV−vis at 23 500 and 24 500 cm^{-1}.[25,33]

α-$TO^{\bullet}$ has been extensively characterized by electron paramagnetic resonance (EPR) spectroscopy with all the hyperfine coupling constants assigned.[14,25,34,35] The equilibrium in eq 8 shifts toward the protonation reaction in CH_2Cl_2 compared with that in CH_3CN; thus the cation radical has some stability in pure CH_2Cl_2.[12,16,17] Therefore, chemical oxidation experiments performed in CH_2Cl_2 at low temperatures have allowed the EPR spectra to be obtained of the cation radicals of all the tocopherols,[36,37] as well as EPR spectra obtained in acidic CH_3CN and CH_2Cl_2 conditions.[12,15,17]

The infrared spectra of α-TO^+, (CH_3)α-TO^+, and (COOH)α-TO^+ have been obtained in CH_3CN, either by electrochemical oxidation of the starting materials[15,17] or by chemical oxidation with NO^+.[16] The phenoxonium cations show three strong bands in the carbonyl region at 1605, 1650, and 1670 cm^{-1}, which molecular orbital calculations predict correspond to an asymmetric C=C ring stretch, a symmetric C=C ring stretch, and a C=O stretch.[16] An infrared spectrum was obtained of (CH_3)α-$TOH^{+\bullet}$ in CH_2Cl_2 containing 1 M CF_3COOH, which was very similar to the spectrum of the starting material with no absorbancies detected in the carbonyl region (1500−1800 cm^{-1}).[17] A resonance Raman study on (COOH)α-$TOH^{+\bullet}$ in aqueous acid solution detected a single strong band in the 1300−1800 cm^{-1} region at 1620 cm^{-1}, which was assigned to a C=C ring stretching mode.[38]

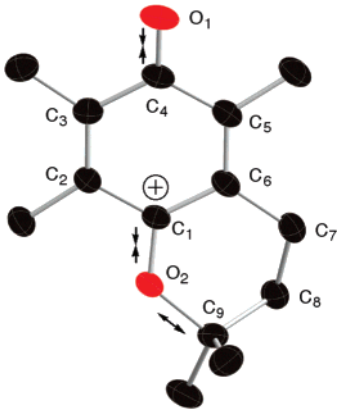


FIGURE 2. ORTEP plot for the molecular structure of (CH_3)α-TO^+ (crystallized with the $[B(C_6F_5)_4]^-$ counteranion). Thermal ellipsoids are drawn at the 50% probability level and hydrogen atoms are omitted. Modified from ref 18.

A sample of (CH_3)α-TO^+ in CH_3CN at 233 K was characterized by ^1H and ^{13}C NMR spectroscopy.[16] The quaternary carbon (C_9 in Figure 2) shifted from 73.1 ppm in the neutral molecule to 99.9 ppm in the cation, with the large downfield shift consistent with increased positive charge on C_9, suggesting that the chromanol structure is important in stabilizing the phenoxonium cation.[16,18] The ^1H and ^{13}C NMR spectra were obtained of the *o*-quinone methide (Scheme 2), which was trapped with *N*-methylmorpholine-*N*-oxide.[31]

The crystal structure of (CH_3)α-TO^+, stabilized with the non-nucleophilic $B(C_6F_5)_4^-$ and $CB_{11}H_6Br_6^-$ anions, has recently been obtained confirming the phenoxonium structure of the cation (Figure 2).[18] The C_4-O_1, C_2-C_3, and C_5-C_6 bond lengths in (CH_3)α-TO^+ are typical of compounds with a quinone structure and the C_1-O_2 bond length in (CH_3)α-TO^+ is between what is expected for a single and what is expected for a double bond. The C_9-O_2 bond length in the phenoxonium cation (1.520 Å) is longer than that expected for a C−O single bond (1.44 Å); thus, the high stability of the phenoxonium cation can be rationalized by the chromanol ring maintaining structural integrity around C_9 despite the long and, therefore, weak C_9-O_2 bond.

2.4. Concerted versus Consecutive Reactions. If a chemical reaction occurs simultaneously to an electron-transfer step, the process is termed a "concerted" single-step reaction, whereas a "consecutive" or "gated" process involves the transformation (such as isomerization) or chemical step (such as proton transfer) occurring after (or before) the electron transfer.[39] The one-electron oxidation of α-TOH to α-$TOH^{+\bullet}$ (eq 3) followed by the loss of a proton to form α-$TO^{\bullet}$ (eq 8) occurs *via* a consecutive process. This conclusion is based on spectroscopic measurements (EPR, UV−vis, and attenuated total reflection−Fourier-transform IR spectroscopy (ATR−FTIR)),[12,15,17] which unambiguously identified the cation radical, confirming it has substantial stability (in dry acidic conditions, it is stable for at least several hours). However, it has been assumed that the proton-transfer reaction only occurs through the phenolic hy-

drogen atom. It is possible that the initial proton loss in the cation radical or dication occurs through one of the methyl groups adjacent to the OH group, with subsequent tautomerization to reprotonate the methyl group. Such a process would not be easily voltammetrically detectable if it occurred very quickly, in a concerted fashion. Voltammetric experiments on phenols containing amine groups in the ortho-position (which allow hydrogen bonding to the nitrogen) have concluded that the one-electron oxidation occurs through a concerted process.[40,41] Therefore, the electron−proton transfer mechanism that occurs during the oxidation of phenols needs to be ascertained on a case-by-case basis, preferably by correlating a range of voltammetric and spectroscopic techniques.

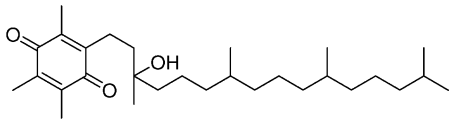
It is not clear whether the formation of the phenoxonium cation *via* oxidation of the cation radical to the dication (eqs 4 and 9) occurs through a concerted or consecutive mechanism, since there is presently no spectroscopic data confirming the identity of the dication. Some uncertainty also remains in the case of eq 1 as to whether a simple hydrogen atom transfer reaction occurs or additional chemical and electron-transfer steps are involved. A series of reactions not involving direct hydrogen atom transfer have been proposed to account for the reaction between α-TOH and superoxide, $O_2^{-\bullet}$ (which also forms α-TO$^\bullet$).[13] Superoxide is a reasonably powerful reducing agent ($E^\circ = -1.23$ V vs Fc/Fc$^+$ in CH_3CN[15]) and nucleophile, so its ability to react with α-TOH to form the "oxidized" product, α-TO$^\bullet$, is very interesting.

2.5. Using Cyclic Voltammetry To Determine Antioxidant Capacity. It has been suggested that cyclic voltammetry experiments can be useful for measuring antioxidant capacity in phenols,[42,43] although such a procedure is flawed for two reasons. First, the accepted mechanism for antioxidant activity involves the formation of TO$^\bullet$ and occurs through eq 1, which is a hydrogen atom abstraction mechanism and not necessarily an oxidation process. TO$^\bullet$ can form by an oxidation reaction, but it must occur either through the starting material being oxidized to the cation radical, then losing a proton (concertedly or consecutively; Scheme 1, pathway 2; under those conditions TO$^\bullet$ should be immediately further oxidized) or through the one-electron oxidation of the phenolate anion (Scheme 1, pathway 3). Second, the peak potentials measured by voltammetry during the oxidation of phenols do not necessarily correspond to the formal potentials because they are affected by homogeneous proton-transfer reactions that accompany the heterogeneous electron-transfer steps. In some circumstances, it may be possible to extract the electrode potentials *via* digital simulation techniques,[24] but it is still not clear how these potentials relate to eq 1.

3. Concluding Remarks and Outlook

The electrochemical and spectroscopic data outlined in this review indicate that there are other intermediate forms of vitamin E that are worthy of consideration in a biological context, in addition to the natural phenol (TOH) and its phenoxyl radical (TO$^\bullet$). It is noteworthy that the mechanism in Scheme 1 is thought to be typical of the general electrochemical behavior of all phenols.[23,44] However, vitamin E appears to be unusual in the high persistence of the cation radicals (of all the tocopherols) in low acid conditions[17,36,37] and the very high persistence of the phenoxonium cation of α-TOH in pure CH_3CN or CH_2Cl_2.[15−18] It is also interesting that the transformation between phenol and phenoxonium cation is completely chemically reversible on the subseconds and hours time scales (^{13}C NMR experiments indicate that α-TO$^+$ is formed in 100% yield) but only for the fully methylated vitamer (α-TOH), which is also the form that is preferentially retained in mammalian systems.[5,6,10]

Chart 2. α-Tocopherol Quinone



In the absence of acid, the electrochemically induced transformation of α-TOH to α-TO$^+$ (*via* the loss of two electrons and one proton) occurs at approximately +0.5 V vs Fc/Fc$^+$, which is sufficiently low to theoretically occur within biological systems. The oxidation of α-TOH is known to occur *in vivo*, with one of the products identified as α-tocopherol quinone (Chart 2).[45] Conversion of α-tocopherol quinone back to α-TOH is not known to occur in mammals.[45] Since phenoxonium cations are thought to be involved as intermediates in the oxidation of all phenols,[23,44] it is reasonable to propose that the long-term oxidation products (such as α-tocopherol quinone) should also be formed through reaction of the phenoxonium cation intermediate. It has been speculated that the major function of α-TOH in mammalian systems is to act as a precursor to α-tocopherol quinone, which is then able to function as a coenzyme.[45] It is highly unlikely that the dication has any biological importance since it is only observable as a transient in strongly acidic conditions and requires a significantly higher potential to be formed *via* one-electron oxidation of the cation radical (approximately +1.4 V vs Fc/Fc$^+$).[12,15,17]

The voltammetric and spectroscopic experiments that were used to establish the identities of their cationic species (Scheme 1) were all performed under conditions where their stabilities were at the highest. For the transformations in Scheme 1 to be important biologically, it is necessary to determine whether the reactions can occur within lipid bilayers, under conditions where the solubility and stability of cationic complexes are likely to be considerably less. Determining the location of vitamin E within membranes has been an area of substantial theoretical and experimental interest.[3,4] The general consensus is that vitamin E adopts a highly specific orientation within membranes with the hydrophobic phytyl chain pointing into the lipid bilayer, and the hydrophilic phenolic head positioned close to the aqueous interface. It is feasible that with such an orientation, a cationic charge can be stabilized by the penetration of a counteranion at the aqueous−lipid interface or by the phosphate group itself.

However, vitamin-E-based cationic compounds are also reactive with water, so penetration of the chromanol head out of the phospholipid membrane will significantly decrease the stability of the phenoxonium ion or cation radical. Future work on this project is, therefore, directed at examining the oxidative behavior of vitamin E within phospholipids using spectroelectrochemical methods.

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