of these peaks vary depending on the position of the GC peak subjected to the MS analysis due probably to different GC retention time for the protio and deuterio compounds. The reproducible intensity at each m/z value was obtained by adopting a summation procedure in which the sample gas from GC was continuously introduced into MS, and the intensity of each m/z value was integrated for the whole GC peak. Deuterium KIEs were calculated by compring the deuterium contents of 1-3 and 4-6 samples.[10] Analysis of the deuterium content for the LBH reduction was carried out with NMR (Bruker-WM 360) instead of GC/MS. In this case, crude benzhydrol was separated from unreacted benzophenone if necessary by preparative TLC and purified by recrystallization from hexane. The relative intensity of the methine proton of benzhydrol to that of the aromatic protons was used to calculate the deuterium content.

Determination of Carbon-14 Kinetic Isotope Effects. A solution of benzophenone labeled with ^{14}C at the carbonyl carbon (1.0 M, 10 mL) was divided into five parts and transferred with a stainless steel needle into flame-dried test tubes capped with rubber septa. To these solutions preset amounts of standardized solutions of reducing agents were added; the molar ratio of active hydride to ketone was in the range 0.3-0.7. After standing overnight in a constant-temperature bath at 25.0 ± 0.1 °C, the reaction solutions were worked up, and the actual fractions of reaction were determined by GLC (PEG HT, 2-m glass column at 190 °C). The product benzhydrol and the unreacted benzophenone were separated by preparative TLC and purified by repeated recrystallizations from hexane. Radioactivities were determined by a liquid scintillation counter (Beckman LS 9000) as reported previously.[8] KIEs were calculated for each fraction of reaction by using four equations of Tong and Yankwich.[9] These equations allows KIE calculations in four ways by using any three of the measured parameters, fraction of reaction, f, radioactivity of the starting ketone, R_0, activity of the recovered ketone, R_r, and activity of the product alcohol, R_p. Agreement among the KIEs calculated by the four different equations was excellent in all cases, and the isotope effects thus obtained showed no trend with the fraction of reaction. These facts indicate the high reliability of the results.

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Registry No. C-14, 14762-75-5; Dz, 7782-39-0; $NaBH_4$, 16940-66-2; $LiBH_4$, 16949-15-8; $LiAlH_4$, 16853-85-3; benzophenone, 119-61-9.

Supplementary Material Available: Tables of calculated MMI, EXC, ZPE, VP, imaginary frequencies, KIE, and EIE for each model (1 page). Ordering information is given on any current masthead page.

Kinetics of Ozonation. 4. Reactions of Ozone with α-Tocopherol and Oleate and Linoleate Esters in Carbon Tetrachloride and in Aqueous Micellar Solvents

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Abstract: Vitamin E (α-tocopherol; α-T) is known to protect animals against the deleterious effects of ozone in polluted air; one such effect is the ozone-initiated autoxidation of polyunsaturated fatty acids (PUFA) that occur in membranes. In order to assess the possibility of a direct reaction of ozone with α-T competing with the very fast ozone-PUFA reaction, we have measured the rates of reaction of ozone with α-T, oleic acid, and linoleic acid. In CCl_4 as solvent, α-T reacts with ozone with a rate constant of about 5500 M^{-1} s^{-1}; methyl oleate and methyl linoleate react 2 orders of magnitude faster. In aqueous micellar solutions the rate constants for α-T and the fatty acids are more similar. The k for the ozone/α-T reaction is about 1×10^6 M^{-1} s^{-1} at pH 7, but decreases as the solution becomes more acidic; the k's for oleic acid and linoleic acid are ca. 1×10^6 M^{-1} s^{-1} and exhibit no significant pH dependence. Since the ratio of fatty acids to α-T in membranes is typically at least 100-1000 to 1, we conclude that the direct reaction of ozone with α-T is unlikely. Thus, the protection that vitamin E provides to animals breathing ozone-containing air must result from vitamin E acting as a free radical scavenger. We have also detected the α-tocopheroxyl radical as an intermediate from the reaction of ozone with α-T both in CCl_4 and aqueous micelles using electron spin resonance spectroscopy. We suggest that the observation of this intermediate is consistent with an initial electron transfer from α-T to ozone.

Ozone is among the most important of atmospheric oxidants, causing damage to a wide variety of biological target molecules in vivo.[1-3] In particular, when ozone in polluted air reacts with polyunsaturated fatty acids (PUFA) in pulmonary lipids, free radical intermediates are produced and autoxidation is initiated.[2] We have shown from studies of PUFA autoxidation[2c,3] and by spin-trapping techniques[4] that free radicals are produced from the reaction of ozone with PUFA or simple olefins in vitro. Evidence for the in vivo production of free radicals includes observations of increased production of conjugated dienes via lipid peroxidation,[5] expiration of ethane and pentane in the breath of animals exposed to ozone,[6,7] and the decreased sensitivity to the

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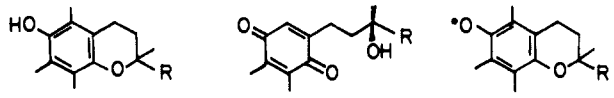
effects of ozone in animals administered free radical scavengers.[7,8] Although the efficiency of radical production from the reaction of ozone with PUFA may be fairly low,[4] the ultimate damage caused by this process may be magnified because PUFA autoxidation is a chain reaction. Since the extent of ozone damage is moderated by antioxidants, it is usually assumed that ozone reacts with PUFA to form free radicals and that endogenous antioxidants present in pulmonary membranes trap these radicals to stop the autoxidation chain reaction and lessen the extent of ozone damage.

The most important lipid-soluble antioxidant found in biological systems is *d*-α-tocopherol (**1**; α-T).[9,10] Animals fed diets enriched



1; α-T **2**; α-TQ **3**; α-T-oxyl

in α-T are less sensitive to ozone exposure than are animals deficient in α-T;[7,11] thus, it is apparent that α-T can serve a protective role against the effects of ozone.

Also of potential importance, however, are the direct ozonation of antioxidants (in competition with PUFA ozonation) and the reaction of antioxidants with nonradical products such as ozonides that are formed during the ozonation of PUFA. The occurrence of these pathways has not been evaluated; however these reactions could result in a more rapid depletion of antioxidants, rendering the tissue more sensitive to autoxidation chain reactions. For these reasons we report here a comparison of rates of ozonation of α-T and of PUFA, as well as an analysis of the interactions between antioxidants and nonradical ozonation products.

The kinetics of ozonation of alkenes have been studied under a variety of conditions including both aqueous[12-14] and aprotic, lipophilic environments.[15-18] The rates of ozonation of some phenols that can be viewed as simple models of α-T have been measured in aqueous solution,[12] and we recently reported the rate constants for the reactions of ozone with some biologically relevant substrates in aqueous micellar solutions, including α-tocopherol, ascorbic acid, and oleic and linoleic acids.[1] The present report is an extension of our earlier study.

Experimental Section

Materials. Sodium dodecyl sulfate (SDS), oleic and linoleic acids and their methyl esters (Sigma), hexadecane, 1,4-pentadiene (Aldrich), carbon tetrachloride (reagent grade, Mallinckrodt), acetonitrile (reagent grade, Baker) Chelex-100 (Bio-Rad laboratories), and di-*tert*-butyl peroxide (Lucidol) were used as purchased. The α-tocopherol was a generous gift from Henkel Corp. and was assayed by Henkel to be 94.7% *d*-α-tocopherol, 95.7% total tocopherols, by GC. α-Tocopherylquinone (**2**; α-TQ; ICN) and α-tocopherol acetate (α-TAc; Sigma) were used as received.

Table I. Rates of Reaction of Alkenes with Ozone in CCl_4

substrate	concn, mM	k, M^{-1} s^{-1} × 10^{-5}
methyl oleate[a]	4.90	5.7 ± 1.1
	1.47	6.4 ± 1.8
methyl linoleate[a]	0.56	7.0 ± 0.5
	1.12	6.9 ± 1.2
1,4-pentadiene	3.88[b]	0.69 ± 0.07
	1.94[c]	0.77 ± 0.05
	1.94[d]	0.99 ± 0.05

[a] 25.0 °C. [b] −20.3 °C. [c] −10.1 °C. [d] −0.1 °C.

Stopped-Flow Kinetics. Stopped-flow kinetics were carried out as described earlier[15] on a Nortech stopped-flow spectrophotometer (Model SF-3L, 0.2-mm path length, mixing/dead time 6 ms) interfaced to an On-Line Instrument Systems Model 3820 data system.[19] All runs were replicated 5–12 times with at least two different concentrations of substrate. Substrate was always present in large excess relative to ozone so that pseudo-first-order conditions were maintained.

Ozone solutions were prepared by bubbling a stream of ozone in oxygen into either CCl_4, acetonitrile, or acidified water (H_2SO_4) and were then diluted with more of the same solvent. Equal volumes of appropriate substrate solutions were mixed with the ozone solutions, and the loss of ozone was followed spectroscopically. In the case of α-T, the absorbance change was due to the loss of both ozone and α-T as well as the formation of a transient intermediate (see below). Runs in CCl_4, CH_3CN, and wet CH_3CN were done at 25.0 ± 0.1 °C, unless noted otherwise, by following the loss of ozone at 275–295 nm. Due to its low boiling point, 1,4-pentadiene was studied at several lower temperatures, and the rate of reaction was extrapolated to 25 °C.

For experiments in aqueous media, solutions were prepared in phosphate-buffered water containing 40–300 mM SDS. Some of the phosphate/SDS solutions were passed through a Chelex column to remove metal ions; others were used without removing metal ions, and no differences were observed. The substrates were added as a solution in methanol, and the resulting mixtures were either shaken or sonicated until they appeared to be completely transparent. The molar ratio of SDS to substrate was at least 70:1. These solutions were mixed in the stopped-flow apparatus with acidified solutions of ozone, and the pH was measured on the effluent. The temperature was maintained at 25.0 ± 0.1 °C except for some runs with α-T, for which the temperature was 37.0 ± 0.1 °C. For fatty esters and some runs with α-T the reactions were followed at 255 nm. Other runs with α-tocopherol were followed at either 292 nm (λ_{max} for α-T) or 283 nm (the isobestic point for α-T and the quinone).[20] The observed rates did not vary with wavelength.

Product Analysis. The product mixture of the reaction of ozone with α-T in CCl_4 was examined by HPLC on a Varian 5000 instrument equipped with an IBM octadecyl (C_{18}) column and a UV detector, using methanol containing 4.5% water. UV-visible spectra were taken on a Hewlett-Packard 8451A diode array spectrophotometer. A partial characterization of the products was carried out by HPLC analysis and by taking UV spectra of the fractions collected. Additionally, portions of these fractions were acidified with a few drops of 10% HCl, and the UV spectra were retaken after ca. 1 h.

The ESR Spectrum of the α-T/Ozone Reaction Mixture. ESR spectra were collected at ambient temperature on an IBM 100D spectrophotometer equipped with a flow cell with a mixing/dead volume of about 0.25 mL. Deoxygenated solutions of α-T (0.5–0.6 mM) and ozone[21] (ca. 0.1 mM) in CCl_4 were pumped through the cell with a dual peristaltic pump at a combined flow rate of 100–130 mL/min; thus the mixing/dead time was at least 120–160 ms. Similarly, 0.3 mM α-T in aqueous SDS (35 mM) containing 25 mM phosphate buffer was allowed to react with ozone (ca. 0.05 mM) in acidified water in the ESR cell at a combined flow rate of 125 mL/min. The pH of the mixed solution was 3.0. For comparison, a solution of α-T and di-*tert*-butyl peroxide in SDS micelles was irradiated in an ESR cell while the spectrum was collected.

Competition Studies. A semiquantitative study of the reactivity of ozone toward α-T in the presence of excess methyl oleate was carried out. To 100 mL of 500 mM SDS was added 2 mL of a methanolic solution of α-T (1.6 mM), methyl oleate (20.1 mM), and benzophenone (6 mM as internal standard). A 5-mL portion of the resulting solution, and then 5 mL of water, was passed through a short reverse phase column to remove the SDS. The methyl oleate was also removed in this process. The column was then washed with two 5-mL portions of methanol, and the methanolic solution was analyzed in triplicate by HPLC, as described

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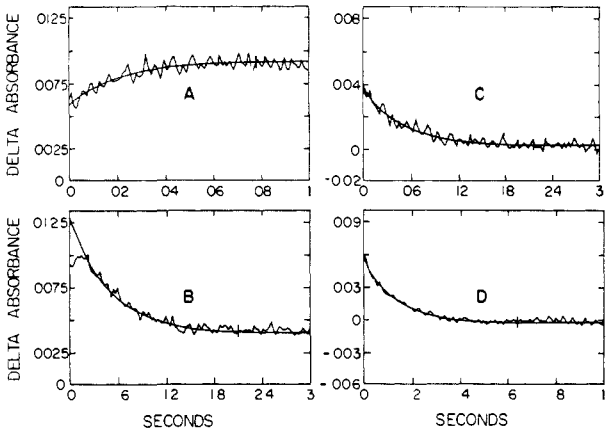

Figure 1. Traces recorded at 295 nm for the reaction of α-tocopherol with a limited amount of ozone. The smooth lines are fitted curves. (A) 13.4 mM α-T in CCl_4. (B) 3.8 mM α-T in CCl_4. (C) 0.14 mM α-T in water/SDS, pH 6.3. (D) 0.14 mM α-T in water/SDS, pH 4.8.

Table II. Rates of Reaction of α-Tocopherol, α-Tocopherylquinone, and α-Tocopherol Acetate with Ozone in CCl_4[a]

entry	substrate	[substrate], mM	k, M^{-1} s^{-1}[b] first phase	second phase
1	α-T	17.8	6100 ± 800	-
2		17.2[c]	-	110 ± 12
3		13.4	5100 ± 400	250 ± 50
4		7.1[d]	4900 ± 500	150 ± 15
5		7.1[c]	5600 ± 1800	240 ± 40
6		3.8	5700 ± 1000	960 ± 35
7		3.8		1100 ± 75
8	α-TQ	8.6	11200 ± 500	-
9		2.6	11700 ± 850	-
10		1.1	11500 ± 250	-
11	α-TAc	21.9	144 ± 20	-
12		7.1	131 ± 3	-
13		2.1	132 ± 3	-

[a] All data were collected at 25.0 °C, monitoring the change in absorbance at 295 nm. [b] These values are based on the amount of substrate present, assuming pseudo-first-order kinetics. When values are given for two phases, the reaction was found to be biphasic and to yield two rate constants. See discussion in the text. [c] The CCl_4 was saturated with water before the reaction. [d] The α-T solution was filtered through $MgSO_4$ and stored over molecular sieves.

above, with UV detection at 295 nm. At this wavelength only two significant peaks were observed: α-T and benzophenone. The ratio of the two was identical with that of a sample of the initial methanol solution; i.e., it was unchanged by the above procedures. Portions of the SDS solution were treated with either 1 or 2 equiv of ozone relative to the amount of α-T present. After removal of SDS (as above) HPLC analysis showed no detectable loss of α-T due to ozonation.

Addition of α-T to Ozonized Methyl Oleate. A stream of ozone in oxygen (0.3 mmol min^{-1} by iodometric titration) was bubbled through 4.0 mL of a solution of methyl oleate (200 mM) in CCl_4 at room temperature for 30 s. Thus 0.15 mmol of ozone was added to a 5-fold excess of methyl oleate.

A 2.0-mL portion of a CCl_4 solution of α-T (79 mM) and benzophenone (27 mM) was added to the ozonized methyl oleate solution. Another 2.0-mL portion of the α-T solution was added to 4.0 mL of the methyl oleate solution without ozonation. These solutions were allowed to stand at room temperature for 1–2 h. After this time the solutions were analyzed by HPLC. The relative area of the α-T and benzophenone peaks was unchanged by the addition of ozonized methyl oleate.

Results

Data collected from stopped-flow measurements of the reaction of ozone with alkenes in CCl_4 are shown in Table I. Typical traces from the reaction of α-T with ozone are shown in Figure 1. The rate of change of absorbance in the ozone/α-T reaction is biphasic, as can be seen from Figure 1b; an initial increase in absorbance is followed by a slower decrease. The rate of the first phase, Figure 1a, is proportional to the concentration of α-T, while the rate of the second phase, Figure 1b, is not. The rate data for both phases

Table III. Rates of Reaction of α-Tocopherol with Ozone in Acetonitrile, with and without the Addition of Water

entry	[α-T], mM	k, M^{-1} s^{-1} first phase	second phase
1	21.7	-	26 ± 4
2	9.6	1550 ± 300	54 ± 10
3	4.6	1420 ± 250	126 ± 13
4	2.2	1370 ± 480	-
5[a]	3.7	34000 ± 6400	2450 ± 410
6[b]	3.7	33000 ± 8300	4848 ± 920

[a] Acetonitrile containing 5% water. [b] Acetonitrile containing 10% water.

Table IV. Rates of Reaction of Ozone with α-Tocopherol and Oleic and Linoleic Acids, and Their Methyl Esters, in Aqueous SDS Solution[a]

substrate	[substrate], mM	[SDS], mM	pH	k, M^{-1} s^{-1} × 10^{-5}
α-tocopherol	0.09	60	2.3	0.13 ± 0.01
	1.05	75	2.8	0.15 ± 0.03
	0.46	60	2.9	0.22 ± 0.03
	0.14	150	4.8	1.1 ± 0.5
	0.14	150	6.3	3.2 ± 0.5
	0.14	300	6.8	6.6 ± 0.5
	0.09	100	7.0	7.5 ± 1.5
	0.09	60	7.1	7.3 ± 1.7
	1.05[b]	75	2.3	0.31 ± 0.04
	1.05[b]	75	4.7	1.4 ± 0.15
	1.05[b]	75	5.8	3.2 ± 0.5
α-tocopherol acetate	0.31	250	2.3	0.13 ± 0.01
	0.31	250	5.8	0.15 ± 0.02
	0.31	250	6.9	0.18 ± 0.01
oleic acid	0.36	140	2.5	9.1 ± 1.6
	0.36	140	2.8	10.0 ± 4.0
	0.18	50	3.1	10.4 ± 2.8
methyl oleate	0.46	150	2.0	8.6 ± 0.9
	0.23	150	4.4	10.0 ± 1.7
	0.46	150	5.8	8.5 ± 0.8
	0.46	150	6.4	8.5 ± 0.8
	0.54	100	6.8	7.7 ± 1.6
linoleic acid	0.17	40	2.6	11.0 ± 0.6
	0.17	40	2.7	9.9 ± 2.3
	0.17	160	2.8	11.0 ± 0.8
methyl linoleate	0.32	150	4.2	11.0 ± 1.7
	0.32	150	5.7	11.3 ± 0.9

[a] Unless otherwise noted, data were collected at 25.0 °C. [b] These data were collected at 37.0 °C.

of this reaction are collected in Table II. Entries 2 and 5 were carried out in CCl_4 saturated with water, while the CCl_4 used for entry 4 was filtered through $MgSO_4$ and further dried over molecular sieves. Neither of these treatments significantly altered the rate of the first phase and all three runs give rate constants for the second phase that are below the average of runs using untreated CCl_4.

Rate data for the reactions of α-TQ and α-TAc with ozone also are shown in Table II. A continuous decrease in absorbance rather than a biphasic curve is exhibited in these reactions. The reaction of α-TQ was faster than that of α-T, while α-TAc reacted with ozone more slowly.

The reaction of α-T with ozone in anhydrous acetonitrile appears to be similar to that in CCl_4; these data are collected in Table III. When water is added to the solvent the rates of both phases are accelerated, as indicated in entries 4 and 5 of the table. The addition of 5% water increases the observed rate of the first phase by a factor of 20, but the addition of twice as much water does not increase the rate further. The rate of the second phase appears to be roughly proportional to the concentration of water.

In aqueous SDS solutions, a monophasic reaction is observed for the reaction of α-tocopherol with ozone. Typical traces are illustrated in Figure 1, parts c and d. Representative data collected in aqueous media are compiled in Table IV, and a plot of all of the data collected at 25 °C vs. the pH of the reaction mixture is shown in Figure 2. The observed rates are proportional to the concentration of substrate and, in the case of α-tocopherol, vary

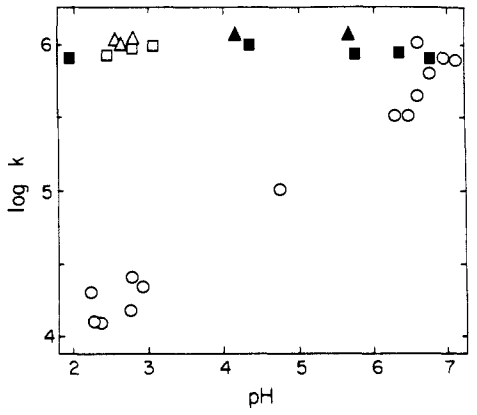

Figure 2. A plot of log k vs. pH for the reaction of ozone with various substrates in water/SDS at 25.0 °C. Substrates include α-tocopherol (circles), oleic acid (open squares), methyl oleate (closed squares), linoleic acid (open triangles), and methyl linoleate (closed triangles).

with pH. The concentration of SDS does not appear to affect the rates of reaction.

The ESR spectra of α-T/ozone mixtures in CCl_4 and SDS/water are illustrated in Figure 3, parts a and c, respectively, as are the simulated spectra of the α-tocopheroxyl (**3**; α-T-oxyl) radical (Figure 3b)[22] and the spectrum of α-T-oxyl produced by the irradiation of di-*tert*-butyl peroxide in the presence of α-T in SDS micelles (Figure 3d). The spectra of the mixtures of α-T and ozone (Figure *3a,c*) also show evidence (arrow) of another species that has a g-value characteristic for a peroxyl radical, although it is certainly unusual for high concentrations of peroxyl radicals to accumulate under these conditions. When ESR spectra of the α-T/ozone mixture in CCl_4 were obtained at a slower flow rate, a much weaker α-T-oxyl radical signal and a more intense signal with a g-value like that of a peroxyl radical were observed.

HPLC analysis of the product mixture resulting from the reaction of α-T and O_3 in CCl_4 indicates that only a trace of α-TQ is present, along with other products eluting in a single band before α-T on a reverse-phase column. The UV spectrum of this band contains a broad absorbance from 225 to 265 nm, and its treatment with HCl resulted in the formation of α-TQ. Similar results are obtained when aqueous methanol is used as the solvent.

When a solution of α-T and benzophenone was added to a solution of methyl oleate that had been previously ozonated, the area ratio of α-T to benzophenone was 3.31 ± 0.10, as compared to the ratio of 3.23 ± 0.15 measured for the nonozonated control mixture. This implies that there is no significant reaction between α-T and the ozonation products of methyl oleate. The competition experiments indicate that, in the presence of a large excess of methyl oleate, α-T does not react to a significant extent with ozone.

Discussion

Fatty Acids. From the data presented in Table I it is apparent that methyl oleate and linoleate react with ozone in both CCl_4 and aqueous micellar solutions with rate constants that are typical of those for ozone–alkene reactions. In CCl_4 our measured value for the rate constant for the reaction of methyl oleate with ozone (6×10^5 M^{-1} s^{-1}) is in reasonable agreement with the value reported by Razumovskii for both methyl oleate and oleic acid (1×10^6 M^{-1} s^{-1}).[17] Although our value for methyl oleate is 3 times the rate constant (extrapolated to 25 °C) for *cis*-4-methyl-2-pentene with ozone,[15] this difference might be expected on stereoelectronic grounds. As noted earlier,[16] 3-methyl-1-pentene reacts 2.5 times more slowly with ozone than does 1-hexene due to the greater steric bulk at the carbon adjacent to the double bond. By this same argument, methyl oleate would be expected to react faster than *cis*-4-methyl-2-pentene, and by about the same factor.

The rate constant for the reaction of methyl linoleate with ozone in CCl_4 is 7×10^5 M^{-1} s^{-1}. This value is less than twice that

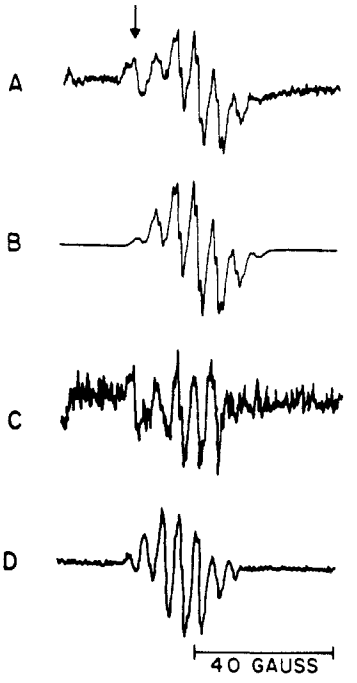

Figure 3. (A) The ESR spectrum of the solution obtained by mixing CCl_4 solutions of ozone (ca. 0.1 mM) and α-tocopherol (0.6 mM) at a combined flow rate of 100 mL min^{-1}. The ESR conditions are microwave frequency, 9.7745; microwave power, 20 mW; modulation frequency, 100 kHz; modulation amplitude, 0.8 G; gain, 1×10^6; center field, 3484.6; sweep width, 80 G; sweep time, 200 s; time constant, 500 ms. The g-value is 2.0047. The arrow designates a signal with a g-value of 2.015 that we identify as a tertiary peroxyl radical. This is an accumulation of 8 scans. (B) Simulated spectrum of the α-tocopheroxyl radical with hyperfine coupling constants of 5.98 (3 H), 4.57 (3 H), 0.94 (3 H), and 1.47 G (2 H) (see ref 22); sweep width, 80 G; line width, 0.4 G. (C) The ESR spectrum of the solution obtained by mixing aqueous solutions of ozone (ca. 0.1 mM, acidified) and α-tocopherol (0.3 mM in 35 mM SDS, 25 mM sodium phosphate) at a combined flow rate of 125 mL min^{-1}. The pH after mixing was 4.0. The ESR settings were as described in (A). This is an accumulation of nine scans. (D) The ESR spectrum of an aqueous solution of α-tocopherol (3.5 mM), SDS (140 mM), and di-*tert*-butyl peroxide (ca. 100 mM) irradiated continuously in the ESR cavity with a low-pressure mercury lamp.

obtained for oleate, but this difference is consistent with the rate differences obtained for model compounds. For example, the rate constant for 1,4-pentadiene (extrapolated to 25 °C) is 1.3×10^5 M^{-1} s^{-1}, just 1.3 times the rate constant for 1-hexene under the same conditions.[16] Pentadiene reacts less than twice as fast as 1-hexene because of the electron-withdrawing effect of allylic double bonds ($\sigma^* = +0.13$ for $CH_3CH{=}CHCH_2{-}$).[23,24] For the same reason, methyl linoleate would be expected to react about 1.3 times as fast as methyl oleate; we observe that it reacts 1.2 times as fast.

The rate constant for the reaction of methyl oleate or linoleate with ozone in SDS micelles is about 1×10^6 M^{-1} s^{-1}. This is intermediate between the rate constant for methyl oleate in CCl_4 and the rate constant of 3-hexenoic acid, a PUFA model, in water, suggesting that the rate constants of ozonation of unsaturated fatty acids vary only slightly with changes in solvent. This is not unexpected since the rates of ozonation of simple alkenes in water are just 2–4 times faster than the same substrates in CCl_4.[13,14] The free acids react with ozone at the same rate as do their methyl esters, as was observed earlier for oleic acid and its methyl ester in CCl_4.[17]

While rate constants determined in aprotic solvents are informative, aqueous micellar solutions are a better model for the

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(24) The rate of reaction of 1,4-pentadiene, when corrected statistically, fits on the line of σ^* vs. log k presented in ref 16.

membranes in which α-T and the fatty acids occur in biological systems. These micellar solutions may be described as a loosely associated aggregation of SDS molecules with both substrate and ozone rapidly partitioning between the micelle and the bulk aqueous phases. Although the kinetics of reactions in micelles are complex,[25] they can be simplified by assuming that the substrate (PUFA or α-T) resides totally in the micelles[26] while ozone is partitioned roughly equally between the micellar and bulk aqueous phases.[27] If these conditions are met, the observed rates will equal the rate of reaction in the micelles.[25] The similarity of the rates measured in SDS micelles to those of model compounds in water[13,14,28] attests to the validity of these assumptions.

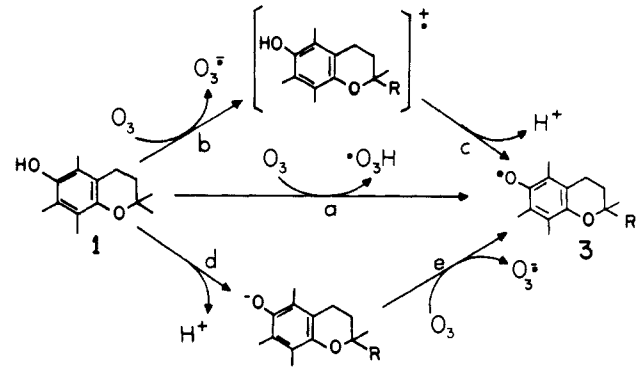
α-Tocopherol. We observe two distinct rates and types of changes in absorbance in the reaction of α-T with a limited amount of ozone in CCl_4 (see Figure 1a,b) or in CH_3CN. The first change is an increase in absorbance, indicative of the formation of an intermediate; the second absorbance change can reasonably be assigned to the decomposition of the adsorbing intermediate. The first rate varies directly with the concentration of α-T, implying that it is first order in α-T. The rate of the second phase does not appear to be proportional to the concentration of α-T, consistent with the hypothesis that it involves the reaction of an intermediate produced in the first phase.

The reaction of α-T with ozone in SDS micelles exhibits a single phase. The observed decrease in absorbance is first order in α-T and shows pronounced pH dependence as illustrated in Figure 2. We were concerned by the fact that the initial increase in absorbance observed in the ozone α-T reactions in CCl_4 was not present in the aqueous reactions. To rule out the possibility that there was an earlier, faster step in the ozonation of α-T, we carried out the reaction in the presence of a 12-fold excess of methyl oleate. If the rate obtained in the stopped flow experiments were that of the first step, i.e., if no earlier reaction were occurring at a rate too fast to follow by stopped-flow, the methyl oleate should largely protect the α-T from ozonation. In complete agreement with the stopped-flow experiments, α-T is not ozonated to a significant extent in the presence of a large excess of methyl oleate, indicating that no earlier, faster step is occurring.

The low reactivity of α-TAc in CCl_4 (Table II) suggests that the phenolic hydrogen of α-T plays a major role in its reaction with ozone. While α-TAc is less electron rich than is α-T, it is probably not sufficiently different to account for the large difference in reactivity if the phenolic hydrogen of α-T were not involved in the reaction. Also note that the reaction of α-TAc with ozone in SDS micelles exhibits little or no pH dependence (Table IV).

The ESR spectra of the α-T/ozone solutions in either water/SDS or CCl_4, obtained shortly after mixing, indicate the presence of the α-T-oxyl radical **3** and at least one other radical species. On the basis of its *g*-value, persistence, and the lack of any hyperfine splitting, the second radical intermediate is probably a tertiary peroxyl radical. One earlier report also noted the production of phenoxy radicals from the ozonation of phenols,[29] so the formation of **3** is not without precedent. The radical signal observed in water is far weaker than that observed in CCl_4 (see Figure 3c).

Scheme I



While the products of the reaction of ozone with α-T in CCl_4 were not fully characterized, the data are consistent with the α-T-oxyl radical being a key intermediate. The UV spectrum of the early-eluting band observed by HPLC contains broad absorbances from 225 to 265 nm, similar to the dienones resulting from the reaction of α-T with singlet oxygen or radical precursers.[30] These dienones could be derived from the trapping of the α-T-oxyl radical. Treatment of this fraction with HCl resulted in the formation of α-T quinone, as was observed with authentic α-T dienones.[30] Some authors also have reported the formation of dimers and trimers of **3**;[31] although we were unable to observe such products, we cannot exclude the possibility of their formation.

While most of these data were collected at 25 °C, the ozonation of α-T was also investigated at 37 °C; these data are included in Table IV. At this higher temperature the rate of reaction is faster by less than a factor of 2. Assuming an energy of activation of 2.4 kcal mol^{-1} (that of 1,4-pentadiene), the rate of reaction of methyl linoleate should be only 1.2 times as fast at 37 °C as at 25 °C. Thus at neutral pH α-T would react with ozone, on a molar basis, at the same rate or only slightly faster than does methyl linoleate.

The detailed mechanism for the reaction of ozone with α-T is not entirely clear, but several observations can be made. First, two phases are observed in aprotic solvents, indicating that at least one intermediate is present. This intermediate is produced by a reaction that is first order in α-T; the rate of the second step, the destruction of the intermediate, is independent of the α-T concentration.

By contrast, in water/SDS a single smooth absorbance decrease is observed, first order in α-T. Note that the first observed step in water is the disappearance of the reactants while the first observed step in CCl_4 is the appearance of an intermediate. If the same mechanism operates in both protic and aprotic solvents, both steps must be accelerated in aqueous media, but the second step must be accelerated more than the first so that the first step becomes rate determining. The fact that both steps are accelerated in CH_3CN by the addition of water and that the second is increased in rate by a larger factor than the first step (see Table III) is consistent with this hypothesis.

Second, the α-T-oxyl radical, **3**, is observed by ESR spectroscopy in both CCl_4 and in water/SDS, although the signal is stronger in CCl_4. The product mixture is similar in both solvents and appears to be derived from the α-T-oxyl radical. This also is consistent with a common mechanism in all of the solvents studied, involving **3** as a key intermediate.

Third, the ozone/α-T reaction in aqueous micellar media is pH-dependent, increasing in rate with increasing pH. This phenomenon also is observed with simple phenols,[12] where the observed rate is simply the sum of the rates of the ionized and un-ionized forms.[12,13] We expected α-T in SDS micelles to behave

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(26) The partition coefficient between heptane and water for linoleic acid is 1.2×10^5 and for oleic acid is 2.3×10^5 (see: Mukerjee, P. *J. Phys. Chem.* **1965**, *69*, 2821–2827). It is reasonable to believe that those of the esters are similar. We assume that the partition coefficient is at least as large for α-tocopherol, but have found no literature value.

(27) The partition coefficient for oxygen between SDS micelles and water is 2.9. (Matheson, I. C. B.; King, A. D., Jr. *J. Colloid Interface Sci.* **1978**, 66, 464–469), and since ozone is more polar than oxygen, its partition coefficient should be smaller than this value.

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like a simple phenol in water; however, since the pK_a of α-T in SDS micelles is greater than 14,[32,33] the concentration of the anion can not fully account for the pH dependence. Some other factor must be involved. One possible explanation is suggested by the observation that the pK_a of α-T in micellar solutions varies with the nature of the micelle.[33] It is possible that the micellar environment changes with pH, altering the extent of ionization of α-T. A pH-dependent change in the structure of the micelle also might change the rate of ozonation of the un-ionized form of α-T.

At this time we suggest that the available experimental facts on the reaction of ozone with α-tocopherol are most consistent with a mechanism in which ozone oxidizes α-T to the α-T-oxyl radical. Scheme I shows three possible mechanisms by which this oxidation could occur. Thermochemical considerations suggest that the direct hydrogen atom transfer (path *a*) is too endothermic to occur readily. However, the same result can be obtained by either an electron transfer followed by a proton transfer (path b–c) or by a proton transfer followed by an electron transfer (path d–e). One-electron oxidations of phenols are well-known,[34] and the proton transfer from the resulting cation radical (path c) could be fast enough so that it is not observed.[35] There is also ample precedent for charge transfer in ozonation reactions with electron-rich aromatic systems.[36] In either case, additional stabilization could be realized by solvation of the ozone radical anion and the proton. Clearly, the effect of pH suggests that path d–e predominates in aqueous media, although as discussed above, the pK_a of α-T may require that path d–e not be the sole pathway involved; in other words, the actual mechanism may involve a mixture of the two pathways shown in Scheme I.

Biological Significance. In biological membranes, the rate constant for ozonation of α-T would be at most comparable to that of a fatty acid. However, since unsaturated fatty acids are typically present in biological membranes at concentrations 100–1000 times higher than α-T,[37] α-T would not compete for direct reaction with ozone. Additionally, since the ozonation products of methyl oleate do not react with α-T at temperatures of 37 °C and below, α-T is not consumed by secondary reactions of this type.

Conclusions. When animals breath smoggy air, α-T is known to provide important protection.[7,8] Since ozone would be expected to react virtually exclusively with PUFA in membrane lipids and not with the α-T directly, the protection that α-T provides against ozone must arise because α-T scavenges radicals produced from an ozone–PUFA reaction,[3-8] as illustrated in eq 1 and 2. The

$$O_3 + \text{PUFA} \rightarrow \rightarrow \rightarrow \text{ROO}^{\bullet} \quad (1)$$

$$\text{ROO}^{\bullet} + \alpha\text{-T} \rightarrow \rightarrow \text{nonradical products} \quad (2)$$

direct, sacrificial reaction of ozone with α-T in biological membranes containing normal concentrations of unsaturated fatty acids does not occur.

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Registry No. α-T, 59-02-9; α-TQ, 7559-04-8; α-TAc, 58-95-7; α-T-oxyl, 23531-69-3; O_3, 10028-15-6; 1,4-pentadiene, 591-93-5; oleic acid, 112-80-1; methyl oleate, 112-62-9; linoleic acid, 60-33-3; methyl linoleate, 112-63-0.

(32) The pK_a of α-T is about 12 in cationic micelles and about 13 in zwitterionic micelles (see ref 33). In an attempt to measure the pK_a in SDS by the methods outlined in ref 33, we were only able to verify that the value was >13.

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(35) The pK_a of phenoxyl cation radicals is ca. -5 (see: Land, E. J.; Porter, G.; Strachan, E. *Trans. Faraday Soc.* **1961**, 57, 1885-1893) and, by analogy to $HO_2^{\bullet}$ (pK_a = 4.7; see: Bielski, B. H. J. *Photochem. Photobiol.* **1978**, *28*, 645-649), the ozone radical anion can serve as a proton acceptor in the absence of other nucleophiles.

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^{17}O NMR Spectra of Cyclic Phosphites, Phosphates, and Thiophosphates

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Abstract: The ^{17}O NMR spectra of a number of cyclic and bicyclic phosphites, phosphates, and thiophosphates are presented, and, in so far as possible, the ^{17}O chemical shifts are interpreted in terms of conformational factors.

Following the pioneering work of Christ and Diehl,[2] a number of groups have studied the ^{17}O spectra of various phosphorus derivatives including phosphites and phosphates.[3-10] However, despite the importance of cyclic phosphates in biochemical processes and of cyclic thiophosphates as phosphate analogues useful

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