How Phenol and α-Tocopherol React with Ambient Ozone at Gas/Liquid Interfaces

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The exceptional ability of α-tocopherol (α-TOH) for scavenging free radicals is believed to also underlie its protective functions in respiratory epithelia. Phenols, however, can scavenge other reactive species. Herein, we report that α-TOH/α-TO^- reacts with closed-shell O_3(g) on the surface of inert solvent microdroplets in <1 ms to produce persistent α-TO−O_n^-($n = 1-4$) adducts detectable by online thermospray ionization mass spectrometry. The prototype phenolate PhO^-, in contrast, undergoes electron transfer under identical conditions. These reactions are deemed to occur at the gas/liquid interface because their rates: (1) depend on pH, (2) are several orders of magnitude faster than within microdroplets saturated with O_3(g). They also fail to incorporate solvent into the products: the same α-TO−O_n^- species are formed on acetonitrile or nucleophilic methanol microdroplets. α-TO−$O_{n=1-3}^-$ signals initially evolve with [O_3(g)] as expected from first-generation species, but α-TO−O^- reacts further with O_3(g) and undergoes collisionally induced dissociation into a $C_{19}H_{40}$ fragment (vs $C_{19}H_{38}$ from α-TO^-) carrying the phytyl side chain, whereas the higher α-TO−$O_{n\geq 2}^-$ homologues are unreactive toward O_3(g) and split CO_2 instead. On this basis, α-TO−O^- is assigned to a chroman-6-ol (4a, 8a)-ene oxide, α-TO−O_2^- to an endoperoxide, and α-TO−O_3^- to a secondary ozonide. The atmospheric degradation of the substituted phenols detected in combustion emissions is therefore expected to produce related oxidants on the aerosol particles present in the air we breathe.

Introduction

Antioxidants (AOs) prevent, suppress, delay, and/or regulate oxidative damage in vivo.[1] It is generally assumed that ascorbic acid (AH_2), uric acid (UA), reduced glutathione (GSH), and α-tocopherol (α-TOH) are present in biosurfaces, such as the lung epithelium and leaves plasmalemma, to mitigate the harmful effects of reactive oxygen species (ROS).[1−8] The integral functions of AOs, however, remain to be fully elucidated. For example, AOs may not act alone but in combination or concertedly.[9−11] The byproducts of ROS deactivation may participate in regulatory and signaling processes.[1,12−15] AOs reactions with different oxidants in diverse media should not be expected to adhere to a common or fixed pattern.[1,16−18] And, of course, only their fastest reactions would matter in any given environment.[1]

α-TOH consists of a hydrophobic n-$C_{16}H_{33}$ phytyl chain attached to a phenolic chroman-6-ol headgroup (Scheme 1).[19,20] It efficiently inhibits lipid peroxidation in vitro.[21−23] Because phenols are known to trap carbon- and oxygen-centered radicals into resonance-stabilized phenoxyls via exothermic H-atom transfer,[9,21] the exalted antioxidative efficiency of α-TOH has been generally ascribed to its low O−H bond energy. The molecular parameters that determine reaction pathways under specific conditions, however, vary considerably among phenols.[24] Bond energies: BDE(α-TO−H) = 318 kJ mol^{-1} versus BDE(PhO−H) = 360 kJ mol^{-1},[24−27] make α-TO−H a better H-atom donor. One-electron reduction potentials: E^0(α-TO•/α-TO^-) = 0.06 V versus E^0(PhO•/PhO^-) = 0.86 V (vs SHE),[18,28−32] make α-TO^- also a better electron donor,[29] but the very weak acidity of α-TOH: pK_a(α-TOH) ∼13[29] versus pK_a(PhOH) = 9.9, conspires against this pathway in physiological media.[28]

It has been pointed out that if α-TOH were merely a free-radical scavenger, it would be problematic to explain what differentiates it from related scavengers,[15,19,33,34] or why its effects are realized at concentrations that are insufficient to fully quench reactive radicals.[13] Because the full suppression of ROS (as obligatory intermediates of O_2 metabolism) would be lethal,[1,35,36] AOs obviously act in subtler ways. Furthermore, at least some AOs should control oxidative stress from pervasive nonradical ROS such as $^1O_2$ and O_3.[37,38] The issues of whether α-TOH scavenges ambient O_3(g) and, if so, how, become relevant in the case of biosurfaces exposed to polluted air,[2,4,19,34,39−41] and, more generally, to the atmospheric fate of the complex phenols that have been characterized (out of a significant mass of still unidentified organics) in forest fires and combustion emissions in general.[42−47]

Ozone is a sluggish one-electron oxidant but a reactive O-atom donor that can also behave as a diradical.[48−50] For example, O_3(g) adds to undissociated AH_2 (pK_a = 4.1) yielding an ozonide, but oxidizes ascorbate to dehydroascorbate (a two-electron process),[16,51] and epoxidizes UA.[17] It should be emphasized that ambient O_3(g) interacts primarily with interfacial fluid microfilms containing AOs rather than with the underlying tissues.[41,52] This interaction generates intermediates that can transduce oxidative injury by diffusing through the films within microseconds.[53] These films represent unique reaction media. Enhanced reaction rates relative to the bulk phase,[54] selective anion enrichment,[55,56] and water deficiency[16,57,58] have been demonstrated at air/liquid interfaces.[16,17] Few techniques, however, can instantly monitor the formation of primary products on the surface of fresh microdroplets exposed to ppmv O_3(g) levels at ambient temperature and atmospheric pressure for less than ∼1 ms without further processing, under essentially wall-less conditions, on the basis of a universal, as opposed to species-specific, property such as molecular mass. Dynamic electro/thermospray ionization mass spectrometry seems to meet

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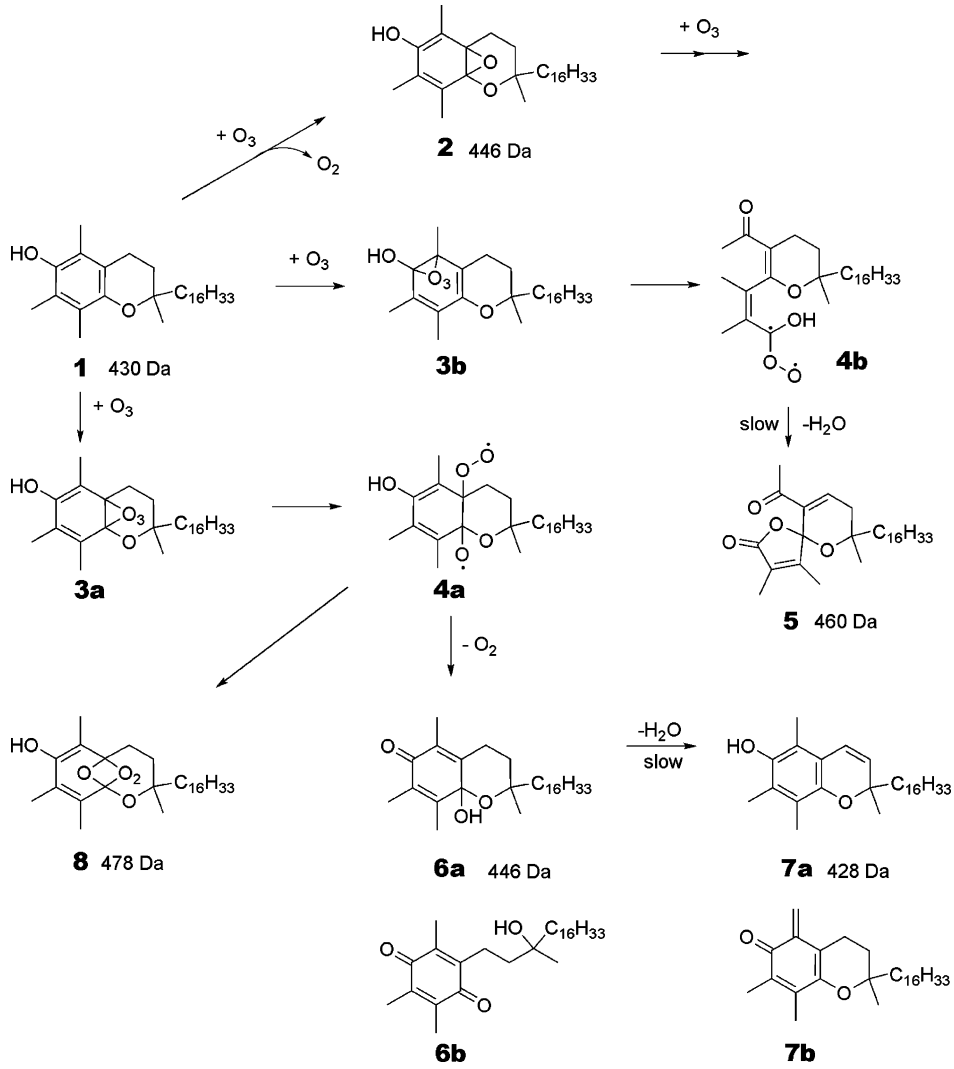
SCHEME 1



these requirements because it combines mass-based detection, high sensitivity, and short integration times.[57,59,60] Herein, we report the online thermospray mass spectrometric detection of the species generated in the interfacial ozonolysis of phenol and α-TOH microdroplets exposed to O_3(g) for ∼1 ms and on the cooperative detoxification of ozone by AH_2/α-TOH mixtures. Our results are compared with previous studies in O_3(g)-sparged α-TOH bulk solutions.[61−63]

Experimental Section

Our experiments simulate reactive O_3(g)/surface interactions in microdroplets, generated by spraying α-TOH solutions into dilute O_3(g)/N_2 mixtures, whose interfacial composition is continuously monitored via online thermospray ionization mass spectrometry (TSMS) after sub-millisecond contact times.[16,57,59] Solutions are pumped (50 μL min^{-1}, Harvard Apparatus) into the spraying chamber (maintained at atmospheric pressure) of a commercial electrospray ionization mass spectrometer (Agilent 1100 MSD Series, modified with an O_3 injection system) through a grounded stainless steel needle coaxial with a sheath issuing nebulizer N_2(g).[64] The fast nebulizer gas (2.65×10^4 cm s^{-1}) shears the liquid jet (10.6 cm s^{-1}), and separates ions already present in solution, into charged microdroplets carrying excess anions or cations.[65,66] The terminal velocities of the microdroplets thus created exceed ∼10^3 cm s^{-1},[67] leading to τ < 1 ms transit times across the ∼0.5 cm wide O_3(g) plume. The negatively charged microdroplets generated in this process carry modest excess charges,[66] in contrast with those produced in conventional electrospray sources maintained at high voltage relative to ground.[65,68,69] These microdroplets *subsequently* evaporate solvent in the dry, warm N_2(g) emanating from the electrically polarized inlet to the mass spectrometer while being drawn to it with increasing acceleration: $a = (ze/m)E$, both because the electric field E is more intense near the inlet and droplets lose mass m, but retain their excess charges ze. The spraying chamber is not, therefore, a conventional well-stirred reactor with a normal distribution of microdroplets residence times but a unidirectional flow reactor that shrinking microdroplets traverse at exponentially increasing speeds and shorter residence times. The strong direct correlation between residence time and droplet size, ensures that O_3(g) mostly reacts with the liquid jet and/or nascent microdroplets. Solvent evaporation ultimately leads to charge crowding, whereby microdroplets shed their interfacial films carrying (from electrostatics) the excess charges into smaller offspring.[64] These events ultimately produce nanodroplets from which bare ions are electrostatically ejected into the gas phase and analyzed by mass spectrometry.[70,71] Thus, the ion composition of the interfacial liquid layers that are incorporated into nascent charged microdroplets is transduced without distortion into the recorded mass spectra.[72] This

technique therefore probes, within ~1 ms, the composition of nanodroplets created from the interfacial layers of the liquid jet and/or nascent microdroplets that had just reacted with O_3(g), prior to warming up, and solvent evaporation. Short exposures minimize secondary reactions and online detection optimizes the search for short-lived species. Typical instrumental parameters were as follows: drying gas flow rate, 10 L min^{-1}; drying gas temperature, 250 °C; nebulizer pressure, 2 atm; collector capillary voltage, +3.5 kV; fragmentor voltage, 17 V. α-TOH (>97%) and L-ascorbic acid (>99%) were dissolved in methanol (>99.9%) or acetonitrile (>99.9%). All chemicals were obtained from Sigma-Aldrich except for acetonitrile (Fisher). Solution pH was adjusted by adding HCl or NaOH and measured using a calibrated pH meter (VWR). Reported pH values correspond to pH measured in methanol using a H^+-electrode calibrated with standard aqueous buffers (see Appendix S1 of the Supporting Information for further details).[73] We verified that anion concentrations [A^-] measured by TSMS in sprayed aqueous solutions of carboxylic acids (pK_a ~4.8) vary with bulk pH according to: $[A^-]/([AH] + [A^-]) = 1/(1 + 10^{pK_a - pH})$, that is as in bulk solution.[74] Further experimental details and validation tests can be found in previous publications.[16,17,54,57,59,70]

Results

Products of α-TOH Ozonation at the Air/Liquid Interface. Figure 1 shows negative-ion mass spectra of 2 mM α-TOH:MeOH microdroplets at pH 10.9 in the absence/presence of O_3(g). α-TO−O^- (429 Da) reacts with O_3(g) within ~1 ms to produce α-TO−O_n^- species at 445 ($n = 1$), 461(2), 477(3), and 493(4) Da. The sum of ion signals from 50 to 800 Da remains constant (±10%) in the absence/presence of O_3(g), ruling out the significant formation of MS-silent products. The same α-TO−$O_{n=1-4}^-$ products, albeit in slightly different proportions, are formed in the ozonolysis of α-TOH solutions in acetonitrile (Figure S1 of the Supporting Information), revealing that these solvents may influence the course of reaction but are not incorporated into the products.

As indicated in the Experimental Section, the detected products are deemed to be formed in reactions between O_3(g) and α-TOH at the air/water interface, rather than in the gas or bulk liquid phases, for the following reasons. The possibility of a (α-TO^- + O_3) ion−molecule reaction in the gas phase[75] is obviously excluded by the fact that α-TO^- conversions depend markedly on solution pH (Experimental Section, and Appendix SA in the Supporting Information). Similar dependences were found in previous ozonation studies in this experimental setup.[16,17,54,57,59] Furthermore, the rate constant for O_3(g) dissolution in an inert microdroplet of radius a is given by eq I:

$$k = \frac{3\alpha\langle c\rangle}{4a} \qquad \text{(I)}$$

By adopting reported mass accommodation coefficients $\alpha > 0.1$ for O_3(g),[76,77] we evaluate a rate constant $k_{diss} = \sim 3 \times 10^7\ s^{-1}$ for the dissolution of O_3(g) in microdroplets of radius $a = 1 \times 10^{-4}$ cm ($\langle c\rangle = 3.9 \times 10^4$ cm s^{-1} is the average molecular velocity of O_3 at 300 K).[78] Therefore, microdroplets should be saturated with O_3 within a small fraction of ~1 ms exposure times. Under 5 ppmv of O_3(g), about 1.5×10^7 O_3 molecules collide and stick to the surface of each microdroplet in these experiments,[76] and the same number ultimately desorbs, rapidly saturating neat methanol or acetonitrile microdroplets with $[O_3]_{sat} = \sim 0.5\ \mu$M (Henry's law constant for the dissolution of O_3 in acetonitrile is ~10 times larger than in water).[54,79] From reported rate constants for the (α-TOH + O_3) reaction in bulk solvents at neutral pH: $k_R < 1 \times 10^5\ M^{-1}\ s^{-1}$,[80] the 1/$e$ reaction time of O_3 dissolved in [α-TOH] = 1 mM solutions is >10 times longer than residence times, implying that chemical scavenging in the bulk does not significantly compete with the physical dissolution of O_3(g). Hence, α-TOH 1/e reaction times in the bulk of microdroplets maintained at $[O_3]_{sat} = \sim 0.5\ \mu$M throughout are >20 s, that is, orders of magnitude longer than that in part A of Figure 2, where 50% α-TOH is ozonized in microdroplets exposed to 5 ppmv O_3(g) for ~1 ms. We infer that our experiments monitor the rapid ozonation of α-TOH on the surface of microdroplets. Because half of the 800 α-TOH molecules (molar fraction, $x = 1.8 \times 10^{-5}$; cross section, $\sigma = \sim 3 \times 10^{-15}$ cm^2 $molecule^{-1}$) present on the surface of fresh $a = 1 \times 10^{-4}$ cm microdroplets (area = 1.25×10^{-7} cm^2) react with 1.5×10^8 colliding O_3(g) molecules in ~1 ms, the reactive uptake coefficient of O_3(g) on α-TOH solutions (assuming a

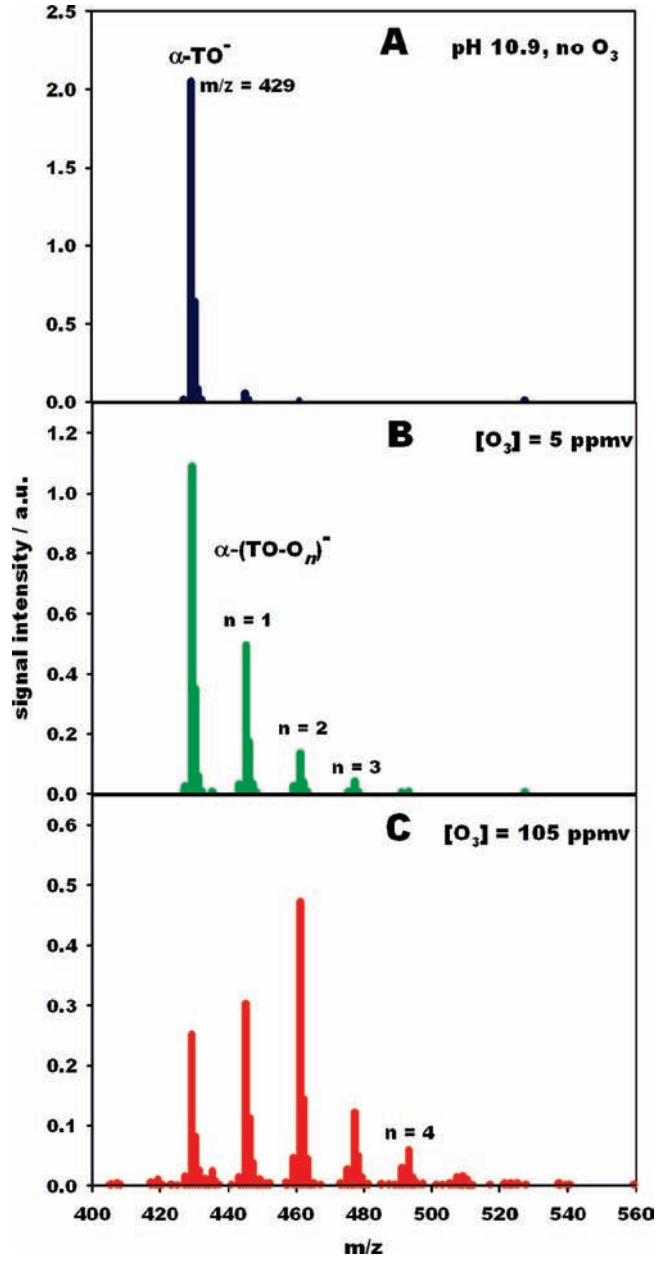


Figure 1. Negative-ion mass spectra of the products of α-TOH + O_3(g) in 2 mM α-TOH:MeOH microdroplets at pH 10.9, in the absence (**A**), and presence (**B** and **C**) of O_3(g).

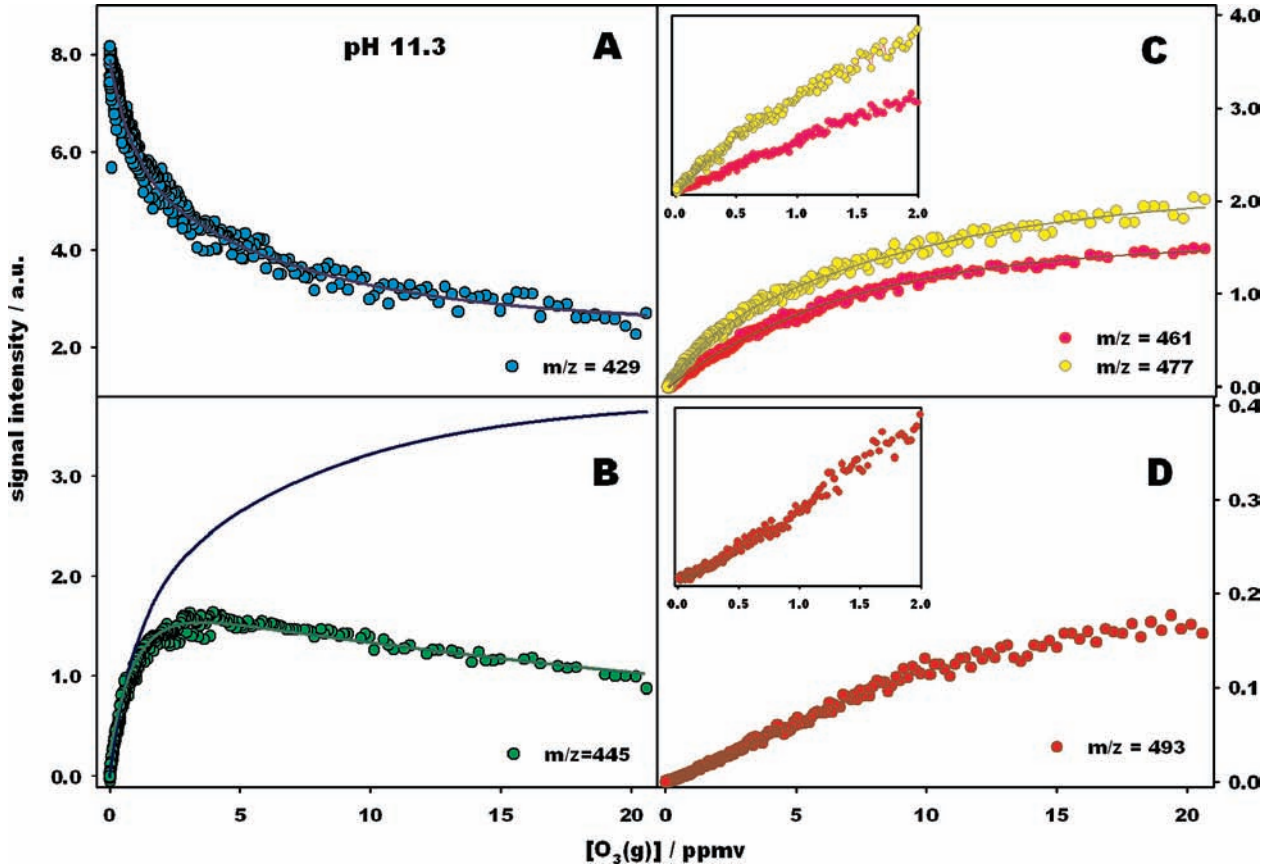


Figure 2. Reactant and products of α-TOH + O_3(g) as functions of [O_3(g)] in the ozonolysis of 1.0 mM α-TOH:MeOH microdroplets at pH 11.3. **A**: m/z = 429 (α-TO^-); **B**: m/z = 445 (α-TO−O)$^-$, **C**: 461 (α-TO−O_2)$^-$ and 477 (α-TO-O_3)$^-$; **D**: m/z = 493 (α-TO−O_4)$^-$.

1:1 stoichiometry) is: $\gamma \sim 400/1.5 \times 10^8 = 2.5 \times 10^{-6}$, or ~40 times larger than on aqueous anthracene solutions.[81] We recently found that O_3(g) oxidizes I^- at identical rates on water and methanol microdroplets,[56] despite the dissimilar structures of their aerial interfaces and an estimated 10-fold larger solubility in methanol than in water, suggesting that rates are controlled by I^-/O_3(g) encounters at the air/liquid interface.[79] We conclude that the observed decay of α-TO^- signals and the concomitant evolution of α-TO−O_n^- signals in the presence of O_3(g) takes place in the gas/liquid interfaces monitored by TSMS.

Figure 2 shows the dependence of reactant and product signals on [O_3(g)] in the ozonolysis of α-TOH:MeOH microdroplets at pH 11.3. α-TO^- decays monotonically (part A of Figure 2), but products display dissimilar behaviors with increasing [O_3(g)] (parts B, C and D of Figure 2). α-TO−O^- peaks at [O_3(g)] ~5 ppmv and declines afterward (part B of Figure 2 and Figure S2 of the Supporting Information), whereas α-TO−$O_{n\geq 2}^-$ signals steadily increase with [O_3(g)] to plateau above ~20 ppmv O_3(g) (Figure S2 of the Supporting Information). However, the yields of α-TO−$O_{n=2,4}^-$ adducts relative to α-TO−$O_{n=3}^-$ increase with [O_3(g)] (Figure S3 of the Supporting Information), implying that they are also generated in secondary reactions.[80] The evolution of α-TO−$O_{n=3}^-$ (part C of Figure 2 and Figure S2 of the Supporting Information) is therefore consistent with the one-step addition of O_3 to α-TOH to produce a relatively unreactive ozonide.

Figure 3 shows positive-ion mass spectra obtained in the interfacial ozonolysis of α-TOH: MeOH microdroplets at pH 1.6. In this case, α-TOH_2−O^+(m/z = 447) is the dominant signal throughout (parts B and C of Figure 3 and part B of Figure 4). The absence of α-TOH_2−$O_{n\geq 2}^+$ signals is clearly due to the inability of α-TOH−$O_{n\geq 2}$ species to protonate at pH 1.6 because they appear as α-TOH−O_n−Cl^-(n = 0−4) clusters in negative-ion mass spectra (parts D−F of Figure 3). Note the presence of a weak m/z = 430 signal, which is isobaric with protonated tocopheryl α-$TOH^{\bullet+}$ (part B of Figure 3). The nearly identical decays of α-TOH_2^+ and α-TOH−Cl^- signals with increasing [O_3(g)] (parts A and C of Figure 4) proves that the course of α-TOH ozonation is independent of the sign of the excess charge of reacting microdroplets. More importantly, they show that the overall α-TOH/α-TO^- reactivity toward O_3(g) in acid media is significantly lower than in basic solutions (cf., the x scales of part A of Figure 2 and parts A and C of Figure 4).

MS/MS of α-TOH Ozonation Products. Tandem mass spectrometry (MS/MS) of α-TO^-, α-TO−O^-, α-TOH−O_2^-, α-TOH−O_3^-, and α-TOH−O_4^- yields m/z = 163, 177, 419/417, 433/407, and 447/449 Da daughter ions (plus their neutral complements) along their lowest energy collisionally induced dissociation (CID) channels (Table S1 of the Supporting Information). Note that none of the parent ions undergo neutral O_n losses, that is α-TOH−O_n^- ions are bona fide products of α-TOH ozonation rather than mass spectral fragments of heavier homologues. Significantly, only α-TO^- and α-TO-O^- split 266 Da ($C_{19}H_{38}$) and 268 Da ($C_{19}H_{40}$) neutrals that carry the phytyl side chain (Table S1 of the Supporting Information). The addition of two or more O atoms to the chroman-6-ol ring opens up lower-energy decomposition channels leading to the extrusion of smaller neutrals such as CH_2CO (42 Da) and CO_2 (44 Da). This is interpreted as evidence that α-TO−O^- results from the epoxidation of the (4a, 8a) double bond shared by the phenyl and (phytyl-)pyranyl moieties (Scheme 1). This process formally releases $^1O_2$ (Scheme 1) that should rapidly add to α-TOH (k_{add} = ~4 × 10^7 M^{-1} s^{-1} in bulk methanol)[82,83] to produce

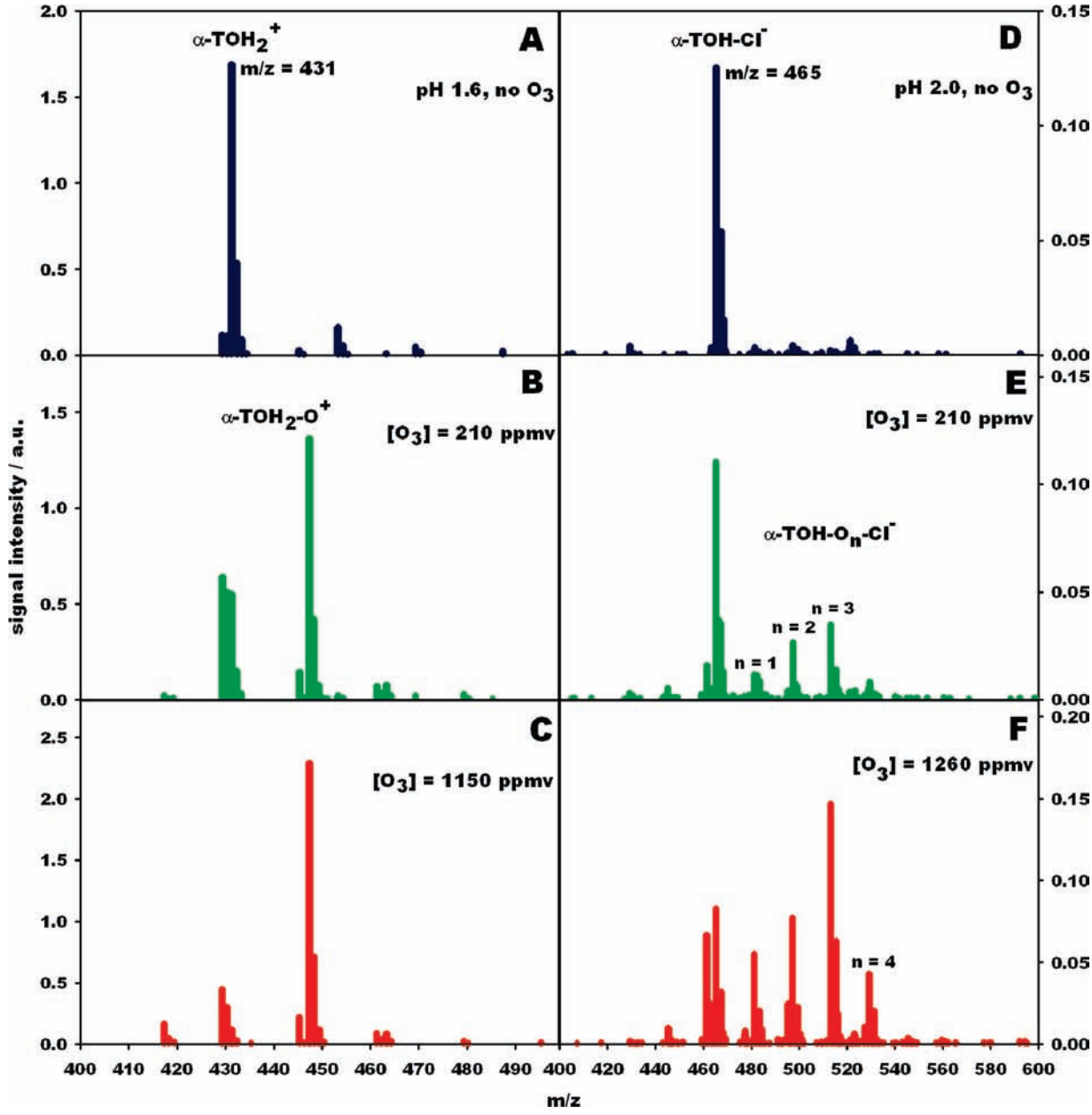


Figure 3. Positive-ion mass spectra of the products of α-TOH + O_3(g) in 1 mM α-TOH:MeOH microdroplets at pH 1.6, in the absence (**A**) and presence (**B** and **C**) of O_3(g). Negative-ion mass spectra of 1 mM α-TOH:MeOH microdroplets at pH 2.0 in the absence (**D**) and presence (**E** and **F**) of O_3(g).

α-TOH$-O_2^-$ in situ. This finding evokes the O_n additions to the purine ring, and the MS/MS fragmentation patterns of the corresponding adducts, in the ozonation of UA.[17] Interestingly, whereas the 429 (α-TO^-) and 445 (α-TO-O^-) split $C_{19}H_{38}$ and $C_{19}H_{40}$ neutrals (Table S1 of the Supporting Information) the conjugated 431 (α-TOH_2^+) and 447 (α-TOH_2-O^+) positive ions split $C_{19}H_{38}$ and $C_{19}H_{38}O$ respectively to generate tropylium ions (Table S2 and Scheme S1 of the Supporting Information).

Phenol Ozonation. We also investigated the interfacial ozonation of the prototype phenol C_6H_5OH in methanolic solution at pH 11.6. In contrast with the experiments of Figure 1, we found that a ~80% decrease of both m/z = 93 (PhO^-) and m/z = 187 ($PhOH-PhO^-$) signals upon exposure to 420 ppmv O_3(g) only yields traces of O-addition products, such as the m/z = 141 putative ozonide (cf. Figures 1 and 5). These results confirm previous reports that the ($PhO^- + O_3 \rightarrow PhO\cdot + O_3^{\cdot-}$) reaction in aqueous solutions largely proceeds via electron transfer into PhO· [E^0(PhO·/PhO^-) = 0.86 V, E(O_3/$O_3^{\cdot-}$) = 1.03 V][32,84] and represent direct evidence that the dominant [$C_6H_5O^- + O_3$(g)] and [α-$TO^- + O_3$(g)] reactions at the air/liquid interface involve electron transfer and O_n addition, respectively. The fate of PhO· at longer times (i.e., beyond the ~1 ms reaction times accessible to present experiments) may involve self-association into (neutral) species and/or ozonation. The $O_3^{\cdot-}$ radical anion will be immediately protonated to HO_3· en route to (·OH + O_2).[85] Because the addition of a large excess of *tert*-BuOH (an efficient ·OH radical scavenger that is otherwise inert to O_3) has no effect on the course of C_6H_5OH ozonolysis (Figure S4 of the Supporting Information), we infer that ·OH radicals, should they be formed in our system, do not play a significant role in the interfacial ozonation of mM C_6H_5OH:MeOH solutions during submillisecond reaction times.

Ozonation of α-TOH/AH_2 Mixtures. Considering that: (1) α-TOH and AH_2 are simultaneously present in plasma (25 and 40 μM, respectively) and epithelial lining fluids (2.5 and 100 μM, respectively)[6] and, (2) AH_2 is >75 times more reactive than

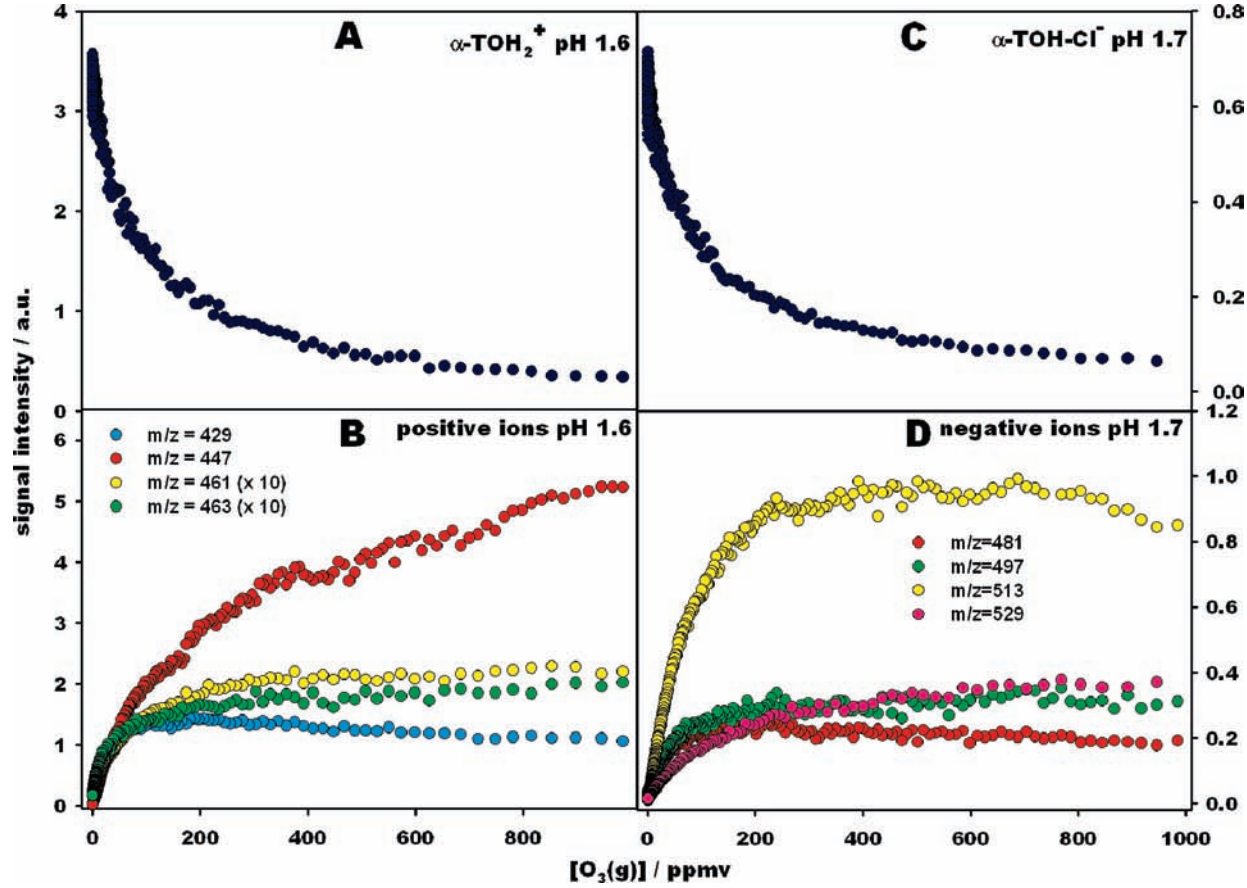


Figure 4. Reactant and products of α-TOH + O_3(g) as functions of [O_3(g)] in the ozonolysis of 1.0 mM α-TOH:MeOH microdroplets at pH 1.6 (**A** and **B**), at pH 1.7 (**C** and **D**). **A**: m/z = 431 (α-TOH_2^+). **B**: m/z = 429 [7a($+H^+$) and/or 7b($+H^+$), Scheme 1], 447 (α-TOH_2-O^+), 461 [**5**(+**H**)$^+$, Scheme 1], 463 (α-$TOH_2-O_2^+$). **C**: m/z = 465 (α-TOH−Cl^-). **D**: m/z = 481 (α-TOH−O−Cl^-), 497 (α-TOH−O_2−Cl^-), 513 (α-TOH−O_3−Cl^-), 529 (α-TOH−O_4−Cl^-).

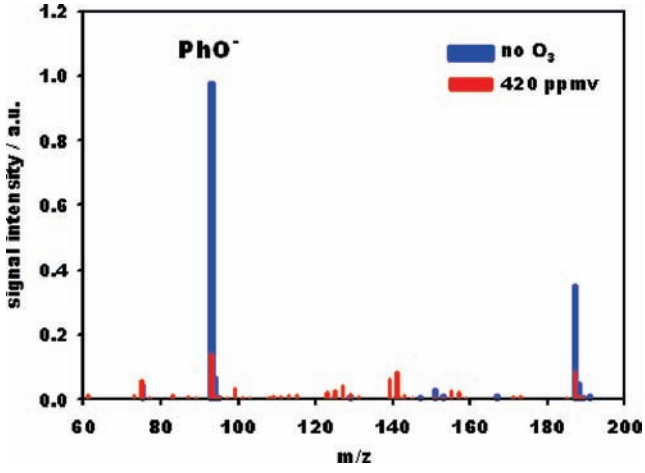


Figure 5. Negative-ion mass spectra of PhOH + O_3(g) in 1.0 mM PhOH:MeOH microdroplets at pH 11.6 in the absence (blue trace) and presence (red trace) of 420 ppmv O_3(g).

α-TOH toward O_3 in bulk aqueous solutions at physiological pH,[86] we explored the potential competition/interactions between α-TOH and AH_2 ozonations at the air/liquid interface. The ozonation of aqueous AH_2 at pH ∼7 in this experimental setup had been found to generate threonate (THR^-, m/z = 135), dehydroascorbate (DHA^-, m/z = 173), and a secondary ascorbate ozonide (AOZ^-, m/z = 223) products that are impervious to further attack by O_3(g).[16] The negative-ion mass spectra of ozonized (α-TOH + AH_2) microdroplets contains, in addition to the aforementioned peaks, prominent signals at m/z = 619, 635, 651, and 667, which are readily assigned to DHA-(α-TO−O_n)$^-$(n = 1−4) clusters. There is no evidence of similar adducts involving THR^- or AOZ^-. Remarkably, whereas AH^- ozonation losses are unaffected by up to 2 mM α-TOH (Figure 6, upper panel), in accord with their relative concentrations and reactivities in bulk solution,[86] the production of both THR^- and AOZ^- is significantly inhibited by the addition of >1 mM α-TOH (Figure 6 and Figure S5 of the Supporting Information). These observations seem to imply that: (1) α-TOH and AH_2 do not compete for O_3(g) on sparsely covered interfaces and, (2) α-TOH intercepts reactive intermediates, possibly the primary 1,2,3-trioxolane ozonide of ascorbate,[17,51] which would otherwise proceed to AOZ and THR.[16] DHA production may also be inhibited by α-TOH, but its extensive association in DHA-(α-TO−O_n)$^-$ clusters precludes a firmer conclusion.

Discussion

Ozone can possibly transfer an O atom to α-TOH (with the simultaneous release of $^1O_2$), accept an electron from α-TOH/α-TO^-, or add to the chroman-6-ol ring (Scheme 1). Thermochemistry and spin conservation, however, preclude direct O_2 transfers (+ ^3O).[87] The prototype aromatic PhO^- appears to react via electron transfer into neutral species that cannot be protonated or deprotonated, such as the phenoxyl PhO•, and remain MS-silent under present experimental conditions (Figure 5):[84,88,89]

$$PhO^- + O_3 \rightarrow PhO\bullet + O_3\bullet^- \quad (1)$$

This finding concurs with previous studies of phenol ozonolysis in bulk water.[84] A similar pathway in the case of α-TO$^-$ would generate the corresponding phenoxyl α-TO• that, in contrast with PhO•, could be protonated at the ether position of the pyran ring (below). The detection of weak α-TOH$^{\cdot+}$(m/z = 430) signals under acidic conditions (part B of Figure 3) indicates, however, that this is a minor channel.[80] α-TO•, lacking the ionizable α-TO−H function, is, of course, MS-silent in the negative-ion mode. It is apparent that the reaction between O_3(g) and α-TOH is dominated by the transfer of one to nine O-atoms under acid or basic conditions (Figures 1 and 3).

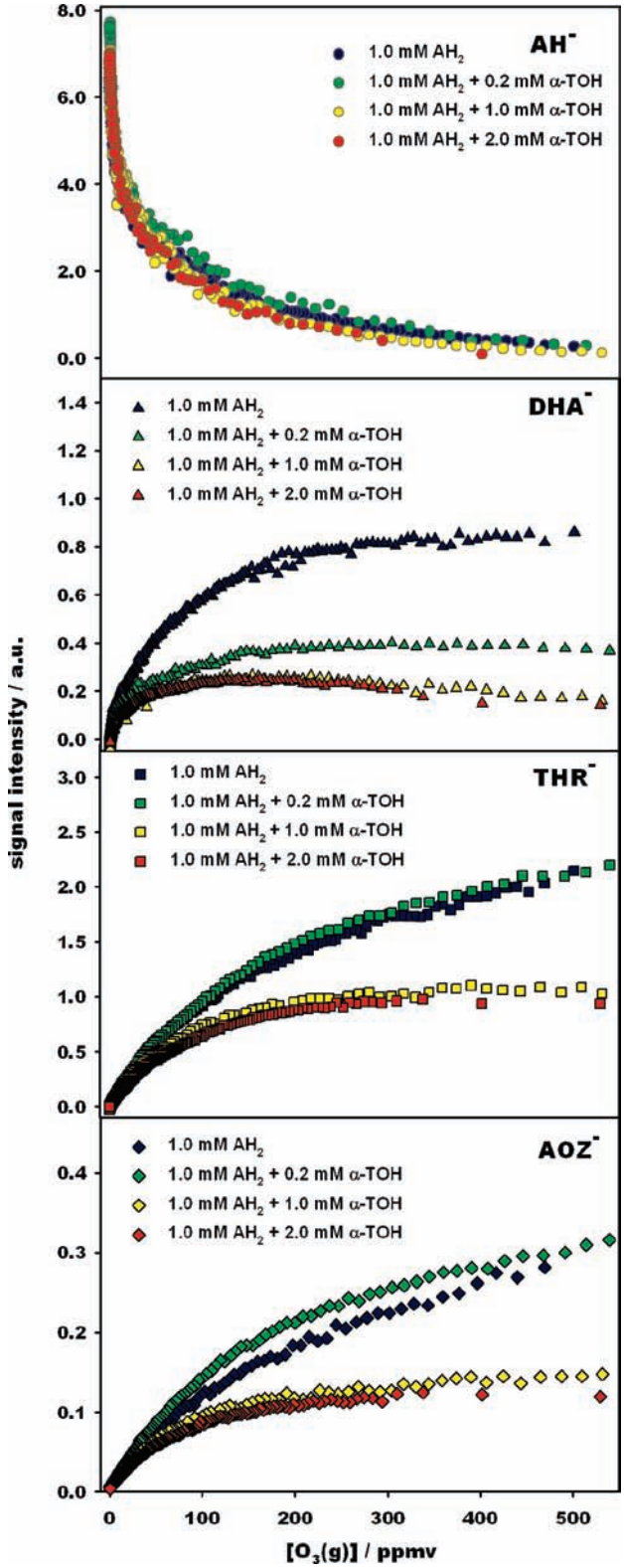


Figure 6. Reactant and products of AH_2 + α-TOH + O_3(g) as functions of [O_3(g)] in the ozonolysis of (1.0 mM AH_2 + α-TOH): MeOH microdroplets at pH 7.2. m/z = 175 (AH^-), 173 (DHA^-), 135 (THR^-), 223 (AOZ^-).

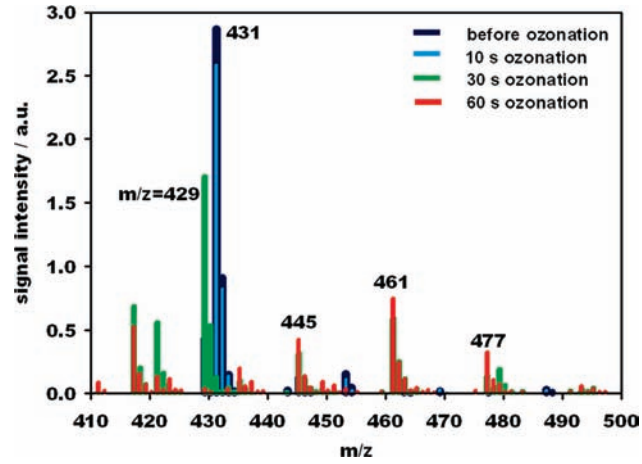


Figure 7. Positive-ion mass spectra of α-TOH + O_3(g) in 1 mM α-TOH:MeOH solutions at pH 1.6 before and after being sparged with 1000 ppmv O_3(g) for variable periods.

At pH > 10, α-TO$^-$ reacts with O_3(g) leading to α-TO−O$^-$ as the main product. α-TO−O$^-$ is as reactive as α-TO$^-$ toward O_3(g) because its concentration peaks at ∼50% α-TO$^-$ conversion (part B of Figures 2 and Figure S2 of the Supporting Information). α-TO−O_2^-, α-TO−O_3^-, and α-TO−O_4^-signals also appear at the onset (parts C and D of Figure 2) increase steadily with [O_3(g)], and level off after α-TO$^-$ is depleted, but their relative yields depend on the extent of α-TO$^-$ ozonation revealing the occurrence of additional chemistry even in this short time scale. The ozonation of α-TOH at pH < 2 follows a similar course leading to α-TOH−O_n species that can be detected as α-TOH−O_n−Cl$^-$ clusters (parts D−F of Figures 3). Note the presence of weak m/z = 429 and 445 signals, which correspond to α-$TOH_2(-H_2)^+$ and α-$TOH_2(-H_2)$−O^+. They are likely produced from α-TOH_2−O and α-TOH_2−O_2 via slow dehydrations that run their full course in experiments in which α-TOH:MeOH solutions are sparged with O_3(g) for up to 60 s and then analyzed by electrospray mass spectrometry (Figure 7).

A reaction mechanism underlying these findings is proposed in Scheme 1. Assignments are based on positive/negative-ion spectra, MS/MS fragmentation patterns, and by analogy with the products previously identified by Liebler et al., in the ozonolysis of α-TOH in sparged acetonitrile and acetonitrile/water (60:40) solutions.[61−63,84] α-TOH (**1**), the 430 Da chroman-6-ol, can be epoxidized to **2** (446 Da), or add O_3 to either of its (5,6) or (4a,8a) double bonds leading to the 478 Da primary ozonides **3a** and **3b**, respectively.[51,90] **3a** and **3b** are expected to open into the Criegee biradicals **4a** and **4b**, respectively. **4a** may lose O_2 prior to its conversion into the 446 Da 8a-hydroxy-chromen-one **6a**, or the quinone **6b**,[62,63] or undergo reclosure into the persistent secondary ozonide **8**. **4b** may lose H_2O and rearrange into the 460 Da dioxaspiro-dien-2-one **5**.[61−63] **6a** slowly dehydrates into the 428 Da chromenol **7a** or the chromen-one **7b** (above). The unsaturations remaining in **2**, **6a**, and **6b** are targets for further O_3 attack leading to α-TOH−O_n (462, 478, or 494 Da, n = 2−4, respectively) species (not shown in Scheme 1). The prominent α-TO−O_2^- product (m/z = 461), however, is deemed to largely arise from concerted O/O_2 transfers: O_3 + 2 α-TOH → α-TOH−O_2 + α-TOH−O, or,

alternatively, by the rapid addition of $^1O_2$ (from O_3 + α-TOH → α-TOH−O + $^1O_2$) to α-TOH. The m/z = 429 and 445, signals in negative-ion electrospray mass spectra are assigned to **1** and **2** (rather than to the isobaric **6a** and **6b**) because they preserve the acidic chroman-6-ol function. Further evidence that α-TOH−O is the chromanol **2** rather than the chromen-one **6a** is provided by the fact that only α-TO−O$^-$ along with α-TO$^-$ lose the phytyl group via CID, and that they are similarly reactive toward O_3 (part B of Figure 2 and Figure S3 of the Supporting Information). Previous reports on the ozonolysis of α-TOH in bulk solutions indicated that **5** and **6a** are initial products and that **6a** is subsequently transformed into **6b** (Scheme 1).[61−63] Note that none of the products appearing in these mass spectra incorporates methanol, as would be expected from the nucleophilic trapping of putative Criegee biradicals, such as **4a** or **4b**.[90]

Positive-ion mass spectra reveal that, among ozonation products at pH ∼2, only α-TOH−O can be significantly protonated into m/z = 447 (α-TOH$_2$−O)$^+$ (parts B and C of Figure 3). The higher *n*-homologues remain neutral and, hence, MS-silent. This observation suggests that only α-TOH-O retains one or more functional groups having sizable proton affinities (PA). Because the PAs of the ether functions of tetrahydropyran (836 kJ mol^{-1}) and 2-methyl tetrahydrofuran (852 kJ mol^{-1}) are considerably larger than those of ketones (823 kJ mol^{-1}), secondary or tertiary alcohols (<810 kJ mol^{-1}), epoxides (774 kJ mol^{-1}), or phenols (753 kJ mol^{-1}),[91] we suggest that α-TOH−O$_{n\geq 2}$ species may have lost the chromane ether function. The only long-lived product that preserves both the phenolic and the ether functions of α-TOH and hence will appear in negative- and positive-mode mass spectra is the epoxide **2**. One can only speculate that the ether group of the secondary ozonide **8** may be considerable less basic and, hence, cannot be protonated. The strong signals at m/z = 429 and 461 in the positive-ion electrospray mass spectra of methanolic α-TOH solutions that had been sparged with O_3(g) for up to 60 s (Figure 7, cf. parts A−C of Figure 3) arise from slowly forming products. The slow dehydrations of **4b** into **5** and **6a** into **7a**,**b**, respectively,[62] meet these requirements (Scheme 1). Note the intense m/z = 513 (α-TOH−O$_3$−Cl$^-$) signal in part D of Figure 4 that reports ozonide yields under acidic conditions vs its weak m/z = 477 (α-TO−O$_3^-$) counterpart at high pH (part C of Figure 2). We had previously found that ozonide yields are also enhanced at low pH in the ozonolysis of AH_2 and UA.[16,17] These findings amount to enhanced production of strong pro-oxidant species on acidified biosurfaces.[92−96]

The lack of solvent incorporation into the products of α-TOH ozonation[49,51,90] may reflect both chemical structure and the unique properties of air/liquid interfaces as reaction media. The fast trapping of Criegee diradicals/zwitterions by nucleophilic methanol into α-methoxy-hydroperoxides has been previously used as a mechanistic test of their participation.[90] The putative Criegee intermediates derived from the primary ozonides **3a**,**b** (Scheme 1), however, seem to forego solvolysis in favor of intramolecular ring closure and decomposition processes.[17] Considering that the interfacial ozonolysis of acyclic unsaturated phospholipids in MeOH[97,98] and DMF/MeOH (90:10)[99] exclusively yields α-methoxy-hydroperoxides, and that the solvent is incorporated even into the products of cholesterol ozonolysis in aqueous dispersions,[100] we infer that the dominance of intramolecular ring closure over solvolysis in the interfacial ozonolysis of the endocyclic double bonds of AH_2, UA, and α-TOH is evidence of media effects *at the air/liquid interface*.[16,17] We have shown elsewhere that the initial slopes of [reactant] versus [O_3(g)] plots are proportional to reaction rate constants.[16] The data of part A of Figure 2 and parts A and C of Figure 4 yield a rate constant ratio for [α-TOH + O_3(g)] in methanol: k(pH 11.3)/k(pH 2.0) of ∼380, which is qualitatively consistent with the pH-dependence of [α-TOH + O_3(aq)] rates measured in bulk water.[86]

Summing up, the flash ozonolysis of α-TOH at air/liquid interfaces generates α-TOH-O$_n$(n = 1−4) species rather than α-TO· as initial products, some of which are potential pro-oxidants.[10] The rapid processing of complex, substituted phenols, such as those identified by Cass et al., in wildfire plumes,[42] by atmospheric ozone should generate similar oxidants on secondary aerosol particulates,[101] in competition with their degradation by ·OH radicals.[102] The fate of these species upon inhalation remains to be explored.[103]

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Supporting Information Available: Additional data, data analysis and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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