AQUEOUS PHOTOCHEMISTRY OF 2-OXOCARBOXYLIC ACIDS

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AQUEOUS PHOTOCHEMISTRY OF 2-OXOCARBOXYLIC ACIDS

DISSertation

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Arts and Sciences at the University of Kentucky

By

Alexis Eugene

Lexington, Kentucky

Director: Dr. Marcelo I. Guzman, Associate Professor of Chemistry

Lexington, Kentucky

2018

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ABSTRACT OF DISSERTATION

AQUEOUS PHOTOCHEMISTRY OF 2-OXOCARBOXYLIC ACIDS

Atmospheric aerosols affect climate change by altering the energy balance of the atmosphere, and public health due to their variable chemical composition, size, and shape. While the formation of secondary organic aerosol (SOA) from gas phase precursors is relatively well understood, it does not account for the abundance of SOA observed during field measurements. Recently it has become apparent that in-aerosol aqueous chemical reactions likely provide some of the missing sources of SOA production, and many studies of aqueous phase processes are underway.

This work explores the fates of the simplest 2-oxocarboxylic acids, glyoxylic acid (GA) and pyruvic acid (PA), under simulated solar irradiation in the aqueous phase. Field measurements have revealed that mono-, di-, and oxocarboxylic acids are abundant species present in atmospheric waters. Of particular interest are 2-oxocarboxylic acids because their conjugated carbonyl moieties result in significant UV-visible absorption above 300 nm, allowing absorption of sunlight in the lower troposphere, thereby initiating radical photochemistry and leading to formation of SOA.

In Chapter 2 of this work, GA is demonstrated to primarily undergo $\alpha$-cleavage, producing CO, CO$_2$, formic acid, and the key SOA precursor glyoxal. Trace amounts of oxalic acid and tartaric acid are also quantified. Additionally, the dark thermal aging of glyoxylic acid photoproducts, studied by UV-visible and fluorescence spectroscopies, reveals that the optical properties of the solutions are altered radically by the glyoxal produced. The optical properties display periodicity during photolytic-dark cycles, reflecting behavior expected for aerosols during nighttime and daytime cycles.

In contrast, Chapter 3 shows that PA photoreacts via a proton-coupled electron transfer (PCET) mechanism that produces CO$_2$ and organic acids of increased complexity with 6 to 8 carbons. A combination of analytical techniques including $^1$H and $^{13}$C NMR; $^{13}$C gCOSY NMR; mass spectrometry; chromatography; and isotope substitutions allows the organic products to be identified as: 2,3-dimethyltartaric acid; 2-hydroxy-2-((3-oxobutan-2-yl)oxy)propanoic acid; and the quasi-intermediate 2-(1-carboxy-1-hydroxyethoxy)-2-methyl-3-oxobutanoic acid.
In Chapter 4, PA irradiation is also shown to consume dissolved oxygen so fast that solutions become depleted within a few minutes depending on reaction conditions. This fast process directly produces the atmospheric oxidant singlet oxygen, which enhances the oxidizing capacity of the atmosphere. Additionally, PA photochemistry only proceeds under very acidic conditions (pH ≤ 3.5), like those in most atmospheric aerosols.

Finally, we require a thorough understanding of the behavior of 2-oxocarboxylic acids at the air-water interface of aerosols because much of the GA and PA present in the atmosphere is produced in the gas phase and needs to partition into the aqueous phase to undergo photoreaction. Therefore, Chapter 5 uses surface sensitive online electrospray ionization mass spectrometry (OESI-MS) to demonstrate that carboxylic acids delivered from the gas phase onto the surface of aqueous microdroplets display enhanced acidities relative to bulk water solutions.

This work demonstrates that aqueous photolysis is a very competitive atmospheric fate for both GA and PA. It also shows that these photoreactions are capable of contributing substantially to SOA formation by building chemical complexity and forming oxidants directly.

KEYWORDS Aqueous photochemistry, pyruvic acid, glyoxylic acid, secondary organic aerosol (SOA), online electrospray mass spectrometry (OESI-MS)
AQUEOUS PHOTOCHEMISTRY OF 2-OXOCARBOXYLIC ACIDS

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December 5, 2018

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Chapter 0. Plain Language Summary

Earth’s atmosphere is made up of not just gas, but contains very small particles known as aerosols. These particles can have very large and important effects on the environment. A well-known example of this is smog formation in large cities where the particle concentration has become too high. Breathing in the smog caused by these particles can aggravate respiratory problems like asthma and even lead to cancer in the long term. The particles also have major effects on the Earth’s climate by directly preventing sunlight from reaching the ground or by forming clouds that block incoming sunlight. Much like how meteorologists can predict tomorrow’s weather, climate scientists can predict what effects the particles will have on Earth’s future climate and on the health of humans. This work aims to improve these predictions by providing an improved understanding of chemical reactions that happen within the particles. Specifically, the work in this dissertation studies what happens to two chemicals commonly found in water based atmospheric particles, called glyoxylic acid and pyruvic acid, when they are exposed to simulated sunlight. The results show that both chemicals undergo complex reaction steps in water under a variety of conditions to form products that likely contribute to an increase in the amount of atmospheric particles on Earth. The efficiency of each reaction measured here is reported and can be used by climate scientists to improve the accuracy of their predictions. In the long run, the improved understanding provided by these results should help protect the health of the human race by preventing large-scale disasters like smog events. The results should also contribute toward guiding decisions about environmental policy.
Chapter 1. Introduction and Background

1.1 Abstract
Atmospheric organic aerosols play a major role in climate, demanding a better understanding of their formation mechanisms by contributing multiphase chemical reactions with the participation of water. The sunlight driven photochemistry of aqueous 2-oxocarboxylic acids is a potential major source of organic aerosol, which prompted the investigations into the mechanisms of glyoxylic and pyruvic acid photochemistry presented in chapters 2, 3, and 4 of this dissertation. Before undergoing aqueous reactions, 2-oxocarboxylic acids must first partition into aerosol droplets, thus chapter 5 examines the acid-base reaction that occurs when pyruvic acid meets the interface of aqueous microdroplets. This introduction chapter describes relevant background information needed to understand the motivations for the studies performed and methods employed in the subsequent chapters.

1.2 Why study 2-oxocarboxylic acids?
The aqueous photochemistry of simple 2-oxocarboxylic acids is a topic that has garnered much attention in the literature recently,1-7 because of the complex mechanisms of reaction involved. The chemical behavior of these small molecules is of interest to a wide variety of disciplines because they are common waypoints in the chain of oxidation of various organic molecules, for example the atmospheric processing of aromatic emissions,8-11 of both biogenic and anthropogenic origin. 2-Oxocarboxylic acids are also key species in the chain of reactions during glycolysis12 and the reductive tricarboxylic acid cycle.13 The prevalence of 2-oxocarboxylic acids across many different chemical environments, from atmospheric water to the inside of cells, demands a thorough
understanding of their transformation pathways. This dissertation will explore the behavior and photochemical transformations of 2-oxocarboxylic acids in the context of atmospheric aqueous chemistry.

1.3 Atmospheric chemistry and secondary organic aerosol

Earth’s atmosphere is a dynamic, chemically rich environment made up of organic and inorganic species partitioned between the gas phase and solid/liquid particles suspended in the air. These particles are called aerosols and vary widely in size (~1 nm to 20 μm) and chemical composition. Aerosols are typically divided into size ranges by their diameters, resulting in two broad classifications: fine aerosol (typically particles smaller than 1 μm), and coarse aerosol (particles larger than 1 μm). Coarse aerosol is primarily composed of inorganic particles like dust or sand that are mechanically dispersed into the air. Fine aerosol is composed of a mixture of inorganics and organic compounds, the ratio of which varies based on the source of the aerosol. The inorganics present in fine aerosol are primarily ammonium sulfate with some nitrate. Fine aerosol also contains liquid water because of the high hygroscopicity of its other components. The organic component of fine aerosol is made up of many different water soluble species containing alcohols, carbonyls, oxidized aromatic compounds, and organic acids including 2-oxocarboxylic acids. The mass fraction of organics in fine aerosol can be anywhere from 20-90% depending on the source of the aerosol. The most abundant 2-oxocarboxylic acids in the atmosphere, and therefore most likely to contribute a significant impact on atmospheric chemistry, are glyoxylic acid (GA, two carbons) and pyruvic acid (PA, three carbons). Both acids are semivolatile species that exist in both the gas phase and the particle phase. However, for both species a majority of the
carbonyl group gets hydrated forming a gem-diol. For GA, the $K_{\text{hyd}}$ for this process is 300 at $25^\circ\text{C}^{20}$ so >99% of GA exists as the gem-diol, while only 68% of PA is hydrated ($K_{\text{hyd}}$ = 2.10 at 25 °C).$^{21}$ The existence of the less volatile gem-diol form enhances partitioning of GA and PA to the particle phase, where the majority is found according to their Henry’s law constants, $K_{H,\text{GA}} = 1.1 \times 10^2 \text{ mol m}^{-3} \text{ Pa}^{-1}$ and $K_{H,\text{PA}} = 3.1 \times 10^3 \text{ mol m}^{-3} \text{ Pa}^{-1}$.$^{1,22}$ Average particle phase concentrations of GA have been observed to be 44.3 ng m$^{-3}$ in urban aerosol$^{15}$ and 4.91 ng m$^{-3}$ in pristine marine aerosol.$^{19}$ In the same measurements, PA was found at levels of 49.7 ng m$^{-3}$ (urban)$^{15}$ and 0.79 ng m$^{-3}$ (pristine).$^{19}$ Similar ranges of GA and PA concentrations have been measured in various studies.$^{23-25}$ For the range of relative humidity 50-90%,$^{26}$ the water content in aerosols is dominated by the deliquescence of ammonium bisulfate. This requires that the liquid particles have a ratio of ~0.6 g H$_2$O/1 g sulfate.$^{27,28}$ Simultaneous measurements of GA, PA, and sulfate reveal possible aqueous concentrations of up to ~250 mM for both GA and PA in acidic urban aerosols.

Figure 1.1 shows a general formation scheme of the different components of organic aerosol. Organic aerosol is further divided into primary organic aerosol (POA), also sometimes called particulate matter (PM), and secondary organic aerosol (SOA) based on the formation mechanism of the aerosol. POA is composed of solids or liquids emitted directly to the atmosphere from anthropogenic sources like combustion engines and cooking fires, or from natural biogenic sources, as shown in Figure 1.1. Traditionally, SOA has been thought of as being formed from the gas-to-particle condensation of lower volatility products from the oxidation of volatile organic compounds (VOCs), or by the aging (oxidation) of POA,$^{16,29}$ thus most of the literature in the past has focused on these
pathways.\textsuperscript{30} However, much recent work has begun to recognize the importance of aqueous chemistry occurring in cloud and fog droplets to the formation of SOA.\textsuperscript{30-34}

![Diagram showing formation pathways of organic aerosol species in the atmosphere.](image)

**Figure 1.1** Pictorial representation showing formation pathways of organic aerosol species in the atmosphere.

Within the framework of Figure 1.1, GA and PA are both formed during the oxidation of aromatic species emitted as POA and organic gases from combustion\textsuperscript{11, 35, 36} and biomass burning.\textsuperscript{8-10, 37} They are also formed during the processing of the isoprene given off by plants,\textsuperscript{38-41} which is the most abundant nonmethane VOC emitted to the atmosphere each year.\textsuperscript{42} It is estimated that 15.72 Tg of glyoxylic acid and 0.85 Tg of pyruvic acid are produced in the atmosphere every year, primarily in the aqueous phase.\textsuperscript{43}

The broad range of sources and chemical compositions of the aerosols discussed above has huge implications for the effect aerosols have on Earth’s energy balance, which is the balance between incoming and outgoing radiation. In the absence of other influences, more incoming than outgoing radiation will produce a warming effect on Earth’s surface and vice versa. Aerosol effects on this energy balance fall into two
categories. The first is through aerosol-radiation interactions, where incoming solar radiation is either absorbed or scattered by the aerosol, depending on the composition and optical properties of the aerosol.\textsuperscript{44} Aerosol that absorbs radiation contributes a net warming effect to Earth’s atmosphere, while aerosol that scatters radiation reduces the amount of radiation that enters the atmosphere, providing a net cooling effect. The second way aerosol affects the energy balance is through the aerosol-cloud interaction. Aerosols act as cloud condensation nuclei, providing seeds where larger cloud droplets can grow and enhancing cloud cover of the Earth, thereby reflecting more solar radiation and causing a net cooling effect on the environment.\textsuperscript{44} Aerosol effects are directly related to the mass loading of particles in the air, dictating that any quantification of these effects needs the most accurate accounting possible of how much SOA is present under various conditions.

The extent to which aerosols contribute to cooling or warming is well understood for many types of aerosols, but the contribution from SOA is less certain. Figure 1.2 shows the estimated effect of aerosols on the atmosphere presented as the radiative forcing due to aerosol-radiation interactions (RFari) with uncertainties and for various types of aerosol. The estimates in Figure 1.2 were synthesized from a large body of peer reviewed literature by the Intergovernmental Panel on Climate Change (IPCC) in their fifth assessment report.\textsuperscript{44} Formally, the radiative forcing in Figure 1.2 is the change in the total downward flux of incoming radiation at the top of the troposphere due to the presence of aerosol particles directly interacting with radiation. This means that a negative RFari denotes a reduction of radiation reaching Earth’s surface and a net cooling effect caused by that type of aerosol. One can observe from Figure 1.2 that the uncertainty in the RFari
for SOA is very large compared to the magnitude, for both modeling methods. It is thought that this uncertainty is due to atmospheric models predicting SOA loadings much lower than what is observed by field studies, and that one of the main reasons for the underprediction is that there are other mechanisms of SOA formation not yet incorporated into the models. In order to improve the models, these unknown mechanisms need to be identified, have their significance to the real atmosphere evaluated, and have their physical parameters such as rate constants and quantum yields measured in the laboratory. The aqueous photochemistry of 2-oxocarboxylic acids is likely one of these missing sources that needs to be characterized and included in the models.

**Figure 1.2.** Annual mean top of the atmosphere radiative forcing due to aerosol–radiation interactions (RFari, in W m\(^{-2}\)) due to different anthropogenic aerosol types, for the 1750–2010 period. Hatched whisker boxes show median (line), 5th to 95th percentile ranges (box) and min/max values (whiskers) from AeroCom II models corrected for the 1750–2010 period. Solid colored boxes show the AR5 best estimates and 90% uncertainty ranges. BC FF is for black carbon from fossil fuel and biofuel, POA FF is for primary organic aerosol from fossil fuel and biofuel, BB is for biomass burning aerosols and SOA is for secondary organic aerosols. Reprinted with permission from Figure 7.18 of reference 44.
1.4 Atmospheric photochemistry and 2-oxocarboxylic acids

Sunlight is overwhelmingly the largest source of energy input on the Earth. As such, it is the driving force for most of the chemical transformations that occur in the atmospheric gas and aqueous phases. The majority of organic aerosol can be found in the troposphere where sunlight of wavelengths greater than 290 nm penetrates. The troposphere is the lowest layer of the atmosphere that extends from the Earth’s surface to about 10-16 km depending on the current temperature, underlying surface configuration, latitude, and time of day. The primary reason for the 290 nm cut off in the troposphere is that ozone (O₃) in the layer directly above the troposphere, called the stratosphere, filters out much of the light between 220 and 330 nm. The total solar irradiance that reaches the Earth’s atmosphere is on the order of 1360 W m⁻², a huge amount of energy that allows many varied light-dependent reactions to occur.

In atmospheric chemistry, organic molecules typically undergo two types of photochemical reactions: direct and indirect photolysis. Direct photolysis may occur if the species in question can absorb photons of the wavelengths available in sunlight. Typically, the importance of direct photolysis is evaluated by comparing quantum yields (Φ) which is defined as a number of events per photon absorbed. In this case it would be the number of molecules destroyed or generated per photon absorbed. Φ for many processes in the gas phase and in organic solvents have been reported in the literature, but aqueous photochemical processes have received little attention until recently, leading to a lack of Φ values that can be used in atmospheric models to evaluate the relative importance of each photochemical process. If the molecule cannot absorb sunlight, indirect photooxidation can occur through reaction with photochemically produced
hydroxyl radical (HO’), which is the most important atmospheric oxidant in both the gas phase and the aqueous phase. For most organics, reaction with HO’ is the primary fate because their absorption bands are below 290 nm, or they have such small molar absorption coefficients that the typically fast HO’ reaction outcompetes direct photolysis.34

In this regard, 2-oxocarboxylic acids are a special case. Because of the conjugation of the two adjacent carbonyls, the n→π* transition of 2-oxocarboxylic acids’ absorption profiles are shifted to low enough energy that they can absorb sunlight directly, leading to complex radical chemistry. Figure 1.3 shows the UV-vis absorption spectra of aqueous GA (black trace) and PA (blue trace) overlapped with the solar irradiance spectrum (red trace) recorded on a sunny cloudless day at 230 m above ground level at noon on August 3, 2018 in Lexington, KY, USA (elevation 298 m). The vertical dashed line indicates the minimum wavelength of solar radiation that can penetrate to ground level, 290 nm. The overlap between GA/PA and the solar spectrum indicates that both 2-oxocarboxylic acids should be readily photolyzed to form higher complexity, more oxidized products, contributing to SOA production in the atmosphere. Furthermore, two studies based on a combination of rate constants and absorption profiles have predicted that direct aqueous photolysis should be a significant fate for PA, and may even be the dominant fate for PA rather than gas phase photolysis or gas/aqueous reaction with HO’.1, 34 GA can also be expected to undergo direct photolysis, albeit at a slower rate. However, with the much higher atmospheric production of GA, its direct photolysis should still have a significant impact on atmospheric composition, and both GA and PA should contribute to SOA production.
Thus, given their potential importance to SOA production, this dissertation will identify the products of and propose mechanisms for the aqueous photolysis of GA and PA under atmospherically relevant conditions. The quantum yields lacking in the literature will also be reported so these processes can be incorporated into atmospheric models of SOA production.

1.5 Previous work on the photochemistry 2-oxocarboxylic acids

Before the work in this dissertation was performed, only one study of pure glyoxylic acid photochemistry was available in the literature. Back and Yamamoto conducted this study in the gas phase at multiple wavelengths of 239, 275, 346, 366, and 382 nm. They identified the main reaction products as CO$_2$ and formaldehyde, with secondary CO and H$_2$ production occurring from the photodecomposition of the produced formaldehyde. As shown in Scheme 1.1 They were also able to distinguish a second source of CO independent from the formaldehyde.

**Figure 1.3** UV-visible absorption spectra of the 2-oxocarboxylic acids (black) glyoxylic acid and (blue) pyruvic acid in water, and solar irradiance spectrum from a sunny, cloudless day (August 3, 2018) in Lexington, KY (red) at 230 m above ground level. The vertical dashed line at 290 nm indicates the lower limit of sunlight that reaches the troposphere.
Although they were unable to measure formic acid, they postulated its presence as a coproduct from the production of CO. The primary mechanistic pathways proposed to explain the products were all internal H atom transfer processes followed by dissociation to give the following: CO$_2$/formaldehyde; CO/formic acid; and CO$_2$/CO/H$_2$, as shown in Scheme 1.2. Quantum yields were reported relative to the CO$_2$/formaldehyde pair as 3% for CO/formic acid and 16% for CO$_2$/CO/H$_2$ at 275 nm.\textsuperscript{50}

In the 400 nm irradiation of gas phase GA adsorbed on TiO$_2$, GA was photocatalytically converted to CO$_2$, carbonate and formate.\textsuperscript{51} Hydrated glyoxylate underwent a one electron oxidation to form an acyloxy radical which then decarboxylated to give CO$_2$ and HC(OH)$_2$ which underwent an additional one electron oxidation coupled with deprotonation to make formate as shown below in Scheme 1.3.
Scheme 1.3. Photocatalytic Oxidation of Glyoxylate on TiO$_2$.\textsuperscript{51}

\[
\begin{align*}
\text{HC(OH)$_2$CO}_2^- + h^+ & \rightarrow \text{HC(OH)$_2$CO}_2^- \\
\text{HC(OH)$_2$CO}_2^- & \rightarrow \text{HC(OH)$_2$}^- + \text{CO}_2 \\
\text{HC(OH)$_2$}^- + h^+ & \rightarrow \text{HCO}_2^- + H^+
\end{align*}
\]

Finally, a very recent paper describes the gas phase photochemistry of glyoxylate when embedded in cationic sodium chloride clusters.\textsuperscript{52} When the clusters were excited by 310-380 nm light, mass spectrometry was able to show that homolytic cleavage caused CO and HCO$^-$ to be eliminated from glyoxylate, leaving behind formate and carbon dioxide radical anion (CO$_2^-$) adducts with sodium chloride. The authors postulate that this type of reaction likely occurs in the troposphere.

The first study of the aqueous phase mechanism of GA direct photolysis constitutes chapter 2 of this dissertation (and has been published in reference 3), which describes the aqueous photochemistry ($\lambda \geq 305$ nm) of 250 mM GA solutions at pH 1 doped with sodium chloride and sodium sulfate in amounts that mimic sea spray aerosols. Surprisingly, irradiated aqueous GA formed many of the same products that are observed in the gas phase chemistry, including CO$_2$, CO, and formic acid indicating similar reactive pathways are available in the gas and aqueous phases. However, glyoxal, oxalic acid and tartaric acid were also formed in solution. Overall, oxalic and tartaric acid formation was explained by a proton coupled electron transfer or hydrogen abstraction mechanism (indistinguishable), while the other products were formed through homolytic cleavage of the C-C bond. Of particular interest is the formation of the most abundant product, glyoxal, because it is thought to be a major driver of SOA formation.\textsuperscript{53-56}
quantum yield of GA loss to direct aqueous photolysis was reported to be 0.17%, which correlates to an atmospheric lifetime of about 11 days, while the lifetime against indirect aqueous photolysis with HO• was about 5 days, indicating that whereas, oxidation by HO• is still the primary fate, direct photolysis of GA in water is still competitive.

To date, no other studies of aqueous GA photochemistry are available in the literature, except what was published from this dissertation, but there are a few related studies with similar conclusions. For example, upon 355 nm irradiation, iron (III) glyoxylate complexes in water were found to undergo electron transfer from the carboxylate oxygen in GA to the iron atom, resulting in iron (II) and an acyloxy radical which quickly decarboxylated to give formyl radical. This formyl radical was then converted to formic acid by another iron (III) glyoxylate complex. The mechanism proposed for this process was strikingly similar to the catalytic decomposition described earlier. Indeed all the processes involving glyoxylic acid seem to revolve around direct cleavage of the C-C bond.

In contrast to GA, PA photochemistry has been studied far more, though more work still remains undone, especially in the context of atmospheric reactions. In the gas phase, the first study of PA photochemistry was by Vesley and Leermakers, who were able to detect CO₂ and acetaldehyde in the 366 nm photolysis of PA gas. A quantum yield for the decomposition of PA of 1.02 was reported, which was verified (as 0.9) decades later by Yamamoto and Back. Both sets of authors contend that the primary photochemical decomposition pathway proceeds via an internal hydrogen atom transfer utilizing a cyclic transition state and methylhydroxycarbene as the intermediate yielding acetaldehyde. Yamamoto and Back also imply that there were other products of the reaction that were
not volatile enough for them to identify using their gas chromatography (GC) method. Subsequently, Berges and Warneck were able to identify acetic acid and small amounts of CO and methane.\textsuperscript{60} More recently Reed Harris et al. were also able to detect methanol and formic acid among the gas phase products.\textsuperscript{61} In the study, which was performed in a small glass reactor with irradiation of $\lambda = 290$-380 nm, the quantum yield of PA decay was measured to be $0.24 \pm 0.05$ for $\sim 100$ ppm of PA in 600 torr of air.\textsuperscript{61} Reed Harris et al. later reported an updated quantum yield of $\Phi = 3.2 \pm 0.5$ for the photodecomposition of 3 ppm of gas phase PA irradiated at $\lambda \geq 300$ nm inside a 4.2 m$^3$ chamber at atmospheric pressure in dry air.\textsuperscript{62} This much larger yield was attributed to the much lower PA concentration used than in the earlier studies, as well as an enhancement due to reactions with O$_2$ that were not observed before even though both studies were performed in air that is 21% O$_2$ by volume, hence significantly more concentrated than PA in the reactors.

In opposition to the gas phase chemistry of PA, there is no consensus among researchers about the aqueous phase chemistry of PA with regard to both the products and the mechanism of reaction. Leermaikers and Vesley initiated the first serious study of the aqueous photochemistry of pyruvic acid in 1963, using 285 mM solutions at natural pH irradiated at $\lambda \geq 280$ nm and 25°C.\textsuperscript{63} The same reaction was carried out in a variety of organic solvents including, methanol, benzene, and chloroform for comparison. The authors state that in water, the primary products are CO$_2$ in the headspace over the reaction and acetoin (H$_3$CC(O)C(OH)CH$_3$) in the liquid phase, which was identified by GC at 90°C after removing the water and redissolving in methanol.\textsuperscript{63} In all organic solvents except benzene, they report that the main product was 2,3-dimethyltartaric acid.
(DMTA), identified by infrared (IR) and nuclear magnetic resonance (NMR) spectroscopies. The reaction did not proceed in benzene, and the purported explanation is that while the other organic solvents are hydrogen donors, benzene is not, indicating a key role of the solvent in the solution phase mechanism. An additional experiment in water using sodium pyruvate instead of the acid, yielded decomposition “…at least an order of magnitude slower,” but the products of this reaction were not characterized. A quantum yield of 0.79 was reported for the aqueous reaction of pyruvic acid, which was “more than 20 times larger” than for the conjugate base. Finally, Leermakers and Vesley speculated that the triplet excited state of pyruvic acid was the reactive species responsible for this chemistry based on the very large phosphorescence they observed upon irradiation (unpublished). Later, Kendall and Leermakers performed quenching experiments that further indicated triplet excited state PA was the reactive species.64

A decade later, Closs and Miller undertook a detailed CIDNP study of the mechanism of PA photoreaction in water.65 CIDNP, or chemically induced nuclear polarization, is an NMR technique used to study radical processes wherein the signals of triplet radical pairs can be observed. In these experiments, the NMR spectra of the 100 mM solutions of PA were able to be recorded during in situ irradiation at \( \lambda \geq 310 \) nm. They proposed that in both hydrogen donating solvents and nonreducing solvents, i.e. water, the mechanism involved free radical recombinations due to the observation of steady state signals only during irradiation, which disappeared after the light was shuttered. Using the chemical shifts of the radical pairs, they tentatively assigned the identities of the radicals present in water to the formation of acetyl radical and PA ketyl radical (also called lactic acid...
radical). They also indicate that these radicals form via H atom transfer from an –OH (unlikely) and go on to generate 2-acetolactic acid and acetoin as shown in Scheme 1.4.

**Scheme 1.4.** Mechanism of PA(aq) photolysis proposed by Closs and Miller.\(^6^5\)

Additional experiments with added naphthalene, a known triplet quencher, again indicated that the triplet state was involved. Finally, the authors postulate that the acetyl and ketyl radicals simply recombine to form 2-hydroxy-2-methylacetoacetic acid (also called 2-acetolactic acid), observed in their solutions as \(\sim 30\%\) of the organic products. They go on to describe that their attempts to synthesize 2-acetolactic acid failed because it decarboxylated into acetoin too quickly to record the NMR spectrum of the parent 2-acetolactic acid, begging the question, if the primary photoproduct really was 2-acetolactic acid, why didn’t it also hydrolyze immediately into acetoin? These results also bring in to question whether the acetoin observed earlier by Leermakers and Vesley\(^6^3\) could also be a decomposition product of the true photochemical product since they only identified it after subjecting the sample to GC at 90 °C.
A few years after the study by Closs and Miller, Davidson et al. were able to determine that the triplet excited state yield of the 350 nm PA photolysis in water was 0.22 with a lifetime of 74 ns.\textsuperscript{66} They also theorized that since the earlier studies showed that quantum yields were dependent on the type of solvent used during the photolysis, homolytic cleavage was unlikely to be the mode of reaction that forms the ketyl and acetyl radicals.\textsuperscript{66} Instead, for the 350 nm photolysis, they proposed that bimolecular electron transfer occurs between the excited state of PA and a ground state PA molecule, shown in Scheme 1.5. The solvent influence on the reaction was then explained by highly polar solvents stabilizing the radical intermediates. However, they explain that this reaction should be encouraged by the presence of pyruvate anion, which is at odds with the documented suppression of photoreactivity in solutions of sodium pyruvate.\textsuperscript{63} In a related publication, the same authors proved the presence of electron transfer during the reaction by demonstrating an enhancement of the yield of CO\textsubscript{2} when electron acceptors were added to the solution.\textsuperscript{67}

\textbf{Scheme 1.5.} Initial step of PA(aq) photolysis from Davidson et al.\textsuperscript{66, 67}

\begin{center}
\includegraphics[width=\textwidth]{Scheme1.5.png}
\end{center}

After a long hiatus, research on the photochemical mechanism of PA in water was revived by Guzman et al. who were able to trap the radicals produced during \(\lambda = 320\) nm \(\pm 10\) nm photolysis of pyruvic acid by irradiating in frozen (77K) aqueous solutions.\textsuperscript{68}
They were then able to use electron paramagnetic resonance spectroscopy to directly observe the organic radicals, which showed that the ketyl and acetyl radicals existed as radical pairs, rather than isolated monoradicals, with each unpaired spin separated by $\geq 0.5$ nm, which again rules out the possibility of homolytic cleavage as a reaction pathway. The radical pairs observed were stable toward temperature changes below 180 K, but upon warming above the sublimation temperature of CO$_2$ (140 K) the frozen sample began to release appreciable amounts of CO$_2$.

**Scheme 1.6.** Initial proton-coupled electron transfer step of aqueous PA photolysis proposed by Guzman et al.$^{68}$

This was taken as an indication that CO$_2$ was produced by the fast and efficient decarboxylation of an acyloxy radical immediately following photon absorption, resulting in the detectable radical pair. The large separation of the unpaired spins was rationalized to result from long range electron transfer occurring between a photoexcited PA and a ground state PA molecule (shown in Scheme 1.6), coupled with a proton transfer to form
a PA ketyl radical and an acyloxyl radical. The acyloxyl radical subsequently decarboxylated to yield CO$_2$ and an acetyl radical.

Next, Guzman et al. characterized the organic products of the oxygen-free reaction in both water$^5$ at 313 nm, 25°C, and pH 1 and in ice,$^6$ using liquid chromatography with UV-vis and mass spectrometric (MS) detection and isotope labeling.$^5$ In this study, only two organic products were identified, the first being a six-carbon carboxylic acid with a neutral mass of 178 amu. The number of carbons was easily identified by using uniformly labeled $^{13}$C PA for the reaction and then analyzing by MS. The MS fragmentation spectrum of this species was consistent with a dicarboxylic acid. Additionally, the MS spectra from a reaction in H$_2$O using PA deuterated only at the methyl group indicated this six carbon dicarboxylic acid contained two methyl groups. All together the data for this product was consistent with the 2,3-dimethyltaratric acid identified by Leermakers and Vesley.$^6$ The other organic product was more complex. Using the same methods the authors were able to determine that the other product was a seven-carbon carboxylic acid of mass 176 amu with one carbonyl functionality proposed to be 2-(3-oxobutan-2-yloxy)-2-hydroxypropanoic acid. The formation of these products from the ketyl and acetyl radicals is shown in Scheme 1.7. Acetoin was not detected in this reaction, and it was postulated that the organic products with masses 176 and 178 amu were thermally labile species that decomposed into acetoin during the high temperature GC analysis used.$^6$ The same products were observed in water and in ice. Based on the evidence for acetyl and ketyl radical formation, the authors proposed a mechanism that proceeds through a proton-coupled electron transfer between the excited PA and a ground state PA that yielded acetyl and ketyl radicals. The initial proton-
coupled electron transfer step, shown in Scheme 1.6, is indistinguishable from H atom abstraction. However, none of the hydrogen atoms in PA are good candidates for H atom abstraction. Thus, a proton-coupled electron transfer, in which the electron and proton come from different sources (instead of together as a neutral H atom), was proposed.

**Scheme 1.7.** Formation of the organic products of PA photolysis in water identified by Guzman et al from ketyl and acetyl radicals.5,69

Recombination of two ketyl radicals was used to explain the formation of 2,3-dimethyltartaric acid. When the acetyl and ketyl radicals combined with a ground state PA molecule, a β-ketocarboxylic acid intermediate was predicted to be formed (undetectable here), which then decarboxylated into the species with molecular weight 176 amu. The formation and subsequent decarboxylation of the β-ketocarboxylic acid also explained experiments the authors did using radical scavengers and varying the temperature during frozen phase photolysis,69 which indicated that the CO₂ formed in the reaction was from two different sources, the other being the initial decarboxylation of the acetyl radical precursor.69 Figure 1.4 shows how the evolution of CO₂ from the aqueous
photolysis could be only partially inhibited by the radical scavenger TEMPO, indicating that a second source of CO\(_2\) was so fast and efficient it could not be prevented.\(^5\)

**Figure 1.4.** Rates of CO\(_2\)(g) evolution (\(R_{\text{CO}_2}\)) vs [TEMPO]\(_0\) for the 313 nm photolysis of deaerated 0.1 M PA aqueous solutions at 293 K. Reprinted with permission from Guzman, M. I.; Colussi, A. J.; Hoffmann, M. R., Photoinduced oligomerization of aqueous pyruvic acid. *J. Phys. Chem. A* **2006**, *110* (10), 3619-3626. Copyright 2006 American Chemical Society.

Finally, Guzman et al. demonstrated the hyperbolic dependence of the reaction on the initial [PA] which supported the multistep reaction mechanism presented.\(^5\)

The extensive work on the mechanism by Guzman et al. was far from being the final word on aqueous PA photolysis. Recently, acetoin was again proposed as a product along with lactic acid, acetic acid, and the 176 and 178 amu carboxylic acids identified by Guzman et al.\(^5\) in the aqueous photolysis under atmospheric conditions based on gas phase IR of the headspace over the reaction and 2D NMR of the postphotolysis mixture.\(^4\)

The formation of acetoin proceeded via the same reaction proposed by Closs and Miller indicated in Scheme 1.4, and the formation of acetic and lactic acids is shown in Scheme 1.8. Unfortunately, the FTIR in this study was complicated by water vapor that
overlapped the signals of interest, making it truly impossible to tell if the new products were really there. In the 2D NMR, signals assigned to acetoin, lactic acid, and acetic acid by the use of external standards were once again overlapped by other components of the mixture. The issue of the formation of these species is addressed at length in Chapter 3 of this dissertation (published in references 2 and 70), where it was shown by more conclusive chromatographic methods that acetoin and lactic acid are not formed photochemically and that the signals attributed to acetoin and lactic acid were most likely contributed by the seven-carbon species identified by Guzman et al., and its β-ketocarboxylic acid precursor.

**Scheme 1.8.** Formation of lactic acid and acetic acid during PA photolysis in water proposed by Griffith et al.⁴

![Scheme 1.8](image)

The work in Chapter 3 also quantified the organic reaction products and was able to detect the unstable β-ketocarboxylic acid intermediate (see Scheme 1.7) for the first time. Additionally, kinetic isotope effects in D₂O as solvent provided more support in favor of a proton-coupled electron transfer mechanism of photoreaction. Despite the results of chapter 3,²,⁷⁰ the incorrect identification of acetoin and lactic acid has persisted in the literature,⁷,⁷¹,⁷² and most recently, an alternative structure and formation mechanism for the product with mass 176 amu was published that proposes the formation of this second
most abundant photoproduct from an impurity in undistilled PA.\textsuperscript{72} The unlikelihood of this happening is discussed in Chapter 4 along with an in depth study of the sensitivity of the reaction to pH and oxygen content. The results in Chapter 4 also show for the first time that PA forms singlet oxygen upon irradiation, which can contribute to atmospheric oxidations when occurring in aerosols.

One final study was performed on the direct photolysis of long chain 2-oxocarboxylic acids in water.\textsuperscript{73} In these reactions, the long chain oxoacids were observed to form recombination products in a mechanism resembling the ketyl radical recombination mechanism that results in 2,3-dimethyltartaric acid first described by Guzman et al.\textsuperscript{5} They also observed \textit{m/z} (mass to charge ratios) of other products they purport to be formed through an analogous mechanism to that of acetoin discussed above and first proposed by Closs and Miller.\textsuperscript{65} Finally, they proposed a new pathway of reaction available to C\textsubscript{4} and longer chain 2-oxocarboxylic acids due to the availability of more easily abstracted hydrogens from a -CH\textsubscript{2} group.

**1.6 Surface behavior of a 2-oxocarboxylic acid at the air-water interface**

As an atmospherically important aside, Chapter 5 of this dissertation describes the acid-base behavior of PA (and acetic acid as a reference) in the gas phase as it approaches aqueous droplets, simulating interaction with atmospheric aerosols. This behavior is important to understand because some pyruvic acid is formed in the gas phase and partitions to the aqueous phase of aerosols. Chapter 5, published in reference 74, used a surface sensitive MS technique called online electrospray ionization mass spectrometry (OESI-MS) to show that titration curves of gaseous PA and acetic acid molecules impinging on the surface of the ESI droplets had an inflection point at far lower pH than
PA or acetic acid in the bulk phase. The effect was more extreme for PA as a 2-oxocarboxylic acid, resulting in a surface pKa of 0.65 vs. 2.43 in the bulk. This shifting was attributed primarily to the increased proton affinity of the interface, while the difference between PA and acetic acid was explained as a consequence of the different hydration configuration of the acid molecules on the surface of water. Even though the focus of this study was for atmospheric chemistry, any process involving PA or other carboxylic acids crossing an air-water interface will be affected by this phenomenon, making these results far reaching.
Chapter 2. Aqueous Photochemistry of Glyoxylic Acid

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Scheme 2.1 Graphical abstract

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2.1 Abstract

Aerosols affect climate change, the energy balance of the atmosphere, and public health due to their variable chemical composition, size, and shape. While the formation of secondary organic aerosols (SOA) from gas phase precursors is relatively well understood, studying aqueous chemical reactions contributing to the total SOA budget is the current focus of major attention. Field measurements have revealed that mono-, di-, and oxocarboxylic acids are abundant species present in SOA and atmospheric waters. This work explores the fate of one of these 2-oxocarboxylic acids, glyoxylic acid, which can photogenerate reactive species under solar irradiation. Additionally, the dark thermal aging of photoproducts is studied by UV-visible and fluorescence spectroscopies to reveal that the optical properties are altered by the glyoxal produced. The optical properties display periodicity in the time domain of the UV-visible spectrum of chromophores with absorption enhancement (thermochromism) or loss (photobleaching) during nighttime and daytime cycles, respectively. During irradiation, excited state glyoxylic acid can undergo α-cleavage or participate in hydrogen abstractions. The use of $^{13}$C nuclear magnetic resonance spectroscopy (NMR) analysis shows that glyoxal is an important intermediate produced during direct photolysis. Glyoxal quickly reaches a quasi-steady state as confirmed by UHPLC-MS analysis of its corresponding (E) and (Z) 2,4-dinitrophenylhydrazones. The homolytic cleavage of glyoxylic acid is proposed as a fundamental step for the production of glyoxal. Both carbon oxides, CO$_2$(g) and CO(g) evolving to the gas phase, are quantified by FTIR spectroscopy. Finally, formic acid, oxalic acid, and tartaric acid photoproducts are identified by ion chromatography (IC).
with conductivity and electrospray (ESI) mass spectrometry (MS) detection and $^1$H NMR spectroscopy. A reaction mechanism is proposed based on all experimental observations.

### 2.2 Introduction

Recently, aqueous phase chemistry was suggested to contribute to the total SOA budget,\(^{32, 75}\) e.g., a simple 2-oxocarboxylic acid such as pyruvic acid can initiate the formation of oligomers in the aqueous phase and even in ice.\(^{5, 68, 69}\) Simultaneously, inorganic electrolytes such as ammonium and sulfate are now known to play a role during dark processes.\(^{76, 77}\) Among all the species found in aqueous SOA (aqSOA), 2-oxocarboxylic acids, such as glyoxylic acid (GA, pK\(_a\) = 3.13),\(^{78}\) are widely present in different environments. Photooxidative processing of isoprene\(^{38-40}\) and aromatics\(^{8, 9, 37, 79}\) produce first generation products such as methylglyoxal, glyoxal, phenol, catechol, and benzaldehyde. In more detail, glyoxylic acid can be directly produced from HO$^\bullet$ attack on glyoxal, glycolic acid or hydroperoxy acetic acid,\(^{80}\) and during the oxidation of oxygenated aromatic species.\(^{8, 9}\) Glyoxylic acid is the most abundant 2-oxocarboxylic acid present in organic aerosols in diverse environments.\(^{25, 81, 82}\) The annual net production of glyoxylic acid in atmospheric waters totals \(2.95 (\pm 0.89) \times 10^{10}\) mol year\(^{-1}\) \(\equiv\) 0.71 (\(\pm 0.21\) ) TgC year\(^{-1}\),\(^{43}\) with up to 85% concentrated in the particle phase.\(^{83}\) The small contribution of the aldehyde form of glyoxylic acid to its total speciation in water provides a tail for absorbing actinic radiation, allowing photoreactions to occur. Interestingly, the direct photoreaction of glyoxylic acid in water has remained previously unexplored and is investigated herein.

In this research, the identification of missing mechanisms of SOA production is investigated by monitoring the sunlight photolysis of aqueous glyoxylic acid, which is
followed by thermal reactions during dark periods. The work 1) explores how atmospheric chemical reactions of interest proceed in atmospheric water mimics, affecting the optical properties of model aqSOA; 2) identifies the reaction products by ion chromatography with dual conductivity and electrospray (ESI) mass spectrometric detection (IC-MS), ultrahigh pressure liquid chromatography mass spectrometry (UHPLC-MS) of derivatized samples with 2,4-dinitrophenylhydrazine (DNPH), and $^{13}$C nuclear magnetic resonance spectroscopy (NMR); and 3) reports the time series of products involved in the reaction mechanism. The research contrasts the importance of dicarbonyl intermediates affecting the optical properties of model aqSOA during photochemical and thermal processes. Based on the information gathered, a mechanism that agrees with all experimental observations for the photoreaction of glyoxylic acid is presented.

2.3 Experimental Section

2.3.1 Preparation of Experiments and Controls

In a typical experiment, solutions of glyoxylic acid (Sigma-Aldrich, 52.1 wt. % in H$_2$O) were prepared daily in water (Elga Purelab flex, 18.2 MΩ cm$^{-1}$) at a concentration of ca. 250 mM. Additionally, experiments studied the effect of glyoxylic acid concentration covering the range 5-250 mM (Supporting Information). The solution of glyoxylic acid was doped with the most abundant inorganic electrolytes found in seawater as a precursor of sea spray:\textsuperscript{84} $[\text{Na}^+] = 468$ mM, $[\text{Cl}^-] = 545$ mM and $[\text{SO}_4^{2-}] = 28.2$ mM. Electrolytes were provided similarly as described before\textsuperscript{85} from NaCl (Fisher, 99 %), Na$_2$SO$_4$ (Fisher, 99 %) and HCl (EMD, 38 %). The absence of NH$_4^+$ in the mixture allows for focusing the work on the effects caused by photoirradiation instead of reiterating the reported
catalysis by ammonium\textsuperscript{86} that can lead to decarboxylation.\textsuperscript{5, 69, 76} After adjusting the pH of the solution of glyoxylic acid to 1.0 with [HCl] = 2.0 M, 180 mL of the solution were transferred to a fused silica photoreactor (220 mL capacity) with a jacket for temperature control at 298 K (Thermo Scientific A25 circulating bath), magnetic stirring, and a septum for sampling gases. The sealed reactor underwent continuous sparging (unless indicated otherwise) with 100 mL min\textsuperscript{-1} air (Scott-Gross, UHP) starting 30 min before photolysis. Experiments were performed under 1 atm air and the conditions of ionic strength, temperatures and photon flux are chosen to simulate those encountered by nascent sea spray aerosols mixing with pollution plumes at coastal regions.

Control experiments (Table 2.S1) were designed to study the effect of inorganic electrolytes and [O\textsubscript{2}(aq)] on the photoreaction of glyoxylic acid. Table 2.S1 indicates which gas was used and if electrolytes were not present in the controls: Control A under 1 atm N\textsubscript{2}(g) (Scott-Gross, UHP), control B under 1 atm O\textsubscript{2}(g) (Scott-Gross, UHP), control C without electrolytes, control D without electrolytes under 1 atm N\textsubscript{2}(g), and control E without electrolytes under 1 atm O\textsubscript{2}(g). From Henry’s law ($K_H = 1.28 \times 10^{-3}$ M atm\textsuperscript{-1} for O\textsubscript{2} at 298 K),\textsuperscript{48} [O\textsubscript{2}(aq)]\textsubscript{1 atm air} = 0.26 mM and [O\textsubscript{2}(aq)]\textsubscript{1 atm O\textsubscript{2}} = 1.24 mM.

\textbf{2.3.2 Photochemical Experiments and Thermal Treatment of Photolyzed Samples}

The photochemical setup (Newport) employed was previously described.\textsuperscript{87, 88} Briefly, it includes a 1 kW high pressure Xe-Hg lamp, a water filter to remove infrared radiation, and a cut-off filter for wavelength \( \lambda \geq 305 \) nm to provide actinic radiation in the solar window. The incident light intensity, \( I_0 = 3.892 (\pm 0.001) \times 10^{-5} \) Einstein L\textsuperscript{-1} s\textsuperscript{-1}, was quantified by chemical actinometry using the concerted photodecarboxylation of phenylglyoxylic acid at pH 1.0.\textsuperscript{89} A calibrated polarographic probe (081010MD)
connected to a meter (both Thermo Scientific) was used to monitor dissolved oxygen ([O$_2$(aq)]) levels during irradiation. During the first stage of processing (Stage I in Scheme 2.2), samples of the experiment and controls (Table 2.S1,) were irradiated for 8 h and 5 mL aliquots were withdrawn from the reactor at 0, 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 h. A sample volume of 3.5 mL was transferred to a Suprasil cuvette (Starna Cells, 10 mm optical path length) to monitor its aging (Stage II in Scheme 2.2) in the dark by UV-visible spectroscopy for 15 h at 298 K followed by 9 h at 323 K. The remaining aliquot was frozen at 253 K and stored in the dark for later analyses by IC-MS, UHPLC-MS, and NMR spectroscopy. The remaining 130 mL of photolyzed from the original solution was stored in the dark for 24 h (Stage II in Scheme 2.2) and then rephotolyzed (Stage III in Scheme 2.2). Rephotolyzed samples underwent a second aging process in the dark, monitored by UV-visible spectroscopy (Stage IV in Scheme 2.2).

**Scheme 2.2** Representation of the processing of aqueous glyoxylic acid solutions. Stages I and III are both 8 h photolysis periods. Stages II and IV sequentially refer to (dark) thermal aging during 15 h at 298 K followed by 9 h processing at 323 K.
2.3.3 Analysis of Products

Samples were analyzed by 1) UV-visible spectroscopy (Evolution 220 spectrophotometer, Thermo Scientific) and fluorescence spectroscopy (FluroMax-4 fluorimeter, Horiba Scientific); 2) IC-MS with a Dionex ICS-2000 instrument (IonPack AS11-HC, 2 mm analytical column) equipped with a conductivity detector and a mass spectrometer (MS) interfaced with an ESI probe (Thermo Scientific, MSQ Plus); 3) UHPLC-MS (Accela 1250 with MSQ Plus detector, Thermo Fisher Scientific) using a C18-selectivity column (Hypersil GOLD, 1.9 µm, 50×2.1 mm, Thermo Scientific) for separation of hydrazones from carbonyls derivatized with freshly prepared 2,4-dinitrophenylhydrazine (DNPH, Sigma Aldrich, HPLC grade); 4) 1H (using a WET suppression method) and 13C NMR experiments (Varian AS600, 150 MHZ for 13C) recorded at 298 K using 5-mm NMR tubes (Wilmad); and 5) Fourier transform infrared spectroscopy (FTIR) for evolving gases in a 2.4 m path length infrared gas cell with ZnSe windows (PIKE) mounted in an iZ10 FTIR module connected to an infrared microscope (Thermo Scientific Nicolet iN10). Data from all these methods is reported as the average of duplicate experiments with error bars corresponding to one standard deviation. Detailed information of all these experimental methods, instrumental parameters, samples and standards preparation, and quantification methods is given in the accompanying Supporting Information.

2.4 Results and Discussion

2.4.1 Optical Properties

UV-visible spectroscopy is used to study the optical property changes of glyoxylic acid photolyzed under the conditions listed in Table 2.S1. Figure 2.1 displays the UV-visible
absorption spectra of 252.1 mM glyoxylic acid before and after photolysis in the presence of electrolytes under 1 atm air (experiment in Table 2.S1) for the four stages listed in Scheme 2.2. The reversible hydration of glyoxylic acid into dihydroxyacetic acid is shifted toward the hydrate form, as indicated by the large equilibrium constant $K_{\text{Hyd}} = [(\text{OH})_2\text{CH-COOH}]/[\text{O=CH-COOH}] = 300$ (at 298 K).²⁰

![Figure 2.1.](image)

**Figure 2.1.** UV-visible absorption spectra during stages (A) I-II and (B) III-IV in Scheme 2.2. Spectrum (red line) before irradiation, after 8 h photolysis (green line) for the end of stages I or III), followed by thermal aging during 15 h of at 298 K (blue line) and 9 h at 323 K (purple line), for the end of stages II or IV). Experimental conditions: Solution of [glyoxylic acid] = 252.1 mM, [Na⁺] = 545.0 mM, [SO₄²⁻] = 28.2 mM, and [Cl⁻] = 468.0 mM, at pH = 1.0 under 1 atm air.

Because the hydration equilibrium is shifted toward 2,2-dihydroxyacetic acid, this species partitions to aerosols and clouds, and it is unlikely to return to the gas phase.⁹⁰ Although $K_{\text{H}}$ is large for glyoxylic acid, the carbonyl group is still observed in the spectrum before irradiation (red line in Figure 2.1A). Contrarily, other 2-oxocarboxylic acids such as pyruvic acid display a large fraction of carbonyl form in equilibrium.²¹ The
carbonyl absorption of glyoxylic acid at $\lambda = 276$ nm in Figure 2.1A comprises an absorbing tail that extends above $\lambda = 305$ nm. After 8 h of irradiation, the green line in Figure 1A shows that for stage I photobleaching occurs. This change reflects the loss of the carbonyl functionality absorption band of the reagent and its associated tail in the UV. For the following 24 h of thermal aging in stage II, the blue and purple lines with increased absorbance demonstrate the effects of thermochromism in the sample. Figure 2.1B presents the results from a second irradiation period, such as a second daytime cycle (stage III) following the nighttime period for the last aged sample in Figure 2.1A. The mixture of products at time zero before the second photolysis is shown in red in Figure 2.1B, which undergoes photobleaching (green line) producing a similar spectrum to that observed at the end of stage I in Figure 2.1A. Similarly, moderate thermochromism occurs in stage IV, during a second dark cycle, recovering a slightly higher absorption than after stage II.

The observed evolution of carbon species formed after stage I is related to the presence of coexistent aldehydes and gem-diols, alcohols, and carboxylic acids that are characterized below by several spectroscopic techniques. In the dark under mild atmospheric conditions, it is anticipated that glyoxal possesses the key aldehyde functional group that displays a continuum of absorption toward longer wavelengths. Interestingly, organic products absorbing in the 300-400 nm range were also observed during the thermal processing of solutions with $[\text{glyoxal}] = 220$ µM-2.21 M (pH 4) in the presence of (NH$_4$)$_2$SO$_4$ or NH$_4$NO$_3$. Because our experiment was purposely performed in the absence of ammonium ion, its catalytic effect in the self-condensation of aldehydes, its possible direct reaction with carbonyls resulting in heterocyclic
products,\textsuperscript{92} as well as the possible formation of alkenes from alcohols,\textsuperscript{93} cannot be recalled to provide an explanation for the data in Figure 2.1. Beer’s law was obeyed between 280 and 500 nm within the experimental accuracy. An example of the loss of linearity between 280 and 450 nm due to typical instrumental limitations is given in Figure 2.S1 (Supporting Information).

Figure 2.2A shows the normalized areas –to initial values– integrated under the UV-visible absorption spectra in the wavelength interval $295 \leq \lambda \leq 500$ nm during the four stages in Scheme 2.2 for the experiment and controls (Table 2.S1). Control F in the absence of light remained stable over the four stages and is not included in Figure 2.2A. There is a large contrast between photobleaching stages I and III \textit{vs.} thermochromism stages II and IV.\textsuperscript{77} Each half cycle, simulating daytime \textit{vs.} nighttime periods, shows a dependence on the presence of inorganic electrolytes and [O$_2$(aq)].

Although some dispersion is observed in the dataset at the end of stage I in Figure 2.2A, thermochromism during stage II provides a better picture of how electrolytes and O$_2$(aq) act. Once stage II is complete, a comparison of the effect of [O$_2$(aq)] (experiment, control A, and control B in Table 2.S1) in the presence of electrolytes, reveals that when O$_2$ is present thermochromism is lower than in an inert atmosphere. The magnitude for the areas at the end of stage II follows the order O$_2$(g) \textless air < N$_2$(g): $0.38 \lessapprox 0.40 < 0.62$. A similar qualitative conclusion is valid for the subset of experiments in the absence of electrolytes (controls C, D, and E) for the areas in air \textless O$_2$(g) < N$_2$(g): $0.58 \lessapprox 0.59 < 1.23$. 

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Figure 2.2. (A) Normalized area \((295 \leq \lambda \leq 500 \text{ nm})\) under the UV-visible absorption spectra for (red ○) an experiment, (blue ▲) control A in N\(_2\), (pink ▼) control B in O\(_2\), (violet □) control C without electrolytes, (orange ♦) control D without electrolytes in N\(_2\), and (black ✴) control E without electrolytes in O\(_2\). Experimental conditions defined in Figure 2.1. Dashed lines are to guide the eye and do not represent a model fit. (B) Fluorescence emission spectra under variable excitation wavelengths \((\lambda_{\text{exc}})\) for the experiment in Figure 2.2A at the end of Stage II. (C) Position of emission maxima \((\lambda_{\text{em}})\) for spectra in (black ▲ and blue solid line) Figure 2.2B vs. \(\lambda_{\text{exc}}\) compared to (red □ and dashed black line) a 24 h aged solution of \([\text{glyoxal}] = 11.8 \text{ mM}\) with the same electrolytes.

The direct effect of electrolytes during thermochromism for stage II can be contrasted for the pair of subsets in air (experiment vs. control C), in N\(_2\)(g) (controls A vs. D), and in O\(_2\)(g) (controls B vs. E). The results show that the lack of electrolytes in the sample similarly enhances thermochromism in air and O\(_2\)(g), which increases further in N\(_2\)(g),
suggesting that the concentration of at least one species contributes to enhance the optical properties of the sample even in the absence of inorganic electrolytes in this simple system. After the second photobleaching and thermochromism periods in stages III and IV, the area of all samples start to merge but control D without electrolytes in N₂(g) is the only one showing a different behavior. In general, the normalized areas follow a periodic function in the time domain with slightly decreasing amplitude for the different stages, an important observation to model the evolution of absorption properties of atmospheric brown carbon.⁹⁴

A recent chromatographic analysis of a nitrogen containing brown carbon mixture concluded the red tail observed in the absorption spectrum could be explained as a linear combination of absorbing molecules.⁹⁵ In order to assess if this was the case for present experiments, fluorescence spectra for the aged photoproducts are presented in Figure 2.2B. The composition of the mixture of photoproducts at the end of stage II (Scheme 2.2) was characterized (see analysis of products below) to include 11.8 (± 0.1) mM glyoxal, 2.00 (± 0.03) mM formic acid, and 493 (± 25) µM oxalic acid. For comparison, the individual emission spectra of glyoxylic acid as well as the identified products at the target concentrations in a matrix with electrolytes was also registered. It was confirmed that glyoxylic, formic, and oxalic acids did not contribute to the emission spectra of the aged photolyzed sample in the interval 350 ≤ λ_{exc} ≤ 400 nm. However, Figure 2.2C shows similar linear correlations between the maximum wavelength of emission (λ_{em}) versus the excitation wavelength (350 ≤ λ_{exc} ≤ 400 nm) exist for the mixture of photoproducts and for aged 11.8 mM glyoxal (24 h in darkness) with electrolytes. We conclude the red shifting of λ_{em} for longer λ_{exc} is due to the presence of glyoxal as an individual
fluorophore. Therefore, the absorption tail of aged photoproducts (Figure 2.1) is thought to be related to the behavior of glyoxal in the emission spectra for $\lambda_{\text{exc}} \geq 350$ nm (Figure 2.2B-C). Emission measurements also confirmed no other fluorophore species (or corresponding chromophore) is present in the starting experimental mixture before photolysis, as revealed by the unchanged value of $\text{Max}_{\lambda_{\text{em}}} = 441.3 \pm 2.1$ nm for the excitation range $350 \leq \lambda_{\text{exc}} \leq 400$ nm. For the interval $280 \leq \lambda_{\text{exc}} \leq 345$ nm, the emission spectra of the aged photolyzed experiment with glyoxylic acid only displayed a practically constant value for $\text{Max}_{\lambda_{\text{em}}} = 444.1 \,(\pm 1.8)$ nm. The unchanged $\text{Max}_{\lambda_{\text{em}}}$ for the wavelength interval 280-345 nm is due to the fluorescence of unreacted glyoxylic acid.

In summary, after irradiation and initial loss of absorption, at least one new chromophore appears but the presence of O\textsubscript{2} diminishes its production (Supporting Information). Electrolytes play a significantly different role enhancing thermochromism in stage II, implying interactions of products with electrolytes. It is important to contrast present results with previous experiments for the reaction of pyruvic acid.\textsuperscript{77} While the previous study was focused on the optical properties of complex organic matter originating from pyruvic acid in 2 M ammonium bisulfate, this work investigates the behavior of glyoxylic acid with a mixture of electrolytes ([Na$^+$] = 545 mM, [Cl$^-$] = 468 mM, and [SO$_4^{2-}$] = 28.2 mM) that does not include NH$_4^+$. The effect of electrolytes in present experiments agrees well with previous observations\textsuperscript{77} that electrolytes enhance thermochromism of postphotolyzed pyruvic acid solutions. Similarly, thermochromism increases with higher ionic strength (from inert electrolytes) and temperature, which may promote dehydration reactions.\textsuperscript{77}
The proposed red shift in the emission spectra (Figure 2.2B-C) resulting from the carbonyl moiety of glyoxal provides the new absorption in this kind of model system,\textsuperscript{76, 77} and should contribute to the optical properties of water extracted ambient particulate matter.\textsuperscript{96} Other atmospheric chromophoric species have been proposed to arise from the oxidation of polyhydroxylated aromatic rings into polyhydroxylated quinones absorbing in the 400-600 nm range.\textsuperscript{8} In addition, the direct oxidation of biomass burning and combustion emissions provided a direct link to explain the production of glyoxylic acid under humid conditions such as those found in clouds, fogs, or aqueous secondary aerosols.\textsuperscript{8, 9} The rapid photobleaching of aged solutions of glyoxal by solar illumination\textsuperscript{97} was proposed to result from the loss of imines generated in reactions between carbonyls and nitrogen containing nucleophiles.\textsuperscript{97} Our findings cannot be explained using the same concept due to the lack of imine formation, suggesting that glyoxal itself is a relevant atmospheric chromophore. Thus, photobleaching is interpreted as the disruption of chromophores’ key function of interacting weakly among each other and with electrolytes.\textsuperscript{76, 77}

2.4.2 Identification of Products

Among the several methods available to study the photoreaction products of glyoxylic acid, useful information is provided by analyzing IC-ESI-MS chromatograms of photolysis products. The information from IC-ESI-MS reveals the mass-to-charge ratio (\(m/z\)) for anionic products present in the sample after chromatographic separation. Figure 2.3 shows IC chromatograms with conductivity and ESI-MS detection in the negative ion mode for the same experiment in Figure 2.1 before (A) and after (B) 8 h photolysis. Before irradiation (Figure 2.3A), a shoulder and two peaks elute at 11.86, 12.79 and
19.55 min in the conductivity detector, which are assigned with matching standards and by their m/z values to (1) glyoxylate (C$_2$H$_1$O$_3^{-}$, m/z 72.99), (2) chloride (Cl$^-$), and (3) bisulfate (HSO$_4^{-}$, m/z 96.99), respectively. Although Cl$^-$ could not be detected by MS, its conductivity peak was shown to grow after spike addition with a standard. Clearly, some conductivity peaks correspond to overlapping species based on the distinctive m/z values extracted with the help of the MS detector. Upon 8 h of photolysis (Figure 2.3B), new peaks elute at 18.60 min and 20.33 min, corresponding to two products from the photoreaction of glyoxylic acid, (4) tartrate (C$_4$H$_5$O$_6^{-}$, m/z 149.01), and 5) oxalate (C$_2$H$_2$O$_4^{-}$, m/z 88.99), respectively.

The similar product composition found in the experiment and control C indicates that the presence of inorganic electrolytes does not determine the formation of the low molecular weight products. Therefore, the major new photoproducts identified by IC-MS are oxalic (C$_2$H$_2$O$_4$) and tartaric (C$_4$H$_6$O$_6$) acids with molecular weights of 90.03 and 150.09 amu, respectively. The formation of these two species requires the presence of glyoxylic acid to form the C$_2$- and C$_4$-carboxylic acids. Although formic acid (HCOOH) escaped MS detection at m/z 45, its presence was confirmed by IC separation with a 1.0 mM KOH isocratic gradient and conductivity detection. Instead, the production of formic acid was quantified by $^1$H NMR.

Figure 2.4 shows the production of glyoxal during irradiation, as registered in the extracted ion chromatogram at m/z 417.05 for its (E) and (Z) hydrazones, corresponding to the reaction 2 DNPH + 1 glyoxal. While the hydrazone with m/z 417.05 is absent before irradiation (blue trace in Figure 2.4), its presence is confirmed for the experiment and matched to a standard (red trace) at 7.02 and 7.65 min.
Figure 2.3. Ion chromatogram with conductivity and ESI-MS(-) detection for the experiment in Figure 2.1 (A) before and (B) after 8 h of irradiation. Key for peaks: (1) glyoxylic acid ($m/z$ 72.99), (2) chloride, (3) sulfate ($m/z$ 96.99) (4) tartaric acid ($m/z$ 149.01), and (5) oxalic acid ($m/z$ 88.99).

After irradiation starts, from 30 min to 8 h, the concentration of glyoxal remains relatively stable $[\text{glyoxal}] = 2.02 \pm 0.10$ mM. Therefore, during the experiment glyoxal is an intermediate that remains in steady state. The origin of glyoxal is discussed below, where its presence is also confirmed by $^{13}$C NMR spectroscopy. The formation of formaldehyde ($\text{H}_2\text{C}=\text{O}$) as a primary photoproduct was discarded after performing UHPLC-MS analysis of derivatized samples. The hydrazone from derivatized formaldehyde would have eluted at 2.39 min in the extracted ion chromatogram at $m/z$ 209.03, as confirmed after spike addition of the samples with a 5 µM standard.
Figure 2.4. UHPLC-MS for the extracted ion at m/z 417.05 for (E) and (Z) DNPH-glyoxal hydrazones in the experiment of Figure 2.1. The red trace corresponds to a standard.

The experimental results point to the different photolytic behavior of glyoxylic acid in water and the gas phase, where CO$_2$ was accompanied by formaldehyde generation.$^{50}$ Figure 2.5 shows the major products in the infrared spectrum of the gas collected over the headspace of the reactor containing carbon dioxide (CO$_2$) and a lower level of carbon monoxide (CO). The spectrum shows the asymmetric stretching vibration of CO$_2$ at 2349 cm$^{-1}$, and the well-resolved CO bands centered at 2143 cm$^{-1}$. The presence of formaldehyde was also discarded in the gaseous products because its very strong absorption at 1771 cm$^{-1}$ was missing from the spectrum.
**Figure 2.5.** (Blue) Infrared spectrum of gas phase products after photolysis ($\lambda > 305$ nm) of glyoxylic acid for 8 h. All other experimental conditions are indicated in Figure 2.1. The red trace shows a 1:18 dilution of the spectrum.

The use of $^{13}$C NMR spectroscopy provides additional information from the chemical shifts ($\delta$) of reactants and products in the photolysis mixture. 3-(Trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS) was added to the NMR tube as a reference ($\delta = 0.00$ ppm) in the aqueous solutions for all samples. Figure 2.6 presents the $^{13}$C NMR spectrum for an experiment after 8 h of irradiation. In addition to the reference peak at $\delta = 0.00$ ppm, DSS signals also appear at 17.60, 21.71, and 56.97 ppm in the $^{13}$C NMR spectrum. The abundant gem-diol (–C(OH)$_2$) group of glyoxylic acid, with $sp^3$ carbon, is observed at $\delta$ 88.89 ppm, while its carboxylic acid group (–COOH) is at $\delta$ 175.90. The –COOH of the formic acid product is observed at $\delta$ 179.41 ppm in Figure 2.6, while oxalic acid ($\delta = 168.51$ ppm) was only detectable after spike addition of a standard (Sigma Aldrich, 99.7 %) to the sample. The origin of formic acid can be ascribed to the formyloxyl radical °COOH created during the homolytic cleavage of excited state glyoxylic acid. This °COOH radical is converted to formic acid after abstracting a hydrogen atom, e.g., from dihydroxyacetic acid. However, a comparison of product
yields suggests that the pathway including H abstraction may be as favorable as the channel resulting in CO₂ evolution.

Because tartaric acid is about 10 times less abundant than oxalic acid, as quantified by IC-MS, indeed this species remains below the limit of detection in Figure 2.6. Spike addition of L- (+)-tartaric acid (Sigma Aldrich, 99.8 %) standard to the product mixture indicates its peaks for –COOH and –CH(OH) – should be observed at δ 177.41 and δ 74.72 ppm, respectively. Remarkably, the new peak appearing for glyoxal at δ 93.22 ppm matches that of a spiked standard (Sigma Aldrich, 38.5 %) in the products’ spectrum, indicating the homolytic reaction HOOC-O(H)C + hν → HOOC’ + ’C(H)=O proceeds upon λ ≥ 305 nm irradiation.

Figure 2.6. 150 MHz ¹³C NMR spectrum after 8 h photolysis of glyoxylic acid under the experimental conditions given in Figure 2.1.

The source of glyoxal could be linked to formyl radical recombination through the reaction: O=(H)C’ + ’C(H)=O → O=(H)C-C(H)=O. However, more likely the addition of
O=\text{(H)}C^\cdot to the carbonyl group of glyoxylic acid generates an alkoxy radical that, after homolysis, produces a molecule of glyoxal and a second \text{HOOC}^\cdot. More details of these reactions are provided in the mechanism below.

Figure 2.7 shows the first-order decay of \([\text{glyoxylic acid}]_t\) at each time \((t)\) point relative to its initial value \([\text{glyoxylic acid}]_0\) during 8 h irradiation of stage 1, which is described by the equation \([\text{glyoxylic acid}]_t/[\text{glyoxylic acid}]_0 = 0.998 \times \exp(-8.85 \times 10^{-3} \text{ h}^{-1} \times t)\) with \(R^2 = 0.980\). The right-hand side vertical axis within Figure 2.7 displays the scale for the production of glyoxal, \text{CO}_2, \text{CO}, formic acid, oxalic acid, and tartaric acid for the same time series. Photoproducts can be consumed during reactions, e.g. with carbonyls or reactive radical species generated during photolysis. For example, glyoxal quickly reaches a quasi-steady state in Figure 2.7 with a characteristic time \(\tau = 7.5\ \text{min}\) as described by the exponential rise to maximum fitting \([\text{glyoxal}]\ (\text{mM}) = 2.04 \times (1 - \exp(-5.31\ \text{h}^{-1} \times t))\) with \(R^2 = 0.981\). The production of formic acid, \text{CO}_2(\text{g}), oxalic acid, \text{CO}(\text{g}), and tartaric acid follows zero-order kinetics with rate constants \(k\) (mM h\(^{-1}\)): \(2.39 \times 10^{-1}\) \((R^2 = 0.998)\), \(2.28 \times 10^{-1}\) \((R^2 = 0.985)\), excluding the outlier at the 95% confidence level for \(t = 8\ \text{h}\), \(6.34 \times 10^{-2}\) \((R^2 = 0.980)\), \(4.92 \times 10^{-2}\) \((R^2 = 0.961)\), and \(6.77 \times 10^{-3}\) \((R^2 = 0.984)\), respectively. Therefore, the uniform changes observed in Figure 2.7 indicate the persistent photoproducts formed undergo reversible thermochromism and photobleaching in water. This versatile behavior is mainly provided by the aldehyde functional group of glyoxal and/or its derived material in the mixture. Finally, it is important to indicate that the behavior of dissolved oxygen in this experimental system is quite different to that reported in studies with methyl vinyl ketone.\textsuperscript{98,99} Under irradiation, \([\text{O}_2(\text{aq})]\) in Figure 2.7 (external right hand side vertical axis) only decreases 70% (from 0.26 mM to 0.08 mM)
within the first 2 h, and then recovers close to 80% of the expected equilibrium concentration. In other words, the production of species that consume O$_2$(aq) is never large enough to completely deplete this important gas in solution during the whole experiment.

![Figure 2.7](image)

**Figure 2.7.** (Red ◊) Normalized loss of glyoxylic acid and production of (brown ▽) CO$_2$, (green ●) CO, (gray ×) glyoxal, (black Δ) formic acid, (pink ○) oxalic acid, (blue ■) tartaric acid, and (teal hexagon) dissolved O$_2$ during 8 h photolysis under the conditions given in Figure 2.1.

### 2.4.3 Proposed Reaction Mechanism

From the experiments described in the discussion above, a mechanism for the photoreaction of glyoxylic acid via radical chemistry is proposed in Scheme 2.3. The proposed mechanism, which advances previous knowledge in this field, serves as a first representation that could be further improved in the future. Dissolved organic matter, including triplet-state carbonyls, can play a significant role as a photosensitizer in the environment. For example, the oligomerization of methyl vinyl ketone proceeds after reaction with OH or excited state pyruvic acid in water. In present experiments, as for other carbonyls and due to its absorbing tail for $\lambda \geq 300$ nm, the lowest energy $n \rightarrow$
π* transition is activated by solar light to produce a very reactive singlet excited state of glyoxylic acid. The singlet excited state can be considered to react directly, or undergo intersystem crossing producing a triplet, which can then react, as it has been suggested for the gas phase photolysis of glyoxylic acid.\textsuperscript{50} Therefore, Scheme 2.3 depicts a generic excited state glyoxylic acid GA* to be produced upon photon absorption by reaction R1.

**Scheme 2.3.** Proposed mechanism for the photooxidation of glyoxylic acid (GA) in water.

The excited state GA* can react directly undergoing homolytic cleavage by reaction R2 into formyloxyl radical HOOC’ and formyl radical ´C(H)=O, the simplest existing acyl radical. The alternative fate of GA*, as a triplet, is to abstract a hydrogen atom from
the very abundant hydrate of glyoxylic acid via reaction R3 to generate the radicals of glycolic acid (K•) and dihydroxyacetic acid (G•). Hydrated glyoxylic acid cannot accept energy from GA* but hydrogen abstraction is a characteristic reaction of triplet 2-oxocarboxylic acids. The doublet displayed in electron paramagnetic resonance measurements in the liquid phase shows that K• can also be generated when H abstraction from glycolic acid occurs. Similarly, G• corresponds to a triplet previously registered during the reduction of oxalic acid at low pH.

Reaction R4 (Scheme 2.3) generates carbon monoxide from 'C(H)=O and also provides a pathway to form K• from GA. The acid dissociation constant of HOOC• (pKₐ = 2.3) indicates that 95% of the speciation of this radical at the experimental condition of pH 1.0 is in the undissociated form. Interestingly, the fate of the conjugated base CO₂• for the pH range 3-9, is to form oxalate through bimolecular recombination with a rate constant \( k_{CO_2^- + CO_2^-} = 1.4 \times 10^9 \text{ M}^{-1} \text{s}^{-1} \). However, at lower pH (e.g., pH 1.0) the yield of oxalate from the previous reaction becomes negligible while the yield of CO₂ increases. Therefore, discarding the recombination reaction HOOC• + 'C(H)=O \( \rightarrow \) HOOC-C(H)=O as viable under continuous irradiation, the fate of HOOC• at low pH is 1) to self-disproportionate with rate constant \( k_{HOOC^+ + HOOC^-} = 1.7 \times 10^9 \text{ M}^{-1} \text{s}^{-1} \) (reported at pH 0) into CO₂ and HCOOH by reaction R5 (Scheme 2.3), 2) to react with another radical, i.e., to produce CO₂ and regenerate the hydrate form of glyoxylic acid by reaction R6; or 3) to react with 'C(H)=O to yield formic acid and CO. Carbon monoxide can also be generated from the formyl radical in the presence of dissolved O₂ by reaction R7 that eliminates hydroperoxyl radical (HO₂•). As indicated above, CO₂• formed by reaction R8a can undergo efficient acid catalyzed reaction with glyoxylic acid.
to release CO$_2$(g) and K$'$ by reaction R8b. At very acidic pH, the sequence R8a + R8b proceeds directly from HOOC$'$ + GA. Alternatively, HOOC$'$ could abstract a hydrogen$^{104}$ from dihydroxyacetic acid to produce formic acid and G$'$.

The production of oxalic acid observed can be explained by the direct oxidation of G$'$ after combining with O$_2$ and in situ elimination of hydroperoxyl radical via reaction R9.$^{108}$ Once oxalic acid is formed, it can undergo in situ reduction to form G$'$ again by any of the α-hydroxyalkyl radicals available in the system.$^{102}$ The combination of two K$'$ radicals via reaction R10, with rate constant $k_{K' + K'} = 5.5 \times 10^8$ M$^{-1}$ s$^{-1}$,$^{109}$ results in the formation of tartaric acid, a less likely process for the fate of this radical, as represented by the low level of product quantified. As observed for the production of dimethyltartaric acid from the recombination of lactic acid radicals in the photolysis of pyruvic acid,$^{5,69}$ the pathway for the production of tartaric acid in this work is not fully quenched in the presence of [O$_2$(aq)]$_{1 \text{ atm air}} = 0.26$ mM. The recombination of α-hydroxyperoxyl radicals should become more important at higher radical concentration than $5 \times 10^{-9}$ M,$^{110}$ and also as proposed here for decreasing [O$_2$] found at higher altitudes. The reaction of K$'$ with O$_2$ proceeds by reaction R11, with a rate constant $k_{K' + O_2} = 1.76 \times 10^9$ M$^{-1}$ s$^{-1}$,$^{111}$ to regenerate the carbonyl form of glyoxylic acid after elimination of hydroperoxyl radical with a rate constant $k_{K'O_2} = 122$ s$^{-1}$.$^{110}$

An alkoxy radical is generated following the addition of 'C(H)=O to the carbonyl group of glyoxylic acid (reaction R12, Scheme 2.3). It has been previously proposed that at high pH the semidione radical would be an important intermediate.$^{102}$ The production of glyoxal in reaction R13 is proposed to result from the cleavage of the alkoxy radical formed in reaction R12. Reaction R13 simultaneously generates a second source of
'COOH that contributes CO$_2$, the major reaction product. It is important to highlight that the presence of carbonyls (e.g., glyoxylic acid or in situ produced glyoxal) acting as sensitizers can result in a positive feedback for the production of CO$_2$.\textsuperscript{102} Because the possible recombination of two \( ^1 \text{C(H)=O} \) radicals seems an unlikely process (i.e. the recombination of two \( ^1 \text{COOH} \) yields little oxalic acid), a reaction such as R13 is thought to dominate the production of glyoxal. The carbonyl group of the alkoxy radical formed in reaction R12 likely undergoes fast hydration and actually generates the monohydrate of glyoxal, which incorporates a second molecule of water \( (K_{\text{hyd}} = 2.2 \times 10^5)\textsuperscript{112} \) to produce the unique tetrol signal observed in the $^{13}$C NMR spectrum (equilibrium R14, Scheme 2.3).\textsuperscript{112}

Reaction R15 shows that hydrogen abstraction by any of the radicals present in the system, denoted by the generic radical L’, yields the hydrated glyoxal radical. In reaction R16, the hydrated glyoxal radical M’ combines with O$_2$ forming the glyoxyl peroxyl radical MO$_2^\cdot$ with a rate constant \( k_{M^\cdot + O_2} = 1.1 \times 10^9 \text{ M}^{-1} \text{ s}^{-1} \).\textsuperscript{110} After elimination of HO$_2^\cdot$, also included in reaction R16 \( (k_{MO_2^\cdot} = 122 \text{ s}^{-1})\textsuperscript{110} \) the gem-diol form of glyoxylic acid is generated, which is in equilibrium R17 \( (K_{\text{Hyd}} = 300)\textsuperscript{20} \) with the carbonyl form of glyoxylic acid. Alternatively, disproportionation during the recombination of MO$_2^\cdot$ radicals\textsuperscript{107} produces glyoxal and glyoxylic acid. Different carbonyls have been shown to favor both $\alpha$-cleavage and hydrogen abstraction occurring simultaneously during primary photochemical reactions\textsuperscript{113} and glyoxylic acid is not the exception. For this mechanism, reactions $G^\cdot + G^\cdot$, $G^\cdot + K^\cdot$, $G^\cdot + M^\cdot$, and $K^\cdot + M^\cdot$ were considered and disregarded because no corresponding products with $m/z$ 165 181, 183, and 167 were found in the IC-MS chromatogram. Remarkably, despite the low levels of products generated in the
condensed phase, this mechanism clearly highlights the importance of understanding further mechanistic steps occurring in atmospheric waters. The mechanism provides details of several reactions (i.e., R7, R9, R11, and R16) capable of generating reactive oxygen species in situ from a photoinitiated process previously unknown.

It is interesting to contrast the effect of dissolved O$_2$ in the formation of both CO$_2$(g) and CO(g) in experiments supersaturated in O$_2$(g) ([O$_2$(aq)]$_0$ = 1.24 mM) vs 1 atm N$_2$(g). For this purpose, the evolution of CO$_2$(g) and CO(g) during irradiation was monitored for [glyoxylic acid] = 5, 25, 100, and 250 mM in the electrolyte matrix during irradiation. The ratio of the initial rates of CO$_2$(g) and CO(g) formation to $I_0$ were employed to calculate the corresponding apparent quantum yields $\Phi_{CO_2}$ and $\Phi_{CO}$ displayed in Figure 2.S4 (Supporting Information). The fact that the ratio of quantum yields (Figure 2.S4) in the presence of dissolved oxygen is always larger than the corresponding ratio under 1 atm N$_2$(g), $(\Phi_{CO_2}/\Phi_{CO})_{O_2} > (\Phi_{CO_2}/\Phi_{CO})_{N_2}$, clearly indicates that O$_2$(aq) favors the formation of CO$_2$(g), the fully oxidized product. The values of $\Phi_{CO_2}$ are enhanced for larger [glyoxylic acid], suggesting that some bimolecular processes and/or the participation of reaction intermediates could be important to facilitate CO$_2$ production during irradiation. In contrast, $\Phi_{CO}$ appears to follow a similar growing trend for increasing [glyoxylic acid] with dissolved O$_2$(g) or N$_2$(g), suggesting the production of CO(g) has a much smaller dependence on [O$_2$(aq)] than CO$_2$(g) does. For the two higher [glyoxylic acid] (Figure 2.S4, Supporting Information), the significant difference in $\Phi_{CO}$ under supersaturated dissolved oxygen vs N$_2$(g) might be attributed to reaction R7 (Scheme 2.3) with direct participation of O$_2$(aq). A related conclusion can be drawn from the larger quasi-steady state glyoxal production obtained by extrapolating the [glyoxal]$_{inf}$
as time $\rightarrow \infty$ (Figure 2.S5, Supporting Information) without dissolved O$_2$. Figure 2.S5 (Supporting Information) suggests that O$_2$(aq) in reaction R7 decreases the availability of 'C(H)=O for the glyoxal production channel R12 + R13. Indeed, the growing trends for $\Phi_{\text{CO}_2}$ and $\Phi_{\text{CO}}$ with increasing $[\text{glyoxylic acid}]_0$ provide mechanistic support for direct reactions R4 and R8a + R8b, and indirectly by enhancing the production of G$^\cdot$ in reaction R3, which is available to facilitate the decarboxylation R6. A related discussion of the effect of [O$_2$(aq)] and [glyoxylic acid]$_0$ in the production of formic, oxalic, and tartaric acids is presented in the Supporting Information.

2.5 Conclusions and Atmospheric Implications

Addressing the significance of aqueous photochemistry in the processing of atmospheric particles is an important issue.$^{32, 34, 75}$ However, uncertainties in this field remain because photolysis quantum yields for many compounds are unknown.$^{34}$ The data in this work allow us to report for the first time an overall quantum yield for the photolytic loss of aqueous glyoxylic acid ($\Phi_{\text{GA}}$) in air under solar irradiation. The ratio of the initial reaction rate of aqueous glyoxylic acid in the experiment of Figure 2.7, (-d[GA]/dt)$_0 = 6.75 \times 10^{-7}$ mol L$^{-1}$ s$^{-1}$, to $I_0$ provides $\Phi_{\text{GA}} = 0.017$ for $\lambda = 325$ (± 20) nm. This value can be used to estimate the photolysis rate in the aqueous phase ($j_{\text{aq}}$) from the integral of the variable actinic flux $F_A$ and the cross section of glyoxylic acid $\sigma_\lambda$: $j_{\text{aq}} = \int F_A(\lambda) \Phi(\lambda) \sigma(\lambda) d\lambda$. Available data for surface altitude, a solar zenith angle of 40°, and a surface albedo of 80% is chosen to represent $F_A$,$^{114}$ while $\sigma_\lambda$ is estimated from the known concentration that simulates atmospheric conditions (Supporting Information) and the corresponding absorbance values in Figure 2.1. Thus, under atmospheric conditions a value of $j_{\text{aq}} = 9.9 \times 10^{-7}$ s$^{-1}$ is obtained, the reciprocal of which can be used to estimate a
rough photolysis lifetime in the aqueous phase of $\tau \approx 11$ d. For comparison, the loss of glyoxylic acids by indirect photolysis in the aqueous phase proceeds only two times faster (e.g., $\tau_{\text{aq. } \text{GA} + \cdot \text{OH}} \sim 5$ d). While the rate constant for the gas phase reaction glyoxylic acid + $\cdot \text{OH}$ is unknown, structure activity relationships give an estimated $k_{\text{GA} + \cdot \text{OH}} = 1.28 \times 10^{-11}$ cm$^3$ molecule$^{-1}$ s$^{-1}$. Using an average daytime [OH(g)] $1.6 \times 10^6$ molecule cm$^{-3}$ leads to a lifetime $\tau_{\text{gas, } \text{GA} + \cdot \text{OH}} \sim 0.56$ day. The large gas phase photolysis rate of glyoxylic acid $j_{\text{gas}} = 8.7 \times 10^{-5}$ s$^{-1}$ provides a shorter lifetime $\tau_{\text{gas, } h\nu} = 3.2$ h. However, as the hydration equilibrium of glyoxylic acid is shifted toward 2,2-dihydroxyacetic acid, this species preferentially and quickly partitions to the particle phase. Due to the improvable presence of glyoxylic acid in the gas phase, its fate should not be dominated by reactivity with $\cdot \text{OH(g)}$ radical.

This work studies for the first time the photoreaction of aqueous glyoxylic acid under simulated solar irradiation and thermal processing of the photolyzed in the dark. The mechanism proposed via radicals is initiated by a photoexcited $\alpha$-dicarbonyl species in water and results in the production of species with large O:C ratio. The results provide an explanation to the possible role of glyoxylic acid in the generation of complex organic matter in aqueous aerosols. Although glyoxylic acid is not a major absorber of solar radiation, the tail of its electronic spectrum is extended well into the region of solar actinic radiation available in the troposphere. After light absorption, glyoxylic acid can induce secondary reactivity of other molecules as demonstrated here. This information contributes new experimental evidence to explain the mechanisms of aqSOA production of oxalic acid and establishes a link to glyoxal chemistry. The low production of tartaric
acid, a dimer of a primary radical, is also detected. However, it is clear that glyoxylic acid is also converted to carbon oxides.

Experiments and controls have also explored the effects caused by inorganic electrolytes and [O₂(aq)]. Chemical identification is obtained from a combination of spectroscopies that supports the direct participation of radicals in the mechanism. In addition, this work has contrasted the optical properties showing the periodic behavior of samples during daytime and nighttime cycles of photobleaching and thermochromism, respectively. Overall, the optical properties of atmospheric brown carbon species change periodically in the aqueous phase. O₂(g) in air cannot stop the development of thermochromism, affecting minimally the optical properties of the model organic material produced, which in the atmosphere is influenced by aerosols’ variable and complex size, structure, and composition. These results are directly related to the ulterior oxidative fate of polyphenols emitted during biomass burning and combustion processes, which have been proposed to contribute chromophores and reactive intermediates in route to the formation of the dicarbonyl, oxocarboxylic acid, and dicarboxylic acid components of brown carbon. In particular, oxocarboxylic acids are important species in the atmosphere because they participate in aqueous phase reactions in the presence of electrolytes. The observed behavior of model organic species should be treated in future climate change models taking into account thermochromism and photobleaching parametrization factors.

2.6 Acknowledgements

We thank the National Science foundation for research funding under NSF CAREER award CHE-1255290.
2.7 Supporting Information For Aqueous Photochemistry of Glyoxylic Acid

Detailed Experimental Section

Preparation of experiments and controls. The concentration range for glyoxylic acid used is bracketed by the water to sulfate ratio found in remote and urban aerosols.\(^5\) Considering the deliquescence curve of an ammonium bisulfate solution\(^{27, 116}\) at 50 % relative humidity (RH), fine arctic aerosol particles contain 0.6 g of H\(_2\)O/g of SO\(_4^{2-}\) or up to \([\text{glyoxylic acid}] \approx 250 \text{ mM}^{117}\) under very acidic conditions,\(^5\) while polluted city environments such as Tokyo can exceed this concentration by 3 times\(^{15}\). Therefore, ca. 250 mM glyoxylic acid (Sigma-Aldrich, 52.1 wt. % in H\(_2\)O) solutions were prepared daily in water (Elga Purelab flex, 18.2 M\(\Omega\) cm\(^{-1}\)). Additional experiments studied the effect of glyoxylic acid concentration covering the range 5-250 mM. “

Even though related studies employed glyoxylic acid monohydrate (Fluka, 97%) of lower purity without further purification,\(^{20, 115}\) we discarded the use of the monohydrate (98%) and selected the aqueous solution of higher purity for the work. The selection of the aqueous reagent considered the evaluation of impurities present in glyoxylic acid monohydrate (Sigma Aldrich, 98%) before and after purification by two methods: 1) Purification of the solid by gently warming the monohydrate to 40 °C under vacuum at 17 mTorr was performed for 12 h, as described elsewhere;\(^90\) and 2) recrystallization of the monohydrate in chloroform (Fisher Optima, LC-MS grade) recovering a 55 wt. % as white crystals. However, even after both purification procedures, the purity of the reagent did not appear to be larger than for the aqueous reagent chosen for this work, as revealed using the multiple techniques described in the experimental section. For example, ion
chromatography showed that the aqueous reagent (glyoxylic acid, 52.1 wt. %) used for the work was appropriately selected.

The solution of glyoxylic acid was doped with the most abundant inorganic electrolytes found in seawater,\textsuperscript{84} which form sea spray aerosols. The electrolytes included $[\text{Na}^{+}] = 468$ mM, $[\text{Cl}^-] = 545$ mM and $[\text{SO}_4^{2-}] = 28.2$ mM prepared from NaCl (Fisher, 99 %), Na$_2$SO$_4$ (Fisher, 99 %) and HCl (EMD, 38 %). This mixture does not contain NH$_4^+$ in order to focus the work on the effects caused by photoirradiation instead of reiterating the reported catalysis by ammonium\textsuperscript{86} that can lead to decarboxylation.\textsuperscript{5, 69, 76} After adjusting the pH of the glyoxylic acid solution to 1.0 with $[\text{HCl}] = 2.0$ M, 180 mL were transferred to a customized fused silica photoreactor (220 mL capacity) provided with a jacket for temperature control at 298 K (Thermo Scientific A25 circulating bath), magnetic stirring, and a port for sampling gases through a septum. The sealed reactor underwent continuous sparging (unless indicated otherwise) with 100 mL min$^{-1}$ air (Scott-Gross, UHP) starting 30 minutes before photolysis. Experiments are performed under 1 atm air and the conditions of ionic strength, temperatures and photon flux are chosen to simulate those encountered by nascent sea spray aerosols mixing with pollution at coastal regions.

Control experiments (Table 2.S1) were designed to study the effect of inorganic electrolytes and $[\text{O}_2(\text{aq})]$ on the photoreaction of glyoxylic acid. In more detail Table 2.S1 indicates which gas was used and if electrolytes were not present in the controls: Control A under 1 atm N$_2$(g) (Scott-Gross, UHP), control B under 1 atm O$_2$(g) (Scott-Gross, UHP), control C without electrolytes, control D without electrolytes under 1 atm N$_2$(g), and control E without electrolytes under 1 atm O$_2$(g). From Henry’s law ($K_H =$
1.28 \times 10^{-3} \text{ M atm}^{-1} \text{ for } \text{O}_2 \text{ at } 298 \text{ K},^{48} \left[\text{O}_2(\text{aq})\right]_{1 \text{ atm air}} = 0.26 \text{ mM} \text{ and } \left[\text{O}_2(\text{aq})\right]_{1 \text{ atm O}_2} = 1.24 \text{ mM}.

**Table 2.S1.** Experiments and controls to investigate the photoreaction of glyoxylic acid

<table>
<thead>
<tr>
<th>Conditions</th>
<th>( h\nu )</th>
<th>Electrolytes</th>
<th>Atmosphere</th>
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<tbody>
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</tr>
<tr>
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<td>✓</td>
<td>✓</td>
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<tr>
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<tr>
<td>Control C</td>
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<td>Control D</td>
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<td>Control E</td>
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<td>Control F</td>
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Key: \( h\nu \) indicates the presence of light (\( \lambda \geq 305 \text{ nm} \)). Electrolytes include Na\(^+\), Cl\(^-\) and SO\(_4\)^{2-}.

Photochemical experiments and thermal treatment of photolyzed samples. The photochemical setup (Newport) employed was previously described\(^{87}\) and includes a 1 kW high pressure Xe-Hg lamp, a water filter to remove infrared radiation, and a cut-off filter for wavelength \( \lambda \geq 305 \text{ nm} \) that provides actinic radiation in the solar window. During the first stage of processing (Stage I in Scheme 2.2), samples of the experiment
and controls (Table 2.S1) were irradiated for 8 h and 5 mL aliquots were withdrawn from the reactor at 0, 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 h. A 30% of the aliquot was immediately frozen at 253 K and stored in the dark for later analyses by IC-MS, UHPLC-MS, and NMR spectroscopy. The remaining 3.5 mL of sample was transferred to a Suprasil cuvette (Starna Cells, 10 mm optical path length) to monitor its aging (Stage II in Scheme 2.2) in the dark by UV-visible spectroscopy for 15 h at 298 K followed by 9 h at 323 K. About 130 mL of the remaining aged-photolyzed sample was stored in the dark for 24 h and then rephotolyzed (Stage III in Scheme 2.2). Rephotolyzed samples underwent a second aging process in the dark monitored by UV-visible spectroscopy (Stage IV in Scheme 2.2).

**Analysis of products.** Samples were analyzed by 1) UV-visible spectroscopy; 2) ion chromatography (IC) equipped with a conductivity detector and a mass spectrometer (MS) interfaced with an electrospray ionization (ESI) probe (IC-MS); 3) UHPLC-MS; 4) Fourier transform infrared spectroscopy (FTIR) for gases; and 5) $^1$H and $^{13}$C NMR experiments. Data from all these methods is reported as the average of duplicate experiments with error bars corresponding to one standard deviation. The competition between homolytic cleavage and hydrogen abstraction resulting from excited triplet state glyoxylic acid could be indirectly assessed from the abundance of products generated by each channel.

UV-visible spectra (190 ≤ $\lambda$ ≤ 750 nm) of initial and irradiated samples were recorded immediately after sampling with an Evolution 220 scanning spectrophotometer (Thermo Scientific) equipped with a temperature control multicell holder (Thermo Scientific, SPE 8 W). In addition, spectra were recorded during the aging processing every 30 min.
Actual initial concentrations of glyoxylic acid in the solutions were registered from total absorbance readings using Beer’s Law and the molar absorptivity of glyoxylic acid in water at $\lambda = 240$ nm: $\varepsilon_{GA} = 11.6 \, \text{M}^{-1} \, \text{cm}^{-1}$. An aged sample from the end of stage II (Scheme 2.2) was placed in a 10 mm optical path micro fluorimeter cell (Starna) for registering the fluorescence emission spectra at 298 K using a FluroMax-4 fluorimeter (Horiba Scientific) and FluorEssence software. The excitation wavelength ($\lambda_{\text{exc}}$) was varied over the range 350-400 nm to record emission spectra up to 800 nm. A single accumulation with 0.1 s integration time and 1 nm step size was employed. A sample with 11.8 mM glyoxal (the actual amount quantified at the end of Stage II) with the same electrolyte composition as the experiment was aged for comparison. A solution of electrolytes (no organics) was used as a blank to subtract out the water Raman peak from each spectrum.

Samples for ion chromatography in the low millimolar range of glyoxylic acid were obtained by dilution (0 to 50 times) of the photolyzed aliquots with water, depending on $[\text{glyoxylic acid}]_0$. Chromatographic samples were immediately injected with an autosampler (Dionex AS) in an IC (Dionex ICS-2000) provided with an IonPack AS11-HC (2 mm) analytical column and a conductivity detector. An ESI probe interfaced the output of the conductivity detector to a mass spectrometer (Thermo Scientific, MSQ Plus) as described before. The ESI-MS operates in negative ion mode for the m/z range 50-500 amu at 70 psi N$_2$ nebulizing gas, 450 °C, 1.9 kV needle voltage, and 50 V cone voltage, unless indicated otherwise. Tartaric and oxalic acids were detected as the monoanions at $m/z$ 149.01 and 88.99, respectively. Sodium oxalate (Alfa Aesar, 99.9 %)
and L-(-)-tartaric acid (Sigma Aldrich, 99.7%) were used as standards for the quantification.

An Accela (Thermo Fisher Scientific) UHPLC with a 1250 quaternary delivery pump and a MS detector (MSQ Plus) was employed for the analysis of carbonyls after derivatization with freshly prepared 2,4-dinitrophenylhydrazine (DNPH, Sigma Aldrich, HPLC grade). For the general identification of carbonyls, 26 µL of photolyzed sample were diluted to a volume of 5 mL, mixed with 5 mL [DNPH] = 25.02 mM dissolved in acetonitrile with 4 % H₂SO₄, and incubated at 20 °C during 1 h. The derivatized samples were mixed with an equal volume of methanol (Fisher Optima, LC-MS grade) and injected (25 µL) with an Accela autosampler into a reversed-phase column (Hypersil GOLD C18 column 1.9 µm, 50×2.1 mm, Thermo Scientific) for separation of hydrazones. The carbonyls in the mixture were compared to those of standard hydrazones for glyoxylic acid, formaldehyde (Mallinckrodt, 37.2 %), and glyoxal (Sigma Aldrich, 38.5 wt. %), which were observed by mass spectrometry at m/z 253.02, 209.03, and 417.05, respectively.

Chromatographic separation at a constant flow rate of 800 µL min⁻¹ from 0 to 1 min was isocratic with 70 % (solvent A) 0.10 mM formic acid (Fisher Optima, LC-MS grade) in ultrapure water (18.2 MΩ cm) and 30 % (solvent B) 0.10 mM formic acid in methanol (Fisher Optima, LC-MS grade). Gradient elution from 1 to 7 min reached a 45:55 ratio of solvents A:B and remained isocratic from 7 to 9 min. The UHPLC was interfaced to the MS by an ESI probe (1.9 kV needle voltage, 350 °C probe temperature, 50 V cone voltage, and 70 psi N₂ nebulizing gas) to detect negative ions. Excalibur software was used to control the UHPLC-MS system. Gaussian functions were employed to fit the data.
reported. A calibration curve made with glyoxal was used to correlate extracted ion chromatogram peak area with dihydrazone concentration in samples. Formaldehyde production in the photolyzed was discarded by this analysis as well as by NMR and FTIR methods described below. The careful quantification of glyoxylic acid was only possible after modifying the above protocol by diluting the samples $2.25 \times 10^4$ times ($\text{pH}_{\text{final}} = 2$) prior to DNPH addition.

NMR (Varian 600 MHz) spectra were recorded at 298 K using 5 mm NMR tubes (Wilmad) with 540 μL of sample spiked with 60 μL of 121.44 mM 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS, Aldrich, 97 %) internal standard in D$_2$O (Cambridge Isotope Laboratories, 99.9 %) for field-frequency lock, using a 90° pulse length for $^{13}$C NMR. Although $^1$H NMR is not the preferred choice of analysis because most products of interest appear under the water peak, the production of formic acid could be quantified by this method. For this purpose, the samples described above were spiked with 10 μL of 7.47 mM gadolinium (III) chloride (Aldrich, 99.999 %) in D$_2$O to shorten T$_1$ relaxation times. $^1$H NMR spectra were recorded using a water suppression enhanced through T$_1$ effects solvent suppression (WET) method. Equation 2.1 was used to calculate [formic acid] by comparison to the area of DSS internal standard:

$$[\text{formic acid}] = \frac{[\text{DSS}] \times \text{(area of formic acid)}}{\text{(area of DSS)}} \times \frac{9}{1} \quad (2.1)$$

where the area of formic acid is obtained by integrating the peak at δ 8.241 (s, 1H), which is only shifted -0.016 ppm relative to the spectral database value, and the area of DSS corresponds to the strong singlet at δ 0.000 (s, 9H), and the factor 9/1 represents the ratio of hydrogen atoms in DSS to formic acid.
For the analysis of evolving gases, the same experimental conditions were used with the exception that the sealed reactor filled with air was not continuously sparged. Aliquots of 500 µL of the reactor headspace were withdrawn through the septum with a syringe provided with a stainless steel needle for analysis in a 2.4 m path length infrared gas cell with ZnSe windows (PIKE) and a capacity of 100 cm\(^3\). The cell was mounted in an iZ10 FT-IR module connected to an infrared microscope (Thermo Scientific Nicolet iN10). Equilibration of gases in the cell during 30 min was allowed prior to scanning. The optics were continuously purged with N\(_2\)(g) while recording 128 scans of background and sample spectra in absorption mode with 1 cm\(^{-1}\) resolution over the range 500-4000 cm\(^{-1}\). For the quantification of CO\(_2\) and CO the respective R-branch, centered at 2361 and 2176 cm\(^{-1}\), were baseline corrected and integrated in the absorbance spectra. For calibration purposes, samples of known [CO\(_2\)(g)] and [CO(g)] were generated during the photodecarboxylation of 25 mM aqueous solutions of phenylglyoxylic acid (Alfa Aesar, 99.9 %) at pH 1.0\(^{89,120}\), and the photodecarbonylation of 10 mM solutions of 1,3-diphenyl-2-propanone (Aldrich, 99.7 %) in benzene\(^{121,122}\) (EMD, Omnisolv, 99.7 %), respectively. The same liquid to headspace volume ratio of experiments was kept for these analyses providing concentrations in molar units relative to the liquid volume.

**Evaluation of Beer’s law.** Figure 2.S1A presents the absorption changes and linear regression at selected wavelengths (\(\lambda = 280, 295, 320, 350, 420, 450 \text{ and } 500 \text{ nm}\)) for the same experiment in Figure 2.1 (after stage II) upon successive dilutions with water by 1, 2, 4, and 10 times. For each monochromatic radiation Beer’s law is followed well only for the UV and shorter visible range (\(\lambda \lesssim 400 \text{ nm}\)). However, for \(\lambda > 400 \text{ nm}\) the absorptivity decays monotonically resulting in a nonlinear behavior.
Figure 2.S1. (A) Absorbance of experiment with glyoxylic acid in Figure 2.1 at the end of stage II (Scheme 2.2 and Figure 2.2) vs. the reciprocal of dilution factor at wavelength ($\lambda$): (black $\Delta$) 280, (blue $\circ$) 295 nm, (red $\square$) 320, (pink $\diamond$) 350, (green $\triangleleft$) 420, and (dark red $\times$) 450 nm. (B) Coefficient of determination ($R^2$) for linear regression data in panel A.

Figure 2.S1B shows large changes in the coefficient of determination ($R^2$) of the linear regressions in part A. The progressive loss of linearity at higher $\lambda$ is due to typical instrumental limitations. Therefore, we conclude that Beer’s law was obeyed between 280 and 500 nm within the experimental accuracy. The complex mixture after stage II represents the low molecular weight fraction of organic matter found in atmospheric waters, which affects the optical properties of particulate matter. The multiple functional groups present in the photolyzed and thermally aged samples behave as absorbing species but can also scatter light in the presence of electrolytes.$^{76}$ This issue should be the focus of future combined modeling and experimental efforts, accounting for the fact that the composition of organic aerosols varies with geographical location, altitude, and the time of day.$^{76, 77}$
**Assignment of an absorbing tail to glyoxylic acid.** Figure 2.S2 shows how the absorbance of glyoxylic acid solutions in the presence of electrolytes increases with concentration. At low concentrations, there is little light absorbed above $\lambda = 250$ nm. However, at the concentrations used in this study, the C=O absorption band for the characteristic $n \rightarrow \pi^*$ transition ($\lambda_{\text{max}} = 276$ nm) grows significantly following a perfect linear trend (see inset of Figure 2.S2). If any impurity with a chromophore absorbing at $\lambda = 305$ nm would be present in the sample, its tail in the region of maximum absorption for glyoxylic acid ($\lambda_{\text{max}} = 276$ nm) should have produced a larger deviation from the practically perfect linear fitting with correlation coefficient $R^2 = 0.9998$. Because the latter is not the case, the data in Figure 2.S2 strongly supports the assignment of the absorbing tail to glyoxylic acid. This band has a large tail that extends well into the tropospheric actinic window. The hydration equilibrium of glyoxylic acid demands that 833 $\mu$M of aldehyde form is available to absorb light in a 250 mM total solution.

**Figure 2.S2.** UV-visible spectra of $[\text{glyoxylic acid}] = 1, 5, 10, 25, 50, 100, 150, 200, 250,$ and 300 mM in the presence of electrolytes. Inset: Absorbance at $\lambda_{\text{max}} = 276$ nm vs. $[\text{glyoxylic acid}]$ fitted with a straight line with correlation coefficient $R^2 = 0.9998$. 
Figure 2.S3. 150 MHz $^{13}$C NMR spectrum of glyoxylic acid reagent as received. Chemical shifts are assigned by comparison to the value for glyoxylic acid in Figure 2.6 of the main text.

Figure 2.S3 presents the $^{13}$C NMR spectrum for the reagent used in this study that confirms no other chromophore is present. Only the signals for the gem-diol (–C(OH)$_2$) group at $\delta$ 88.89 ppm and the carboxylic acid group (–COOH) at $\delta$ 175.90 of glyoxylic acid are registered in the spectrum.

Apparent quantum yields of CO$_2$ and CO production under 1 atm O$_2$(g) or N$_2$(g).

Figure 2.S4 shows the apparent quantum yields $\Phi_{\text{CO}_2}$ and $\Phi_{\text{CO}}$ as well as their ratio, which is discussed in the main text.
**Figure 2.S4.** Apparent quantum yields of formation for (top panel) CO$_2$(g) and (center panel) CO(g) vs initial [glyoxylic acid]$_0$ during 2 h photolysis ($\lambda \geq 305$ nm) in electrolytes under (black ○) 1 atm N$_2$(g) or (blue ▲) 1 atm O$_2$(g).

**Figure 2.S5.** Quasi-steady state glyoxal production obtained as time $\rightarrow \infty$ ([glyoxal]$_{inf}$) vs initial [glyoxylic acid]$_0$ during 8 h photolysis ($\lambda \geq 305$ nm) in electrolytes under (black ○) 1 atm N$_2$(g) or (blue ▲) 1 atm O$_2$(g).

**Quasi-steady state glyoxal production under 1 atm O$_2$(g) or N$_2$(g).** Figure 2.S5 presents the quasi-steady state glyoxal production obtained by extrapolating the concentration of glyoxal to infinite time for experiments in the presence or absence of dissolved O$_2$. It is interesting to examine if the glyoxal generated could contribute to the
observed photochemistry by absorbing light. However, glyoxal is largely hydrated in water (overall hydration constant: $K_{\text{hyd}} = 7.22 \times 10^4 - 2.2 \times 10^5$) forming a tetrol. A rough estimate for the amount of glyoxal monohydrate (the intermediate form with one C=O group) of 13.85 ppm is obtained using lower value for $K_{\text{hyd}}$. Therefore, for the stable [glyoxal] = 2.02 mM during stage I, the [monocarbonyl] $\leq$ 28 nM. If a similar molar absorptivity to glyoxylic acid is considered for glyoxal in the experiment of Figure 2.7, then the contribution of glyoxal to the total absorbance is $2.8 \times 10^4$ times smaller than for glyoxylic acid. The number resulting from the [glyoxal monohydrate] estimated suggests an insignificant contribution of glyoxal to the photochemistry observed. For the hypothetical case of photon absorption by glyoxal in our system, we would still expect identical products to the ones observed, which would originate by analogous reactions to those displayed for glyoxylic acid in Scheme 2.3.

Apparent quantum yields of formic acid, oxalic acid, and tartaric acid production under 1 atm O$_2$(g) or N$_2$(g). Figure 2.8A-B shows the enhanced apparent quantum yields for formic and oxalic acids in the presence of dissolved oxygen. In addition to the reactions presented in the main text, the following O$_2$(aq) dependent pathways for formic acid production are suggested: 1) addition of dissolved oxygen to the formyl radical $^1\text{C}(\text{H})=\text{O}$, and a subsequent Russell type disproportionation that generates the formylperoxyl radical in water, as proposed to explain the generation of singlet oxygen ($^1\Delta_g$) during glyoxal oxidation, and 2) addition of HO$_2^*$ to the formyl radical to form unstable performic acid, which immediately decays into formic acid or CO$_2$/H$_2$O as observed in the gas phase. Therefore, the proposed reactions of formyl radical with either O$_2$(aq) or HO$_2^*$ producing HCOOH or CO$_2$ depend on the diradical. The ratio of
quantum yields for formic acid formation under 1 atm O\textsubscript{2}(g) to 1 atm N\textsubscript{2}(g) is ~6 for [glyoxylic acid]\textsubscript{0} = 5 and ~3 for [glyoxylic acid]\textsubscript{0} = 250 mM. Therefore, reaction R5 is favored when there are fewer glyoxylic acid molecules available to scavenge the formyloxyl radical HOOC•. In agreement, oxalic acid production from G• is also enhanced in the presence of O\textsubscript{2}(aq), which is eliminated as HO\textsubscript{2}• in reaction R9 (Scheme 2.3).

![Figure 2.S6](image)

**Figure 2.S6.** Apparent quantum yields of formation for (A) formic acid, (B) oxalic acid, and (C) tartaric acid vs initial [glyoxylic acid]\textsubscript{0} during 2 h photolysis (\(\lambda \geq 305\) nm) in electrolytes under (black ○) 1 atm N\textsubscript{2}(g) or (blue △) 1 atm O\textsubscript{2}(g).

Finally, the quantum yield of tartaric acid (Figure 2.S6C) is only enhanced due to the presence of O\textsubscript{2}(aq) for [glyoxylic acid]\textsubscript{0} = 250 mM. The different reactivities of singlet and triplet excited states of glyoxylic acid can provide an explanation to this enhancement. Under 1 atm O\textsubscript{2}, the dissolved (triplet) diradical acts as a quencher that reduces the population of triplet excited state \(^3\text{GA}\)° and hydrogen abstraction by reaction R3 (Scheme 2.3). Therefore, reaction R2 (Scheme 2.3) is favored as the main producing
channel for K* through two reactions (R4 and R8) and tartaric acid by recombination R10. The presence of 1 atm O₂ enhances the production of HO₂• by reactions R7, R9, R11, R16 (Scheme 2.3) and through the reaction of HOOC* with O₂ which eliminates HO₂*/O₂•− quantitatively.¹²⁸
Chapter 3: Aqueous Photochemistry of Pyruvic Acid

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And


Scheme 3.1. Graphical abstract

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3.1 Abstract

The variable composition of secondary organic aerosols (SOA) contributes to the large uncertainty for predicting radiative forcing. A better understanding of the reaction mechanisms leading to aerosol formation such as for the photochemical reaction of aqueous pyruvic acid (PA) at $\lambda \geq 305$ nm can contribute to constrain these uncertainties. Herein, the photochemistry of aqueous PA (5-300 mM) continuously sparged with air is reexamined in the laboratory under comparable irradiance at 38° N for noon on a summer day. Several analytical methods are employed to monitor the time series of the reaction, including: 1) the derivatization of carbonyl (C=O) functional groups with 2,4-dinitrophenylhydrazine (DNPH), 2) the separation of photoproducts by ultrahigh pressure liquid chromatography (UHPLC) and ion chromatography (IC) coupled to mass spectrometry (MS), 3) high resolution MS, 4) the assignment of $^1$H NMR and $^{13}$C gCOSY spectroscopic features, and 5) quantitative $^1$H NMR. The primary photoproducts are 2,3-dimethyltartaric acid and unstable 2-(1-carboxy-1-hydroxyethoxy)-2-methyl-3-oxobutanoic acid, a polyfunctional $\beta$-ketocarboxylic acid with eight carbons (C$_8$) that quickly decarboxylates into 2-hydroxy-2-((3-oxobutan-2-yl)oxy)propanoic acid. Kinetic isotope effect studies performed for the first time for this system reveal the existence of tunneling during the initial loss of PA. Thus, the KIEs support a mechanism initiated by photoinduced proton-coupled electron transfer (PCET). Measured reaction rates at variable initial [PA]$_0$ were used to calculate the sum of the quantum yields for the products, which displays a hyperbolic dependence: $\sum \Phi_{\text{product}} = 1.99 \times [\text{PA}]_0 / (113.2 + [\text{PA}]_0)$. The fast photochemical loss of aqueous PA with an estimated lifetime of 21.7 min is interpreted as a significant atmospheric fate for this species. The complexity of
these aqueous phase pathways indicates that the solar photochemistry of an abundant α-ketocarboxylic acid can activate chemical processes for SOA formation.

3.2 Introduction

Recently, the contribution of the aqueous phase photochemistry of α-ketocarboxylic acids, e.g., glyoxylic acid and pyruvic acid (PA), to the formation of secondary organic aerosols (SOA) has been recognized as important. The oxidation of aromatic compounds from combustion and biomass burning emissions produces a variety of low molecular weight organic acids, including PA. Similarly, the photooxidation of isoprene results in the production of methacrolein in the gas phase, which is further oxidized to form water soluble methylglyoxal (Henry’s law constant $K_H = 3.71 \times 10^3$ M atm$^{-1}$). After methylglyoxal partitions to condensed phases, it is quickly hydrated ($K_{\text{hydr}} = 2.7 \times 10^3$) and subsequently undergoes efficient oxidation to PA with a 92% yield. Furthermore, out of 92 compounds studied, the direct photolysis of PA is predicted as the most favorable one to outcompete oxidation by HO$^\cdot$ in atmospheric waters.

Aqueous PA ($K_{\text{hydr}} = 2.10$) at room temperature is available as 32% carbonyl (C=O) form in equilibrium with 68% of its hydrate, 2,2-dihydroxypropanoic acid (DHPA). Thus, the strong $n\rightarrow\pi^*$ absorption of PA at $\lambda = 321 (\pm 20)$ nm can induce sunlight driven photochemistry, as shown in Scheme 3.2. Upon photon absorption in water, the produced singlet excited state can undergo intersystem crossing, forming a triplet excited state with a quantum yield $\Phi_T = 0.22$ (for 0.10 M PA), and a lifetime $\tau_{\text{pyruvic acid}} = 74$ ns. The unequivocal interpretation of, (1) electron paramagnetic resonance (EPR) spectra of irradiated aqueous PA glasses
Scheme 3.2. Reaction mechanism of sunlight irradiated aqueous pyruvic acid (PA) with radical intermediates C⁺, K⁺, X⁺, and Y⁺ labeled in blue font and products observed labeled in bold black font.⁵,⁶⁹ The inset shows the compounds that were previously proposed but which we did not observe (acetoin and lactic acid) and proxy standards used for quantification (levulinic and tartaric acids).

together with, (2) a kinetic model and product analyses⁵ and, (3) the use of TEMPO radical scavenger under a broad temperature range⁶⁸,⁶⁹ demonstrated that the dominant condensed phase photochemistry mechanism (for photodecarboxylation, Φ−CO₂ = 0.78 at 293 K)⁶⁹ is promoted by proton-coupled electron transfer (PCET) between a triplet excited state pyruvic acid (PA*) and a ground state PA molecule.⁵,⁶⁶-⁶⁹ The resulting ketyl
(K•) and acetyl (Y•) radicals are key in this process.\textsuperscript{5} In the presence of air, electron transfer from PA\textsuperscript{*} to O\textsubscript{2} may occur, providing a source of superoxide and another pathway to form Y•.\textsuperscript{133}

The proposed reaction mechanism (Scheme 3.2) explains the large evolution of primary and secondary CO\textsubscript{2}(g) during and after irradiation, as well as the low molecular weight polyfunctional major photoproducts in water and ice: 2,3-dimethyltartaric acid (DMTA) and 2-(3-oxobutan-2-yloxy)-2-hydroxypropanoic acid (the oxo-C\textsubscript{7} product).\textsuperscript{5, 69} Conversely, the possibility that other minor photoproducts, particularly acetoin, acetic, and lactic acids, could be generated has recently been reinstated\textsuperscript{4, 7} based on an alternative mechanism supported by two dimensional nuclear magnetic resonance (NMR) spectroscopy for a mixture of products,\textsuperscript{4} via an \(\alpha\)-acetolactic acid intermediate. Remarkably, this alternative reaction mechanism proceeds through the formation of K• and Y• radicals too,\textsuperscript{4} resulting in the same major photoproducts\textsuperscript{134} observed before.\textsuperscript{5, 69} However, acetoin was previously confirmed to be an artifact resulting from the decomposition of the thermolabile oxo-C\textsubscript{7} product at the high temperatures of gas chromatography analysis\textsuperscript{5} performed by Leermakers and Vesley.\textsuperscript{63} Moreover, we have recently reapproached the determination of acetoin as a photoproduct in the gas and liquid phase and quantitatively determined its upper limit concentration would not exceed 0.006\% of the total pyruvic acid converted.\textsuperscript{70} Additionally, arguments supporting acetoin production from an \(\alpha\)-acetolactic acid intermediate\textsuperscript{4, 134} can be discarded based on previous studies, which are summarized below.\textsuperscript{5, 69} Continuous postphotolysis CO\textsubscript{2} evolution well below 4 °C, the minimum temperature for \(\alpha\)-acetolactic acid decarboxylation,\textsuperscript{135} indicated a different intermediate was the dominant primary
photoproduction that undergoes β-ketocarboxylic acid decarboxylation. These decarboxylation tests were carefully performed by quickly starting with stable solutions of α-acetolactic acid kept at 1 °C, in the sealed photochemical reactor, which were acidified and rapidly thermalized to the desired final temperature of experiments, covering the range from -20 to 20 °C. Past experiments below 4 °C assured that α-acetolactic acid was available unaltered during the reaction, discarding any concern raised about the availability of thermally labile α-acetolactic acid in previous work.

In order to assess if results from different analytical techniques could support or contradict each other, affecting the interpretation of the photochemical mechanism, herein we reexamine the direct photolysis of aqueous PA under an environmentally relevant range of concentrations. Low levels of acetic acid are produced in the presence of air and quantified, while the photochemical production of acetoin and lactic acid is shown not to occur by a combination of NMR spectroscopy, liquid chromatography, and mass spectrometry (MS). The work identifies for the first time 2-(1-carboxy-1-hydroxyethoxy)-2-methyl-3-oxobutanoic acid (oxo-C₈ product) as the missing intermediate and precursor to the oxo-C₇ product after β-ketocarboxylic acid decarboxylation. The identification of this intermediate strongly supports a relevant bimolecular mechanism for the photochemistry of aqueous PA.

3.3 Experimental

3.3.1 Preparation of Experiments

All experiments were performed in duplicate. The concentration of PA was chosen based on its reported molar ratio to sulfate ranging from 2.5 × 10⁻⁴ to 10.7 × 10⁻³ in urban aerosols. For the range of relative humidity 50-90%, water uptake by acidic
aerosol is dominated by the deliquesence of ammonium bisulfate, which dictates the liquid particles have a ratio of ~0.6 g H₂O/1 g sulfate. This reasoning indicates that urban aerosols can be highly acidic and contain 5-250 mM PA. Undissociated PA is a remarkably photoreactive molecule in contrast to its conjugate base. Thus, fresh solutions of ca. 5, 25, 50, 75, and 100 mM pyruvic acid (Sigma-Aldrich, 98.5%, distilled under vacuum) in ultrapure water (18.2 MΩ cm⁻¹, Elga Purelab Flex, Veolia) were used in typical experiments. The pH of the solutions was adjusted to 1.0 with hydrochloric acid (EMD, 38%) as monitored with a calibrated pH meter (Thermo Scientific) to provide a fraction of undissociated PA (pKₐ = 2.4 at 298 K) > 0.96. Alternatively, selected experiments used fully ^1³C labeled sodium pyruvate (Cambridge Isotope Laboratories, 99.7%, 99.3% isotope enrichment) acidified to pH = 1.0 and subjected to the same experimental conditions for comparison to the unlabeled isotopologue. The kinetic isotope effect (KIE) was studied for PA solutions in D₂O (Cambridge Isotope Laboratories, 99.9%). Deuterated hydrochloric acid (DCl, Cambridge Isotope Laboratories, 35.6% in D₂O, 99.8% isotope enrichment) was used to adjust to pD = 1.0 (pH meter reading of 0.6) during KIE experiments for comparison to the experiment in H₂O. Solutions were sparged at 30 mL min⁻¹ with air (Scott-Gross, UHP) for at least 30 min before starting irradiation of aliquots in 5 mm borosilicate NMR tubes (Wilmad) of optical path length l = 0.424 cm. Similarly, controls were performed employing 1 atm N₂(g) (Scott-Gross, UHP) and 1 atm O₂(g) (Scott-Gross, UHP) instead of air. Additional experiments under continuous sparging with 1 atm air or controls under 1 atm N₂(g) or 1 atm O₂(g) were also performed in a photoreactor (220 mL capacity) as described in our previous work.
3.3.2 Photochemical Conditions

For irradiation, a 1 kW high pressure Xe-Hg lamp was used\(^3\) in combination with 1) a water filter to remove infrared radiation, and 2) a cut-off filter at wavelength $\lambda \geq 305$ nm to provide near UV and visible radiation. Neutral density filters were employed to measure an attenuated (10.64% of the total) incident photon rate, $I_0 = 1.14 \, (\pm 0.05) \times 10^{-5}$ Einstein L\(^{-1}\) s\(^{-1}\), by chemical actinometry with phenylglyoxylic acid.\(^89\) The irradiance utilized is comparable to that of Earth at 38° N for noon on a summer day. Reported quantum yields were obtained under light attenuation from initial reaction rates and the calculated absorbed photon rates for variable [PA].

3.3.3 Analysis of Products

Analyses report the average value from two independent experiments with its corresponding standard deviation. All plots include error bars even though they could be smaller than the symbols shown. For product analysis in water, fresh samples were immediately analyzed to avoid further thermal processing. IC-MS, UHPLC-MS, high resolution MS (HRMS), and both $^1$H and $^{13}$C NMR were employed. The gas phase product analysis by Fourier transform infrared spectroscopy (FTIR) was described previously and the detailed peak assignment is available in Table 3.S1.\(^3\) The percentage of PA lost during the reaction is referred to by the term conversion. In order to avoid secondary processes from generated photoproducts that could lead to identification of incorrect reaction mechanisms, the photochemical reaction is generally studied for an arbitrary conversion <30%, unless noted otherwise.
3.3.4 IC- Conductivity-MS Analysis

Samples were analyzed by IC-MS using a Dionex ICS-2000 ion chromatograph (IonPack AS11-HC, 2 mm column) equipped with a conductivity detector and an electrospray ionization (ESI) MS (Thermo Scientific MSQ Plus). Anions were detected at exact mass-to-charge ratios \((m/z)\) corresponding to the loss of a proton from the molecular weight of the parent molecule. Importantly, these chromatographic analyses indicated that PA needed to be distilled initially when received and periodically thereafter to remove traces of acetic \((m/z\) 59.01), parapyruvic \((m/z\) 175.02), and zymonic acid \((m/z\) 157.01) impurities (Figure 3.S1 shows the chromatogram and structures of these impurities), which form slowly over time even when stored in the dark below 8 °C and could interfere with analysis of the chemical mechanism. It is worth noting that in samples of pyruvic acid that have not been distilled, our IC-MS analysis allows for a separation of more than ten minutes between peaks for the photoproduction oxo-C\(_7\) and the impurity parapyruvic acid, both with \(m/z\) 175.

3.3.5 UHPLC-UV-MS Analysis

For carbonyl analysis, samples were derivatized with 2,4-dinitrophenylhydrazine (DNPH, Sigma-Aldrich, 99.6%) and separated on a Thermo Scientific Accela 1250 UHPLC equipped with tandem photodiode array (PDA) and ESI-MS (negative mode) detectors. The separation was adapted from previous work\(^3\) using the same C18 column (hypersil gold, 1.9 μm, 50 × 2.1 mm, Thermo Scientific) but using a water/methanol gradient for elution instead of water/acetonitrile. Additionally, the analysis of samples not treated with DNPH was performed (Figure 3.S2) as described in the Supporting Information.
3.3.6 HRMS

Samples were analyzed in a quadrupole Orbitrap (Q Exactive, Thermo Scientific) mass spectrometer operating in ESI negative mode with an internal lock mass for m/z calibration. The following parameters were applied: sheath gas flow 10 L min⁻¹, capillary temperature of 320 °C, and a heater temperature of 30 °C. The AGC target was set to 3 × 10⁶ and the maximum injection time to 250 ms. 3 Microscans were averaged per scan for high resolution measurements at 140,000 at m/z 255.2322. The spray voltage was operated at 3.2 kV. The S-lens RF level was set to 50.0 and the scan range to m/z 50–400. Samples were diluted 44 times in 1:1 acetonitrile:water for direct infusion (5 μL min⁻¹) into an ESI source.

3.3.7 Quantitative Analysis

Chromatographic quantification of products employed commercially available standards to prepare calibration curves. Levulinic (Sigma-Aldrich, 98.6%) and L-(+)-tartaric (Sigma-Aldrich, 99.8%) acids were used as proxies for candidate products that were not available commercially. Chromatographic relative response factors for the proxy molecules and the products were determined by quantitative NMR (qNMR) following literature procedures,¹³⁸ for accurate quantification by IC and UHPLC.

3.3.8 NMR Spectroscopy

Samples for NMR were spiked with 10% v/v D₂O containing 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS, Aldrich, 97%) and gadolinium(III) chloride hexahydrate (Aldrich, 99.999%).³ ¹H NMR spectra were recorded at 400 MHz (Varian 400-MR), unless noted otherwise, utilizing a WET 1D water suppression pulse sequence. Acetic acid (Acros, 100%) and acetoin (Aldrich, 99.1%) were quantified in ¹H NMR samples using a four
point standard addition. The method is capable of providing highly accurate quantifications down to three times the lower concentration spiked, which covered the range 169-732 μM ($r^2 = 0.986$) and 64-1350 μM ($r^2 = 0.999$) for acetic acid and acetoin, respectively. More importantly, these spikes were also used for qualitative comparison of spectral features along with a spike of lactic acid (Sigma-Aldrich, 88.6% in water). $^1$H NMR predictions were performed in NMRpredict (Modgraph Consultants Ltd.) integrated into MestReNova software, using the BEST algorithm. The algorithm uses a combination of NMR tables and parameterized functional groups to calculate probable chemical shifts. The $^1$H NMR assignments were made from a 600 MHz spectrum with enhanced resolution (see table 3.S3) recorded on a Varian Inova AS600. For experiments with $^{13}$C labeled PA, a $^{13}$C-$^{13}$C gCOSY NMR spectrum was recorded at the University of Louisville over 72 h at 298 K on a Varian VNMRS-700 spectrometer equipped with a 5 mm $^1$H[$^{13}$C,$^{15}$N]f carbon enhanced cold probe at 18 K using a standard gCOSY sequence with 90° pulses and a $t_1$ delay of 20 ms. The $f_2$ dimension was acquired with 2048 points while the $f_1$ dimension recorded 512 increments. A total of 96 scans were recorded per FID of a sample prepared with DSS reference and gadolinium(III) chloride hexahydrate as described above.

3.4 Results and Discussion

3.4.1 IC-MS Analysis

Figure 3.1A shows the IC chromatograms for the time series of an experiment with 113.7 mM PA irradiated at $\lambda \geq 305$ nm. The extracted ion chromatograms (EIC) reveal several anionic species with four different molecular weights eluting at 1) 6.36 min ($m/z$ 175.06), 2) 7.74 min ($m/z$ 87.01), 3) 17.89 min ($m/z$ 219.05), 4) 18.39 min ($m/z$ 219.05), 5) 17.90
min \( (m/z \ 177.04) \), and 6) 18.45 min \( (m/z \ 177.04) \). While some of these species coelute, generating a superimposed conductivity peak, the MS detector can deconvolute these carboxylic acid products. Standard solutions of PA are confirmed to elute at 7.74 min \( (m/z \ 87.01) \); all other chromatographic peaks observed correspond to reaction products.

The black trace in Figure 3.1A displays a chromatogram for a mixture of levulinic and tartaric acids (see structures included in the box in Scheme 3.2) eluting at 4.98 and 17.87 min, respectively. The earlier retention time (5-7 min) of simple oxocarboxylic acids such as levulinic acid and PA (with only one –COOH group) than tartaric acid (a dicarboxylic acid) (18 min) indicates that mono- and dicarboxylic acids are well separated in the chromatographic column.

**Figure 3.1.** (A) IC total conductivity and ESI(-)/MS extracted ion chromatograms (EIC) of 113.7 mM pyruvic acid (PA) photolyzed at \( \lambda \geq 305 \text{ nm} \), without neutral density filters, for (red) 0; (blue) 1; (green) 3; (purple) 5; and (gray) 10 min. Levulinic and tartaric acid standards are displayed in a black trace for reference. (B) Time series for (gray circle) PA loss at \( m/z \ 87.01 \), and formation of (red star) the oxo-C\(_7\) product at \( m/z \ 175.06 \), (blue square), DMTA at \( m/z \ 177.04 \), and (green triangle) the oxo-C\(_8\) product at \( m/z \ 219.05 \).
From this information, it can be concluded that the species eluting at 6.36 min ($m/z$ 175.06) is a monocarboxylic acid with neutral mass of 176.17 atomic mass units (amu). In addition, both species eluting later with $m/z$ 219.05 and 177.04 are dicarboxylic acids with neutral masses of 220.18 and 178.14 amu respectively. Despite the power of the MS to resolve both species at $m/z$ 177.04 and 219.05 coeluting in the IC chromatogram, a better time separation was also accomplished by direct UHPLC-MS analysis (Figure 3.S2, Supporting Information).

Previous work identified two carboxylic acid major photoproducts with molecular weight of 176.17 and 178.14 amu in the $\lambda = 320 (\pm 10)$ nm photolysis of PA.\textsuperscript{5} Thus, present results agree with previous findings, as depicted in Scheme 3.2. The dicarboxylic acid with a mass of 178.14 amu species is identified as DMTA, and the monocarboxylic acid with a mass of 176.17 amu is the oxo-C\textsubscript{7} product (Scheme 3.2). A structure for a $\beta$-ketocarboxylic acid intermediate with a neutral mass of 220.18 amu was provided,\textsuperscript{5, 69} and corresponds to the oxo-C\textsubscript{8} product eluting at 17.89 and 18.39 min. The clear identification of the oxo-C\textsubscript{8} product in Figure 3.1A, which is the intermediate undergoing thermal $\beta$-ketocarboxylic acid decarboxylation into the oxo-C\textsubscript{7} product,\textsuperscript{69} strongly supports the mechanism proposed in Scheme 3.2.

The oxo-C\textsubscript{8} product and DMTA display two distinct chromatographic peaks in both the conductivity and the EIC chromatograms, which show the presence of at least two stereoisomers of each species. Given the radical mechanism proposed in Scheme 3.2 and the presence of two stereocenters in each product, four stereoisomers of each product should theoretically be formed with equal probability. DMTA is a meso compound, reducing the number of distinct stereoisomers to only three. Thus, one chromatographic
peak at 17.07 min is proposed to arise from meso-2,3-dimethyltartaric acid and the other at 17.61 min to inseparable (R, R) and (S, S) enantiomers. Close inspection of the broad peak for the oxo-C7 product indicates a small shoulder at the top left-hand side, suggesting that two coeluting peaks from diastereomers are possible.

Figure 3.1B displays the time series of product formation and the loss of PA extracted from the data in Figure 3.1A. The first-order decay of PA fits an exponential function with a coefficient of correlation $r^2 = 0.994$, and rate constant, $k_{PA} = 3.28 \times 10^{-3} \text{ s}^{-1}$.

Similarly, the concentrations of DMTA and the oxo-C7 and oxo-C8 products increase exponentially according to the equation:

$$[\text{product}] (mM) = [\text{product}]_\infty (1 - e^{-k_f t})$$  \hspace{1cm} (3.1)

where the time $t$ is given in min, the $[\text{product}]_\infty$ is a constant (Table 3.1) that represents the asymptotic upper limit that would be reached as $t \to \infty$, and $k_f$ is the formation rate constant. All nonlinear fittings in Figure 3.1B have $r^2 \geq 0.995$. Although the precursor oxo-C8 product is unstable, its expected behavior follows the trend for the daughter oxo-C7 species.

Table 3.1. Exponential Growth Parameters for Products in Figure 3.1 and KIE in D$_2$O

<table>
<thead>
<tr>
<th>Product</th>
<th>$[\text{product}]_\infty$ (mM)</th>
<th>$k_f$ (s$^{-1}$)</th>
<th>KIE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3-Dimethyl tartaric acid</td>
<td>12.79</td>
<td>$1.18 \times 10^{-3}$</td>
<td>$5.61 \times 10^4$</td>
</tr>
<tr>
<td>Oxo-C7</td>
<td>21.35</td>
<td>$1.32 \times 10^{-3}$</td>
<td>$7.23 \times 10^2$</td>
</tr>
<tr>
<td>Oxo-C8</td>
<td>2.08</td>
<td>$2.22 \times 10^{-3}$</td>
<td>$1.70 \times 10^3$</td>
</tr>
</tbody>
</table>
3.4.2 Experiments with $^{13}$C Labeled PA and KIE in D$_2$O

Further confirmation of the structure of products was provided by photolyzing $^{13}$C labeled PA solutions. Figure 3.2A shows the total conductivity chromatograms and EIC for the major species observed in this experiment. As expected, the anion for $^{13}$C labeled PA is observed at $m/z$ 90.02 (Figure 3.2A), 3 amu heavier than for unlabeled PA. The –COOH group for one of the major products of the reaction appears at $m/z$ 182.08 (Figure 3.2). This seven carbon species is 7 amu heavier than the unlabeled oxo-C$_7$ product observed in Figure 3.1. The other major product in Figure 3.2A is observed as two peaks with $m/z$ 183.06, 6 amu higher than the proposed six carbon structure for DMTA. Finally, the intermediate oxo-C$_8$ product is observed at the predictable $m/z$ 227.08 (Figure 3.2), 8 amu heavier than the unlabeled product in Figure 3.1.

Figure 3.2. (A) IC total conductivity and ESI(-)/MS EIC of 97.88 mM $^{13}$C labeled PA photolyzed for (red) 0; (blue) 1; (pink) 2; (green) 3; (purple) 5; and (gray) 10 min. (B) Time series for (gray circle) PA loss at $m/z$ 90.02, and formation of (red star) the oxo-C$_7$ product at $m/z$ 182.08, (blue square), DMTA at $m/z$ 183.06, and (green triangle) the oxo-C$_8$ product at $m/z$ 227.08. All other conditions as presented for Figure 3.1.

Figure 3.2B shows the exponential decay of $^{13}$C labeled PA and exponential rise analogous to the unlabeled products in Figure 3.1B. When comparing the data in Figures
3.2B to 3.1B, an isotope effect is apparent from the lower [products] in the experiment with $^{13}$C labeled PA. Additional experiments (Figure 3.S3, Supporting Information) explored the effect of substituting H$_2$O by D$_2$O in experiments aimed at determining global KIE. The initial slopes in Figures 3.1B and Figure 3.S3 (Supporting Information) are used to calculate the KIE for product formation displayed in Table 3.1. The reported KIE are defined for first order processes by the ratio of initial formation rate constants $k_f$ for experiments in H$_2$O and D$_2$O at pH or pD 1.0 under the full photon rate:\textsuperscript{141}

\[ \text{KIE} = \frac{k_{f,H_2O}}{k_{f,D_2O}} \]  
(3.2)

The KIE in Equation 3.1.2 encompasses all the steps required to form the products, including bond-breaking and bond-making.\textsuperscript{141} From the viewpoint of pathways undergoing KIE, the reaction for the loss of PA must be considered as going through a transition state en route to produce the radicals K$^\prime$ and X$^\prime$. This transition state is stabilized differently by H$_2$O and D$_2$O leading to a primary KIE. A purely primary KIE $\sim$ 6.5 value for $^1$H/$^2$H should be expected at 298 K for homolysis generating two radicals,\textsuperscript{141} such as in the unimolecular Norrish type I cleavage of PA in the gas phase.\textsuperscript{59} However, the initial loss of PA showing a large KIE$_{PA} = 9.09$, supports a mechanism involving PCET, which is equivalent to H atom transfer.\textsuperscript{5} In addition, the large KIE$_{PA}$ disfavors the possibility of a nonlinear transition state as would occur for hydrogen abstraction from the methyl group of PA implied in other mechanisms.\textsuperscript{4, 7} This bimolecular process is energetically favorable due to energy released during the decarboxylation of X$^\cdot$.\textsuperscript{5} The large KIE$_{PA}$ should involve tunneling, i.e., as typical for acid-base reactions. In other
words, we propose the large KIE_{\text{PA}} observed is expected when the acid strengths for ground state PA and the triplet excited state are comparable. Perhaps, if the rate determining step would have proceeded through an activated complex with bent bonds, e.g., for a transition state displaying a considerable hybridization change to a \(C_{sp^2}\) centered radical for H atom abstraction from the terminal methyl group with a \(C_{sp^3}\), a smaller KIE should have been measured.\textsuperscript{141}

The dramatically large KIE values for the generation of DMTA and the oxo-C\textsubscript{7} product (Table 3.1) reveal tunneling also occurs for the conversion of radical intermediates and ground state PA into the final products. The penetration of the wave function of the precursor species through the barrier for the reaction occurs more commonly for molecules with H atoms than for the deuterated version.\textsuperscript{141} Tunneling is typically associated with proton transfer or H transfer or electron transfer, all processes that depend exponentially on the mass of the isotope, and thus decrease when transferring from H\textsubscript{2}O to D\textsubscript{2}O.\textsuperscript{141} Remarkably, the KIE for the generation of the oxo-C\textsubscript{8} product (Table 3.1) should also involve tunneling for the \(\beta\)-ketocarboxylic acid decarboxylation taking place with intramolecular H transfer. These KIE for products have larger activation energy differences between the compounds containing \(^1\text{H}\) and \(^2\text{H}\) than their differences in zero point energy.

3.4.3 HRMS

High resolution mass spectrometry provided confirmation of the molecular formulas that were proposed above. The full HRMS spectrum of a 100.0 mM pyruvic acid solution photolyzed to 20% conversion is shown in Figure 3.3. Both pyruvic acid (\(m/z\) 87.0083) and its hydrate (DHPA, \(m/z\) 105.0192) were observed by this method. Table 3.2 lists the
observed and theoretical \( m/z \) values for pyruvic acid and each of the products detected as well as the mass defect for the predicted formula in the anion identified [M-H]\(^{-}\).

![Figure 3.3. High resolution mass spectrum of 100.0 mM pyruvic acid photolyzed to 20% conversion with inset showing the oxo-C\(_8\) product (\( m/z \) 219.0596).](image)

**Table 3.2. High Resolution MS Analysis for 100.0 mM PA Photolyzed to 20 % Conversion**

<table>
<thead>
<tr>
<th>Species</th>
<th>Observed ( m/z )</th>
<th>Theoretical ( m/z )</th>
<th>Mass defect (ppm)</th>
<th>[M-H](^{-}) formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA</td>
<td>87.0083</td>
<td>87.0088</td>
<td>5.74</td>
<td>( C_3H_5O_3^- )</td>
</tr>
<tr>
<td>DHPA</td>
<td>105.0192</td>
<td>105.0193</td>
<td>0.95</td>
<td>( C_5H_8O_4^- )</td>
</tr>
<tr>
<td>oxo-C(_7) product</td>
<td>175.0605</td>
<td>175.0612</td>
<td>4.00</td>
<td>( C_7H_11O_5^- )</td>
</tr>
<tr>
<td>oxo-C(_8) product</td>
<td>219.0496</td>
<td>219.0510</td>
<td>6.39</td>
<td>( C_8H_11O_7^- )</td>
</tr>
<tr>
<td>DMTA</td>
<td>177.0399</td>
<td>177.0405</td>
<td>3.39</td>
<td>( C_6H_9O_6^- )</td>
</tr>
<tr>
<td>acetic acid</td>
<td>59.0132</td>
<td>59.0138</td>
<td>10.16</td>
<td>( C_3H_5O_3^- )</td>
</tr>
</tbody>
</table>
There is an excellent agreement between the $m/z$ detected and the theoretical values (Table 3.2). It was confirmed that acetoin at $m/z$ 87.0452 (easily distinguished from PA) and lactic acid ($m/z$ 89.0244) were absent in the spectrum of the photolysis products. The analysis of the fragmentation patterns by tandem mass spectrometry (MS/MS) for the species in Table 3.2 is presented in the Supporting Information (Figure 3.S4).

### 3.4.4 Two Dimensional NMR

Figure 3.4 shows the $^{13}$C gCOSY spectrum for the photolysis of 111.3 mM $^{13}$C labeled PA for a 50% conversion, due to the low sensitivity of the NMR experiment. Additional one dimensional $^{13}$C NMR spectra for a 20% conversion is used to guide the assignment of the major product peaks in the spectrum for 50% conversion. Thus, Figure 3.4 is used with confidence for characterizing the major photoproducts, despite any possible contribution of secondary products to the spectrum. The corresponding one dimensional $^{13}$C NMR spectrum for a similar sample is shown in Figure 3.S5 with the peak assignment in Table 3.S2 (Supporting Information).

In theory, the $^{13}$C COSY experiment can show cross-peaks between carbons that are 1 to 3 bonds apart in the molecule depending on the mixing time used. Here the experiment is optimized to observe carbon atoms directly adjacent to one another. As expected, the most intense cross-peaks in Figure 3.4 are from the unreacted PA, presenting $^{13}$C resonances for the carbonyl and hydrate forms at 201.00; 177.82; 167.39; 95.39; 28.40; and 27.64 ppm (see Figure 3.S5, Supporting Information). The new signals are apparent in the –C-O (40-80 ppm), –COOH (155-185 ppm), and C=O (185-220) regions, which is consistent with the functional groups present in the products depicted in Scheme 3.2.
Figure 3.4. 175 MHz $^{13}$C-$^{13}$C gCOSY NMR spectrum for an experiment with 111.3 mM $^{13}$C PA in D$_2$O at pH = 1.0 photolyzed at $\lambda \geq 305$ nm for a 50% reagent conversion under continuous sparging with air. Spectra were recorded during 72 h at 298 K on a Varian VNMRS-700 spectrometer equipped with a 5 mm $^1$H[$^{13}$C,$^{15}$N]f carbon enhanced cold probe at 18 K using a standard gCOSY sequence with 90° pulses and a $t_f$ delay of 20 ms. The $f_2$ dimension was acquired with 2048 points while the $f_1$ dimension recorded 512 increments. A total of 96 scans were recorded per FID of a sample prepared with DSS reference and gadolinium(III) chloride hexahydrate as described in Ref. 2

The carbon backbone of the major oxo-C$_7$ product in the mixture can be mapped out by the cross-peaks labeled in red in Figure 3.4. The most intense carbonyl cross-peak occurs at ~220 ppm and is therefore assigned to the oxo-C$_7$ product. Figure 3.4 shows that this carbonyl is coupled to a –CH$_3$ group at ~29 ppm and a –C–O carbon (~86 ppm). This –C–O carbon (~86 ppm) is in turn coupled to a different –CH$_3$ group at 23.18 ppm. Finally, a second –C–O carbon (~81 ppm) is coupled to both a third –CH$_3$ group at 23.06 ppm and an –COOH at ~180 ppm. A coupling between the –COOH carbon and the last –CH$_3$ carbon (23.04 ppm) is also observable in Figure 3.4. Assembling each of these carbon atoms by their couplings provides a structure consistent with the two halves of the
oxo-C7 product in Scheme 3.2, which is the most abundant photoprodut in the chromatographic analyses. It is worth noting that the –C–O signals could originate from ether or alcohol carbons. However, the lack of C–C coupling between the two –C–O groups (~86 ppm, and ~81 ppm) indicates that an oxygen linkage probably exists between the two –C–O carbons. In consequence, the two carbons are two bonds apart, which diminishes the likelihood of observing the coupling under these conditions. The weak (~ 2-3 Hz) coupling expected for the C-O-C(OH) cross-peak, would require a long delay $t_1$ between pulses to fully manifest; and would be expected to be much weaker than the others since a maximum of 20 ms (ideal for 1-bond couplings) was used for Figure 3.4. Using a long delay of $t_1 = 150$ ms necessary to observe a two bond coupling for the gCOSY experiment was impractical because all the signals of interest in the oxo-C7 product vanished. Rather than belonging to unique molecules, the absence of this coupling only indicates that both sets of peaks belong to different spin systems, which may exist in the same molecule, separated by a heteroatom as for the corresponding oxo-C7 product. Thus, given the intensity of the signals of the two spins systems described above and the fact that only two major photoproducts are detectable by chromatography (DMTA and oxo-C7), the cross peaks from these spin systems are assigned here to the two halves of the oxo-C7 product (Table 3.S1). Moreover, the integrals for the three –CH3 resonances at 29.3, 23.2, and 22.8 ppm in the 1D 13C NMR spectrum are equivalent, confirming that the cross-peaks from both spin systems in Figure 3.4 belong to a unique molecule. Additionally, the three unique carbons in DMTA can be observed in the one dimensional 13C NMR spectrum (Figure 3.S5, Supporting Information) to occur at chemical shifts of 179.14, 84.34, and 21.77 ppm. The
corresponding cross peaks are observable in the gCOSY spectrum of Figure 3.4 independent of those assigned to the oxo-C\textsubscript{7} product.

3.4.5 Analysis of Carbonyls Treated with DNPH

Additional confirmation of the structures of the oxo-C\textsubscript{7} product and dimethyltartaric acid is provided by UHPLC-MS analysis of reaction mixtures after derivatization with DNPH. The derivatization of mixtures with DNPH is the preferred analytical method for the separation and identification of carbonyls. Our previous work established that the mass spectrometer can easily detect the addition of 180.03 amu to the \textit{m/z} value of the underivatized carbonyl containing species.\textsuperscript{3, 70} This mass addition corresponds to C=O group condensation with a bulky DNPH molecule with loss of water. Figure 3.5A shows the EIC from UHPLC-MS analysis of the \textit{E} and \textit{Z} hydrazone isomers of PA at \textit{m/z} 267.04. The hydrazone for acetoin would appear at \textit{m/z} 267.07.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3_5.png}
\caption{UHPLC-MS from DNPH hydrazones of (A) PA (\textit{m/z} 267.04) and (B) oxo-C\textsubscript{7} product (\textit{m/z} 355.09) from an experiment with [PA] = 97.1 mM photolyzed during (red) 0, (blue) 1, (green) 3, (purple) 5, and (gray) 10 min. Inset shows no hydrazone for DMTA (\textit{m/z} 357.07). (C) Time series for (gray circle) PA loss and (red star) oxo-C\textsubscript{7} product formation from hydrazones in panels A and B. All other conditions as presented for Figure 3.1.}
\end{figure}
However, spike additions of acetoin to final concentrations of 10.0 µM before derivatization confirms this molecule is absent in the unspiked samples from photolysis. The EIC for \( m/z \) 355.09 in Figure 3.5B corresponds to the hydrazone of the oxo-C\(_7\) product, while the absence of any peak at \( m/z \) 357.07 (see inset) confirms that DMTA does not possess a derivatizable carbonyl group.

Recently, Griffith et al. reported that acetoin is a major product of the photolysis of aqueous PA, and proposed that its mechanism of formation involves the thermal decarboxylation of 2-acetolactic acid intermediate.\(^4\) This is in contrast with a previous study by Guzman et al., based on experiments performed under similar conditions, which reported the formation of two major products: 2,3-dimethyltartaric acid and 2-(3-oxobutan-2-yloxy)-2-hydroxypropanoic acid (oxo-C\(_7\)) which are also observed here.\(^5,21,69\) The oxo-C\(_7\) can thermally decompose into acetoin, but its carbonyl chromophore absorbs at \( \lambda_{\text{max}} \approx 285 \text{ nm} \) vs. 276 nm for acetoin.\(^5\) In fact, Guzman et al. had shown that the thermal decarboxylation of aqueous 2-acetolactic acid proceeds at significantly slower rates than postphotolysis \( \text{CO}_2(\text{g}) \) emissions, thereby excluding its participation in the mechanism of PA photolysis.\(^5,69\)

A realistic reaction mechanism based on multiple spectroscopic features, such as those detected by Griffith et al. should be able to identify intermediates and explain the reaction kinetics. Assigning spectroscopic features to specific carbonyls in complex mixtures is challenging at best and problematic in general. However, the chromatographic separation used here after derivatization with 2,4-dinitrophenylhydrazine and with mass spectrometric detection, allows for the unequivocal identification of acetoin in our reaction mixtures if it is present. Thus, Figure
3.6 shows the chromatogram of a mixture produced from 1 h photolysis of 50 mM PA at 4 °C (where 2-acetolactic acid should not decompose immediately if any is formed) followed by warming at 25 °C for 1 h, and subsequent derivatization with 2,4-dinitrophenylhydrazine.

**Figure 3.6.** Extracted ion (m/z = 267) chromatogram obtained by UHPLC/ESI(-)/MS (Hypersil GOLD column 1.9 μm, 50×2.1 mm) of unspiked sample (blue line), and 20 μM spiked acetoin for the same sample (red line) prior to derivatization. Retention times for the two hydrazones of PA and acetoin are 4.60, 6.40, and 5.80 min, respectively.

Under the optimized gradient for observing acetoin, the Z and E hydrazones of PA elute at 4.60 and 6.40 min and are seen in samples spiked with acetoin or unspiked. Acetoin is only observed at 5.80 min when it is spiked prior to derivatization ([acetoin]_final = 20 μM). The absence of acetoin in the unspiked sample warrants its yield, if produced, must be below 0.006 %.

The derivatization with DNPH performed on the photolyzed 13C labeled PA is presented in Figure 3.S6 (Supporting Information). With three heavy carbons, the E and Z hydrazones of 13C labeled PA are observed at m/z 270.05, while the hydrazone for acetoin (4 × 13C) should be observed at m/z 271.09. The 13C labeled PA hydrazones with an extra 13C from the natural abundance enrichment expected for DNPH should be observed at
m/z 271.05, which would be indistinguishable from acetoin. However, the percentage ratio of integrating the ion counts \( (I_{m/z}) \) at m/z 271.05 and 270.05 for these perfectly coeluting chromatographic peaks,

\[
R_{271/270} = 100 \times \frac{\int_{0.85 \text{ min}}^{4.28 \text{ min}} I_{271.05} \, dt}{\int_{0.85 \text{ min}}^{4.28 \text{ min}} I_{270.05} \, dt},
\]

in Figure 3.S6 (Supporting Information) yields an average of 1.54(± 0.01)% before and after 10 min of irradiation. Thus, any contribution to the peak with m/z 271.05 must be due to the natural isotopic abundance contribution of DNPH to the \(^{13}\text{C}\) labeled PA hydrazone, which is predicted to be 1.54%, matching \( R_{271/270} \). Finally, a trace production of acetaldehyde at m/z 225.05 (Figure 3.S6, Supporting Information) becomes evident during UHPLC-MS analysis of hydrazones from \(^{13}\text{C}\) labeled PA. Because acetaldehyde is a common ambient air pollutant,\(^{145,146}\) the solution with DNPH can easily scavenge \(^{12}\text{C}\) acetaldehyde forming its hydrazone. Thus, the experiment detecting its \(^{13}\text{C}\) isotopologue during the photolysis of \(^{13}\text{C}\) PA provides additional support for the photochemical production of acetaldehyde. The presence of a trace of acetaldehyde confirms \( Y^* \) is an intermediate radical in the mechanism.

Considering a photochemical mechanism for conversion below 30% in Figure 3.1, the average concentration ratio \([\text{o xo-C}_7]/[\text{o xo-C}_8] \approx 7.4\) can be rationalized to arise from the decarboxylation of the unstable \( \beta \)-ketocarboxylic acid \( \text{o xo-C}_8 \) intermediate into the \( \text{o xo-C}_7 \) product. Furthermore, the derivatization conditions favor the decarboxylative loss of any remaining \( \text{o xo-C}_8 \) product. Figure 3.5C shows how the hydrazones of PA and the \( \text{o xo-C}_7 \) product decay and grow, respectively, in general agreement with Figure 3.1B.

Although acetoin was proposed as a major photoproduct in one of the first studies of PA direct photolysis in water,\(^63\) it was later explained that, under high temperature GC
conditions, the oxo-C₇ and oxo-C₈ products decompose into acetoin. Additionally, we have established an upper limit for the yield of acetoin photoproduction, if any could be detected, to be below 0.006% or at the trace level. Despite the chromatographic results in Figures 3.5 and 3.6 that verify a lack of acetoin in the photoproduct mixture, it is critical to further explore this issue using the gas phase FTIR and liquid phase NMR based techniques used to support its presence. Therefore, the next sections demonstrate spectroscopic evidence against the presence of acetoin in the headspace over the reaction and in the aqueous product mixture. We also use the method of standard addition to accurately reassign the spectroscopic features in the ¹H NMR spectrum of the photolysis mixture inaccurately attributed to acetoin.

3.4.6 FTIR analysis of the headspace over the reaction

Inspection of the inset of Figure 1 from Griffith et al. makes it immediately apparent that the bands assigned to the trace species acetoin (overshadowed by water absorptions) do not match the spectrum of acetoin at multiple wavenumbers. Following a similar protocol to Griffith et al., we obtained in Figure 3.7 a better defined spectrum for the unknown species that partitions into the gas phase (gray line), acetoin (blue line), and for a spike addition of acetoin to the product (red line). The spectrum of acetoin with a peak at 1125 cm⁻¹ does not match the spectrum of the photolysis product with a peak at 1091 cm⁻¹, as is appreciated in the sample spiked with acetoin that causes the shoulder at 1125 cm⁻¹. Therefore, we interpret Griffith et al.’s results in fact confirm that most of PA is converted to the products 2,3-dimethyltartaric acid (m/z = 177) and oxo-C₇ (m/z = 175), as displayed in their Figure S3, which, in addition, lacks signals attributable to detectable amounts of lactate (m/z = 89) in the postphotolysis mixture.
Figure 3.7. Infrared spectrum of (gray line) collected gas over 50 mM aqueous PA after photolysis ($\lambda > 305$ nm) for 1 h, (blue line) acetoin, and (red line) gas product spiked with acetoin.

3.4.7 Qualitative and Quantitative $^1$H NMR Analysis

In order to quantify each product by $^1$H NMR, the entire spectrum must be first assigned. While the chromatographic analysis indicates that only three products largely dominate the composition of the reaction mixture, the $^1$H NMR spectrum is much more complex due to the presence of diastereomers for each product, making the assignment of all resonances difficult. In order to facilitate the assignment of the spectrum, a photolysis with more concentrated reagent was performed for air saturated PA solution of initial concentration $[\text{PA}]_0 = 322$ mM at pH = 1 up to a 20.3% conversion. The assignment of this 600 MHz $^1$H NMR spectrum is available in Table 3.S3.

Figure 3.8 shows $^1$H NMR spectra for an experiment with a solution of 102.4 mM PA before irradiation (panel A), after photolysis for a 15% conversion (panel B), the same postphotolysis sample in panel B after spiking to $[\text{acetoin}]_{\text{final}} = 4.06$ mM (panel C). Figure 3.8D presents a closeup view from the regions where acetoin signals should be observed.
Figure 3.8. 400 MHz $^1$H NMR spectra of 102.4 mM PA solution (A) before photolysis, (B) for 15.5% conversion at $\lambda \geq 305$ nm, and (C) the spectrum in B spiked to $[\text{acetoin}]_{\text{final}} = 4.06$ mM. (D) Close up of spectra in panels A, B and C. (E) Time series of (gray circle) PA loss, (red star) oxo-C$_7$ product, (blue square) DMTA, (green triangle) oxo-C$_8$ product, and (gold diamond) acetic acid. All other conditions as presented for Figure 3.1.

An acetoin standard in D$_2$O referenced to DSS displays three signals: $\delta$ 1.37 (d, $J = 6.9$ Hz, 3 H), 2.21 (s, 3 H), 4.42 (q, 1 H). Figure 3.8D clearly shows that while $\delta$ 2.21 (s) matches quite well with a photoproduct peak, the doublet centered at $\delta$ 1.37 (d) does not, implying that different species are the main contributors to both associated signals under consideration. This is the reason for E&G to have used multiple analytical methods and measured the abundance of different products over time by qNMR.$^2$ Thus, the $^1$H NMR peaks assigned to acetoin by V&C in Reed Harris et al.$^7$ must have actually corresponded to another product.$^2$

Hypothetically, the maximum concentration of acetoin would be constrained by the area from the singlet at $\delta$ 2.21, which yields 238 $\mu$M for 15.4% PA conversion. Given the much lower threshold for the concentration of acetoin set chromatographically,$^{70}$ it is unlikely that the small peak at $\delta$ 2.21 could be due to acetoin. Instead, the resonance at
2.21 ppm can be explained by a methyl group neighboring the C=O in the oxo-C₈ product (see structure in Scheme 3.2 with the -CH₃ group in pink font).

Computational predictions of the ¹H NMR spectrum for the oxo-C₈ product place the analogous –CH₃ singlet at δ 2.16 near the signal of interest. The very intense singlet for the –CH₃ group bound to the C=O of the abundant oxo-C₇ product is found at δ 2.31, which cannot be confused with the intermediate species observed in the spectrum at δ 2.21. Additionally, we must note that the oxo-C₈ and oxo-C₇ products correctly account for the other ¹H NMR peaks at ~δ 4.40 (q) previously assigned to acetoin and lactic acid.⁴ The two oxo products also explain the features in the infrared spectrum of the photolysis products that partitioned to the gas phase (see Table 3.S1),⁷⁰ which were mistakenly assigned to acetoin.⁴ An interesting outcome from the spike addition of acetoin, purposely designed to increase the area under δ 2.21 (s) of the photolyzed samples between 1 and 3 times, is that it can be used to quantify the perfectly overlapping oxo-C₈ photoproduct by qNMR. The quantification of all species by qNMR (Figure 3.8E), including the low production of the oxo-C₈ intermediate, agrees well with the results from IC-MS in Figure 3.1B.

The ¹H NMR study is also capable of evaluating whether acetic acid is a minor photoproduct or not, as proposed elsewhere.⁴,⁷ This analysis has direct implications for assessing if lactic acid is present and at a relevant concentration in the photolyzed mixture. While lactic and acetic acids have been proposed to be produced by a reaction channel in a 1:1 ratio, a second source of acetic acid has been postulated in the presence of dissolved O₂.⁷ Thus, the quantified concentration of acetic acid in Figure 3.8E should serve as an upper limit for the concentration of lactic acid, if any is produced, making the
quantification of lactic acid of lesser importance. Noticeably, a small amount of acetic acid is observed while the [PA] drops. A simple comparison at 3 min of irradiation, corresponding to a 19.8% conversion, indicates that the scarce oxo-C₈ photoproduct is 6.2 times more abundant than acetic acid. In addition, lactic acid was spiked in photolyzed samples for qualitative purposes (Figure 3.S7, Supporting Information). While the chemical shift of peaks in the –CH₃ region (δ ~ 1.40 to 1.44) of the photolyzed mixture do not overlap well with the signal in lactic acid, the area for the photoproducts is also considerably larger than acceptable for the stoichiometric constraint for lactic acid discussed above. The previous observation is consistent with the lack of lactic acid peaks during IC-conductivity-MS analysis.

### 3.4.8 Reaction Mechanism with Formation of the Oxo-C₈ Intermediate

The data presented above showing an oxo-C₈ photoproduct for the first time provides strong support for the mechanism in Scheme 3.2. Briefly, the photochemical process begins with a production of triplet excited state PA* in overall reaction R1a + R1b (Scheme 3.2), which could be thermally lost via reaction R2. PA* undergoes proton-coupled electron transfer (PCET) with a ground state molecule in concerted reaction R3 to yield the ketyl radical K* and an acyloxy radical X*. The rapid decomposition of X* releases CO₂(g) and the acetyl radical Y* in a few picoseconds via reaction R4a, 5, 68, 69 which gets hydrated via reaction R4b with a rate constant $k_{4b} = 2 \times 10^4$ s⁻¹. 147 The primary decarboxylation reaction that generates Y* from an acyloxy radical (Scheme 3.2 of E&G)² propels the process in an irreversible direction. 68 In the presence of dissolved O₂, the strongly reducing Y* radical 147 generates acetic acid and another equivalent of HO₂⁻ in the sequence of reactions R5a, R5b with an overall rate constant $k_5 = 7.7 \times 10^8$
Alternatively $Y^\cdot$ can reduce the abundant ground state PA stoichiometrically producing $K^\cdot$ and acetic acid. The only way to form new C-C or C-O bonds to achieve products of higher molecular weight is by recombination of the prevalent photogenerated radicals from different solvent cages (otherwise the original molecules would be regenerated). The recombination of two $K^\cdot$ radicals in reaction R6 forming DMTA, a major product, proceeds with a rate constant $k_6 \approx 2 \times 10^9 \text{M}^{-1} \text{s}^{-1}$. Reaction R7a also competes for the fate of $K^\cdot$ radicals by generating a peroxy radical $\text{KO}_2^\cdot$ in the presence of dissolved $\text{O}_2$, which rate constant is unknown at pH 1 but has been reported at pH 7: $k_{7a} = 2.6 \times 10^9 \text{M}^{-1} \text{s}^{-1}$. The radical $\text{KO}_2^\cdot$ participates in a null cycle by decomposing back into PA and hydroperoxyl radical, $\text{HO}_2^\cdot$ in reaction R7b.

The high reactivity of the radicals photogenerated tends to deplete $\text{O}_2(\text{aq})$ from the solution quickly. However, up to a 90% regeneration of PA was observed via reaction R7 that traps $K^\cdot$ in the presence of $[\text{O}_2(\text{aq})] = 32 \mu\text{M}$ from continuous air sparging (as monitored before for glyoxylic acid) compared to anoxic conditions. While the recycling of $K^\cdot$ proceeds, $Y^\cdot$ becomes more available for the generation of acetic acid. Similarly, the respective generation of DMTA, the oxo-C$_7$ product, and the oxo-C$_8$ product still proceeds in the presence of air, although slower by 37, 285, and 94 times than under anoxic conditions. Based on Scheme 3.2, the initial photolytic loss rate $R_0 = 1.46 \times 10^{-5} \text{M s}^{-1}$ for 100 mM PA (Figure 3.S9, Supporting Information) produces stoichiometric amounts of $K^\cdot$ and $Y^\cdot$ radicals. Under nonzero $[\text{O}_2(\text{aq})]$ conditions, the measured initial production rates, can be used to estimate $[K^\cdot] = \sqrt{(\text{rate } R_0 - \text{rate } R_8 - \text{rate } R_9)/k_6} = 2.36 \times 10^{-8} \text{M}$. The previous value needs to be multiplied by 9.42 (see the regeneration of PA indicated above under nonzero $[\text{O}_2(\text{aq})]$ conditions) to estimate the total steady state
[K]\text{ss} = 1.71 \times 10^{-7} \text{ M}. 

The competitive pathway for trapping K• in the presence of [O_2(aq)] = 130 \mu\text{M} proceeds with an 80 times drop in oxo-C_7 production. Therefore, the ratio of reaction rates from oxygen scavenging of K• with rate constant k_7a to the production of the oxo-C_7 product must be (d[KO_2•]/dt)/(d[oxo-C_7 product]/dt) = k_7a [K•]_{ss,O_2} [O_2(aq)]/(k_6 [K•]_{ss,O_2}^2) = 79. Thus, by solving for k_7a in equation 3.1.3:

$$k_7a = \frac{79k_6 [K•]_{ss,O_2}}{[O_2(aq)]}$$  \hspace{1cm} (3.3)

a rough estimate of $k_7a = 1.22 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ is obtained at pH 1. Therefore, the advanced quantification methods presented here allow to estimate a reasonable value as compared for similar radicals that scavenge O_2(aq) with a rate constant $k_{sc} \sim 1 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$.\textsuperscript{31} Future efforts should experimentally determine $k_7a$.

The strong oxidant HO_2• from reactions such as R7b can self-disproportionate to form H_2O_2 and O_2 in the absence of transition metal ions. The produced H_2O_2 can be photolyzed at $\lambda \leq 365 \text{ nm}$,\textsuperscript{149} providing a potential source of HO•. Acetic acid could can also result from the reaction of PA + HO• with second order rate constant $k_{PA + HO•} = 3.2 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$, via an alkoxy radical intermediate.\textsuperscript{31} In addition, the reaction of PA with HO• has been proposed to generate acetic, formic, glyoxylic, and oxalic acids.\textsuperscript{38, 41} However, only acetic acid is observed herein, indicating that oxidation by HO• generated in situ is not a competitive pathway during direct photolysis. Ultimately, the fate of any produced HO_2• would be to undergo decomposition into O_2 and H_2O.\textsuperscript{149} A reaction of increasing importance at higher PA concentrations is the addition of K• to the C=O of a neutral PA molecule (reaction R8a), forming the radical C•. Finally, the radicals C• and Y•
must then combine to form the oxo-C$_8$ photoproduct via reaction R8b, which undergoes a typical $\beta$-ketocarboxylic acid decarboxylation with rate constant $k_9 = 1.21 \times 10^{-3}$ s$^{-1}$ into the oxo-C$_7$ product in reaction R9. The formation of the oxo-C$_7$ with a seemingly unstable hemiketal structure is made possible because the transition state of the oxo-C$_8$ product favors the fast $\beta$-ketocarboxylic acid decarboxylation$^{69}$ that generates oxo-C$_7$ prestabilized by intramolecular hydrogen bonds.$^{150, 151}$ Previously proposed pathways to acetoin and lactic acid production$^4, 7$ are not included in this scheme because of the demonstrated lack of evidence for their formation.

Different recombination reactions among the produced radicals have been considered,$^2, 5, 68, 69$ including the recombination of Y$^\cdot$ (and its hydrate) and K$^\cdot$. However, the reaction Y$^\cdot$ + K$^\cdot$ was experimentally discarded because $\alpha$-acetolactic acid standard 1) was confirmed to be absent in the mixture of photoproducts, and 2) remained stable while important evolution of CO$_2$ occurred during the postphotolysis period in the dark below 4 $^\circ$C.$^2, 5, 69$ The key radicals (K$^\cdot$ and Y$^\cdot$) participate in the same mechanism in water and ice,$^2$ which proceeds to form DMTA and oxo-C$_7$ product.$^5, 68, 152$ The generation of the oxo-C$_8$ product from K$^\cdot$ and PA, forming a radical C$^\cdot$ that combines with Y$^\cdot$ as shown in Scheme 3,$^2, 5, 68, 69$ is an important reaction overlooked by proponents of the acetoin pathway.

Instead, the acetoin mechanism also proposes an alternative product with a molecular weight of 176 amu (which was previously ignored$^4, 7, 153$) as 2,4-dihydroxy-2-methyl-5-oxohexanoic acid (DMOHA) without experimental confirmation.$^{72}$ DMOHA is a simple C-C bridge isomer of the oxo-C$_7$ product proposed by Guzman et al. and in this work.$^2, 5, 69$ Assigning the structure of DMOHA to the species with formula C$_7$H$_{12}$O$_5$ is
problematic due to its stability at high temperature GC conditions. Instead, the molecule of C\textsubscript{7}H\textsubscript{12}O\textsubscript{5} is fragile and known to decompose into acetoin in agreement with the structure for the oxo-C\textsubscript{7} product.\textsuperscript{5} Moreover, the formation mechanism of DMOHA from V&C utilizes the same \( \beta \)-ketocarboxylic acid decarboxylation proposed here,\textsuperscript{2} except that the oxo-C\textsubscript{8} precursor is generated from H atom abstraction from parapyruvic acid impurity,\textsuperscript{72} which should not participate in the formation of such an abundant PA photoproduc. We have verified parapyruvic acid is absent before (see Figure 3.S1) and after the photoreaction when starting with freshly distilled PA.\textsuperscript{2} For comparison, Figure 3.9 shows an IC-conductivity chromatogram of impure PA which illustrates the importance of distilling the reagent freshly before using it for photochemical studies, something that was not done for many of the earlier studies of PA photolysis, particularly those that indicate acetoin as a product. Figure 3.9A shows that during dark storage (>3 months) of distilled pyruvic acid in the refrigerator, the production of four impurities (lactic, acetic, parapyruvic, and zymonic acids) proceeds, requiring repeated purification.\textsuperscript{2} Lactic, parapyruvic, and zymonic acids have not been observed as photoproducts when working with freshly distilled PA.\textsuperscript{2} However, some researchers still insist that lactic acid and acetoin are minor photoproducts in the absence of dissolved O\textsubscript{2} based on a series of 2D NMR measurements,\textsuperscript{4,134,154} and assert that “... if sufficient oxygen is present during photolysis, the formation of acetoin and lactic acid is severely inhibited,”\textsuperscript{154} implying that more acetoin and lactic acid would be observed in the absence of O\textsubscript{2}(aq). Therefore, Figures 3.9B and 3.9C show the \(^1\)H NMR spectrum for a photolysis control performed under 1 atm N\textsubscript{2}(g) for the region corresponding to the –CH\textsubscript{3} doublets of lactic acid (~1.42 ppm in Figure 3.9B) and acetoin (~1.36 ppm in Figure 3.9C). Figures 3.9B and 3.9C,
respectively agree with those for the experiment in air displayed in Figures 3.8D and 3.S7.\textsuperscript{2} Indeed, the spike addition of (red line in Figure 3.9B) lactic acid and (pink line in Figure 3.9C) acetoin to the (blue line in Figures 3.9B and 3.9C) photoproducts show the mismatch in the chemical shift position, line shape (appearance of new peaks or shoulders), and intensity ratio for the –CH\textsubscript{3} doublets after spiking to [DL-lactic acid]\textsubscript{final} = 9.34 mM and [acetoin]\textsubscript{final} = 10.02 mM, respectively. For comparison, the corresponding spectral regions for D,L-lactic acid and acetoin standards are shown in Figures 3.9D and 3.7E, respectively. The indistinguishable chemical shifts for 1) the singlet –CH\textsubscript{3} peaks at \approx 2.21 ppm and 2) the weak quartet –CH peaks at \approx 4.40 ppm in both the oxo-C\textsubscript{8} product and acetoin, justified choosing the doublet centered at \approx 1.36 ppm for the comparison provided in Figures 3.9.\textsuperscript{2} Thus, rather than assigning the peaks in question to lactic acid or acetoin, these –CH\textsubscript{3} resonances and those at \approx 2.31 and \approx 2.21 ppm (Figure 3.8 and Table 3.S3)\textsuperscript{2} should be assigned to the oxo-C\textsubscript{7} and oxo-C\textsubscript{8} products, and not to any other species. Figure 3.S10 provides IC analyses supporting the above interpretation of Figure 3.9B.

Furthermore, the –CH\textsubscript{2}– group participating in the C-C bridge in DMOHA proposed by V&C in Rapf et al.,\textsuperscript{72} can be reasonably predicted to appear at \approx 44 ppm in the \textsuperscript{13}C NMR spectrum in water.\textsuperscript{155} However, neither a resonance is observed in the \textsuperscript{13}C NMR spectrum in Figure 3.S5 from at 44 (\pm 10) ppm nor a cross-peak involving such a –CH\textsubscript{2}– in Figure 3.4, justifying the need for an alternative C-O-C bridge in the structure of the oxo-C\textsubscript{7} product. Finally, in agreement with previous findings,\textsuperscript{5} the large kinetic isotope effect KIE\textsubscript{PA} = 9.09 measured in H\textsubscript{2}O relative to D\textsubscript{2}O for the initial loss of PA discarded
the possibility of a nonlinear transition state\textsuperscript{2} as would have occurred for H atom abstraction from the –CH\textsubscript{3} group of PA implied by V&C in their mechanism.\textsuperscript{4, 7, 72}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3_9}
\caption{(A) Ion chromatogram for pyruvic acid (PA, \textit{m/z} 87) stored in the refrigerator for > 3 months after it had been distilled showing its alteration by the development of impurities: lactic (\textit{m/z} 89), acetic (\textit{m/z} 59), parapyruvic (\textit{m/z} 175), and zymonic acids (\textit{m/z} 157), see figure 3.S1 for structures. Bicarbonate (\textit{m/z} 61) and bisulfate (\textit{m/z} 97) are also labelled. All anions were assigned with available standards and/or using the \textit{m/z} values (given in parentheses) obtained by mass spectrometry. These results supplement those from Ref. 2 identifying acetic, parapyruvic acid and zymonic acid impurities and show that only freshly distilled PA can be used as a reagent for photochemistry studies. (B) \textsuperscript{1}H NMR spectrum for (blue) the –CH\textsubscript{3} group doublet centered at \textasciitilde1.42 ppm in a control with 100.1 mM PA at pH 1.0 photolyzed at \(\lambda \geq 305\) nm for a 50\% reagent conversion under continuous sparging with N\textsubscript{2}(g), and (red) the mismatch for this doublet after spiking to [DL-lactic acid]\textsubscript{final} = 9.34 mM. (C) \textsuperscript{1}H NMR spectrum for (blue) the –CH\textsubscript{3} group doublet centered at \textasciitilde1.36 ppm in the same control in panel B, and (red) the mismatch for this doublet after spiking to [acetoin]\textsubscript{final} = 10.02 mM. The corresponding spectral regions for (D) D,L-lactic acid and (E) acetoin standards are shown for comparison of the position, line shape, and intensity ratio for the peaks in these –CH\textsubscript{3} doublets. \textsuperscript{1}H NMR spectra were recorded on a 400 MHz Varian Inova spectrometer using a WET 1D water suppression pulse sequence. Samples were prepared to contain 10\% v/v D\textsubscript{2}O with 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) reference and gadolinium(III) chloride hexahydrate as described in Ref. 2.}
\end{figure}
3.4.9 Evaluation of the Reaction Mechanism from Measured Quantum Yields

The steady state concentration of excited state pyruvic acid, \([\text{PA}^*]_{ss}\), can be inferred by balancing out its measured initial rate of production with an upper limit set by the measured loss of PA, \(k_{hv}[\text{PA}]_0\), against the loss of \(\text{PA}^*\) by thermal processes in reaction R2 and from its bimolecular reaction with ground state PA by reaction R3 (Scheme 3.2). For practical purposes, there is no difference if the (indistinguishable) photoinduced bimolecular process represents a hydrogen atom transfer, an electron transfer, or PCET. Thus, from \(k_{hv} [\text{PA}]_0 = k_2 [\text{PA}^*] + k_3 [\text{PA}^*][\text{PA}]_0\), it is possible to obtain at variable \([\text{PA}]_0\):

\[
[\text{PA}^*]_{ss} = \frac{k_{hv} [\text{PA}]_0}{k_2 + k_3 [\text{PA}]_0}
\]  

(3.4)

Figure 3.S8 (Supporting Information) shows that the photolytic rate in equation 3.1.4, \(k_{hv} [\text{PA}]_0\), depends linearly on the photon absorption by aqueous PA solution \((I_a)\) with a slope of \(~2\). Indeed, Figure 3.S8 indicates that \(\Phi_{PA} \approx 2\) or that each \(\text{PA}^*\) produced per photon absorbed consumes an additional molecule of PA in a highly efficient bimolecular process. The \(\Phi_{PA}\) should not be confused with the photodecarboxylation quantum yield of aqueous PA, \(\Phi_{-\text{CO}_2} = 0.78\). While \(\Phi_{-\text{CO}_2}\) accounts for a \(~50\%) of the evolution of \(\text{CO}_2(\text{g})\) from the primary decomposition of \(X^*\) per photon absorbed, the secondary decarboxylation of the oxo-C8 product must contribute the other \(~28\%) of this gas. Thus, the missing \(~22\%) of produced \(K^*\) radical must recombine to produce DMTA, which does not emit \(\text{CO}_2(\text{g})\). For experiments with \([\text{PA}]_0 \geq 4 \text{ mM}, I_a\) is given by:\(^5\)
\[ I_a = I_0 (1 - e^{-2.303 \varepsilon l [PA]_0}) \]  

\( I_a \) only varies with \([PA]_0\), because the incident photon rate, \( I_0 = 1.14 \times 10^{-5} \) \( \text{M s}^{-1} \), the molar absorptivity of \( \text{PA} \) (\( \varepsilon = 11.3 \) \( \text{M}^{-1} \text{cm}^{-1} \)), and the optical path length of the NMR tube (\( l = 0.424 \) \( \text{cm} \)) are constants. Therefore, by substituting \( k_{hv}[PA]_0 \approx 2 I_a \) in equation 3.1.3:

\[ [PA^*]_{ss} = \frac{2I_a}{k_2 + k_3[PA]_0} \]  

Assuming this simplified reaction scheme, for each photolyzed molecule of \( \text{PA} \) generating the stoichiometric amount of ketyl and acetyl radicals, \( \text{PA} + h\nu \rightarrow \text{K}^* + \text{Y}^* \), provides a means to solve the steady state concentration of ketyl radicals, \([\text{K}^*]_{ss}\) in the system. Thus, balancing out the measured initial rate of \( \text{PA} \) loss to the initial rate of generation of product consuming \( \text{K}^* \) (observed from the channels R6 + R8 + R9 in Scheme 3.2), indicates that:

\[ \sum \text{Rate of product formation} = k_3[PA^*][PA]_0 \]  

The initial rate of generation of product and loss of \( \text{PA} \) are presented in Figure 3.S9 (Supporting Information) for \( a \leq 20\% \) conversion. By substituting \([PA^*]\) from equation 3.1.6 in equation 3.1.7, and reordering, it is apparent that:
Thus, the sum of the corresponding photochemical quantum yields for the oxo-C₇ product \( \Phi_{\text{oxo-C}_7} \), 2,3-dimethyltartaric acid \( \Phi_{\text{DMTA}} \), and the oxo-C₈ product \( \Phi_{\text{oxo-C}_8} \), \( \sum \Phi_{\text{product}} \), depends on \([\text{PA}]_0\) as described by the hyperbola in equation 3.1.8. The \( \sum \Phi_{\text{product}} \) is calculated from the initial formation rates divided by the absorbed photon rate from equation 3.5. Figure 3.10 shows the sum of the quantum yields for the three products \( \sum \Phi_{\text{product}} \) vs \([\text{PA}]_0\) for the interval 5-100 mM measured using neutral density filters that reduce the total photon flux to 10.64%.

\[
\sum \Phi_{\text{product}} = \frac{\sum \text{Rate of product formation}}{I_a} = \frac{2[\text{PA}]_0}{k_2k_3^{-1}+[\text{PA}]_0} \quad (3.8)
\]

**Figure 3.10.** Effect of initial concentration of pyruvic acid ([PA]₀) on normalized sum of the quantum yields for the oxo-C₇ product, DMTA, and the oxo-C₈ product \( \sum \Phi_{\text{product}} \) during the direct photolysis of aqueous solutions at pH = 1 and 298 K.

The general behavior of \( \sum \Phi_{\text{product}} \) vs \([\text{PA}]_0\) in Figure 3.10 is fitted with the hyperbola \( \sum \Phi_{\text{product}} = 1.99 [\text{PA}]_0/(113.2 + [\text{PA}]_0) \) with \( r^2 = 0.952 \) and concentration in mM units. The branching ratio for the production of the oxo-C₇ + oxo-C₈ products...
grows faster than for DMTA for increasing $[\text{PA}]_0$ (Figure 3.9, Supporting Information).

The previous observation is expected for the given competition between the production rate of the oxo-C$_8$ and oxo-C$_7$ products involving radical addition of K$^\cdot$ to a molecule of PA versus the recombination of two K$^\cdot$ radicals forming DMTA.

3.5 Conclusions

The processing of organic species in aqueous secondary organic aerosols is an important matter.$^{32, 75}$ The photolysis rate in the aqueous phase ($j_{\text{aq}}$) can be estimated from equation 3.1.9:

$$j_{\text{aq}} = \int F_A(\lambda) \Phi(\lambda) \sigma(\lambda) \, d\lambda$$

(3.9)

using the measured $\Phi_{-\text{PA}} \approx 2$, the variable actinic flux $F_A(\lambda)$ at the surface for a solar zenith angle (SZA) of 40° and a surface albedo ($\alpha$) of 30%,$^{114}$ and the cross section of PA $\sigma(\lambda)$ extracted from a 100 mM solution. For the environmental conditions defined, the reciprocal of the estimated $j_{\text{aq}} = 7.7 \times 10^{-4}$ s$^{-1}$ can be used to report the lifetime of aqueous PA against loss by photolysis, $\tau_{\text{aq,hv}} \approx 21.7$ min. The aqueous phase loss of PA against expected urban cloud droplets$^{115}$ with $[\text{HO}^-(\text{aq})] = 1 \times 10^{-13}$ to $1 \times 10^{-14}$ M proceeds with a rate constant $k_{\text{aq,PA+HO}} = 1.2 \times 10^8$ M$^{-1}$ s$^{-1},$ with a comparatively long lifetime $\tau_{\text{aq,PA+OH}} \approx 0.96$ to 9.6 days, respectively. Instead, the gas phase photolysis of PA proceeds only 1.9 times faster than in water, as integrated for a unity quantum yield under the same SZA, $\alpha$, and altitude defined above.$^{157}$ However, the reactivity in the gas phase should be of lesser importance because PA partitions quickly to the particle phase.
coexisting in hydration equilibrium with 2,2-dihydroxyproanoic acid.\textsuperscript{21} Combining the rate constant for the gas phase reaction PA + •OH, a \( k_{\text{gas,PA+OH}} = 1.2 \times 10^{-13} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1} \), \textsuperscript{158} with \([\text{HO}^\cdot(\text{g})]_{\text{daytime}} 1.6 \times 10^6 \text{ molecules cm}^{-3}\), yields such a long lifetime, \( \tau_{\text{gas,PA+OH}} \approx 2.0 \text{ months} \), that the importance of this process can be disregarded.

This work has advanced previous efforts by quantifying the generation of 2,3-dimethyltartraric acid, acetic acid, the oxo-C\(_7\) and oxo-C\(_8\) products combining the use of separations by UHPLC and IC coupled to MS, HRMS, the assignment of one dimensional (1D) \(^1\text{H}\) and \(^{13}\text{C}\) NMR and two dimensional (2D) \(^{13}\text{C}\) gCOSY spectroscopic features, as well as by employing quantitative \(^1\text{H}\) NMR (qNMR). The chromatographic separations were performed both with reversed-phase C18 and alkanol quaternary ammonium columns with high specificity to separate monocarboxylic, dicarboxylic, and oxocarboxylic acids prior to UV-visible, conductivity, and MS detection.\textsuperscript{159, 160} In addition, these analyses included the separation, identification, and quantification of carbonyls (C=O) in the mixture of photoproducts treated with 2,4-dinitrophenylhydrazine (DNPH).\textsuperscript{2, 3, 70} The undeniable power of these techniques for the analysis of this class of products,\textsuperscript{2, 70} with low limits of detection (LOD) also makes them excellent for the determination of lactic acid (LOD = 86 nM) and acetoin (LOD = 330 nM)\textsuperscript{2} after chromatographic separation, as exemplified by applications for food industry and clinical settings.\textsuperscript{161-164} This combination of analytical methods supports a photochemical mechanism where PA, K•, and Y• radicals play an important role in water, generating products with large O:C ratios. Particularly, the chemical identification of the oxo-C\(_8\) product proves a radical-radical termination reaction involves the participation of Y•. Additional support for a mechanism with the participation of Y• is provided by the small
production of acetic acid in the presence of dissolved O$_2$(g) and the detection of trace levels of acetaldehyde. In addition, this work demonstrates the importance of quality assurance when characterizing a complex reaction mixture to prevent mischaracterizing spectroscopic features for species such as acetoin and lactic acid. The work also serves as a model study recommending to others that: 1) primary photoproducts should be preferably identified for maximum reactant conversions of ~20-30%, and 2) using more than one method of analysis is a preferred experimental practice.

The high reactivity of the photogenerated radicals tends to deplete O$_2$(aq) from the solution quickly. However, in the presence of dissolved O$_2$, the loss of PA proceeds slower than under anoxic conditions, suggesting the importance of the null cycle via reaction R7 to trap K$^\cdot$ and regenerate the reactant, while Y$^\cdot$ becomes more available for the generation of acetic acid. Finally, simple $\alpha$-ketocarboxylic acids such as PA and glyoxylic acid are a potential source of complex SOA generation with common radical intermediates, which are produced by dissimilar photochemical initiation steps. Overall, the inclusion of direct photolysis for this class of compounds in atmospheric models should reduce the uncertainty associated with SOA sources in current models.

3.6 Acknowledgements

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3.7 Supporting Information

3.7.1 Reactivity of Ketyl and Acetyl Radicals from Direct Solar Actinic Photolysis of Aqueous Pyruvic Acid

Additional Experimental Section:

**Fourier transformed infrared (FTIR) Spectroscopy.** Gas phase products were analyzed by performing the photolysis in a sealed 200 mL quartz reactor. Aliquots of the headspace above the reaction mixture were withdrawn through a septum using a syringe with a stainless steel needle and injected into a 2.4 m path length infrared gas cell with ZnSe windows (PIKE) and a capacity of 100 cm³. The cell was mounted in an iZ10 FTIR module connected to an infrared microscope (Thermo Scientific Nicolet iN10). Absorption spectra (and the background) were recorded at 1 cm⁻¹ resolution after allowing the cell to equilibrate for 30 min and purging the optics continuously with N₂(g). In addition, photoproducts were spiked with acetoin vapor for qualitative analysis.

**UHPLC Analysis of Nonderivatized Samples.** Samples were analyzed with the same Accela 1250 LC-MS described in the main experimental section after diluting them 100 times in ultrapure water. Separation used solvents A (methanol) and B (water) both with 0.1 mM formic acid. Initially a 5% solvent A was kept for 2 min, followed by a 3 min gradient reaching a 50% composition. The samples were spiked with 100 μM salicylic acid (Fluka, 99.6%) as an internal standard. Figure 3.S2 shows good separation of 2,3-dimethyltartaric acid (m/z 177.04) and the oxo-C₈ product (m/z 219.05) confirming the observation made in Figure 3.1 by IC-MS.

**Tandem Mass Spectrometry (MS/MS).** Figure 3.S4 shows the fragmentation patterns for pyruvic acid (m/z 87), the oxo-C₇ product (m/z 175), DMTA (m/z 177) and the oxo-C₈
product \((m/z \ 219)\). Figure 3.S4A shows that pyruvic acid fragments into acetic acid \((m/z \ 59)\) by decarbonylation. Figure 3.S4B shows that the oxo-C\(_7\) product easily produces a pyruvic acid like fragment with \(m/z \ 87\) corresponding to \(\text{C}_3\text{H}_3\text{O}_3^-\), which is easily obtainable by breaking one of the ether bonds in the proposed structure shown in Scheme 3.2 of the main paper. This fragmentation pattern is consistent with what was observed in the past,\(^5\) where it was speculated that the lack of a second ESI(-) detectable fragment indicated there should only be one carboxyl group in the structure of the oxo-C\(_7\) product. The IC-MS analysis presented in Figures 3.1 and 3.2 of the main text confirms this conjecture, and demonstrates that the dicarboxylic acid impurity parapyruvic acid (also with \(m/z \ 175)\) does not contribute to the MS/MS spectrum of the products. Figure 3.S4C shows the MS/MS of DMTA \((m/z \ 177)\) has fragments at \(m/z \ 131, 115,\) and \(87\), consistent with previous work.\(^5\) In addition, this peak at \(m/z \ 177\) loses a water giving \(m/z \ 159\) and the fragment at \(m/z \ 115\) can decarboxylate to form an ion at \(m/z \ 71\). This secondary decarboxylation provides additional support indicating that the product with \(m/z \ 177\) has two carboxyl groups as in the structure of DMTA. In Figure 3.S4D, the oxo-C\(_8\) product shows multiple ions arising from a labile species. The fragment at \(m/z \ 201\) results from water loss from \(m/z \ 219\). The fragments at \(m/z \ 175\) (matching the oxo-C\(_7\) product) and \(157\) result from decarboxylation of \(m/z \ 219\) and \(201\), respectively. Confirmation of the presence of a remaining carboxyl group in \(m/z \ 175\) is shown by its decarboxylation into \(m/z \ 131\). The peak at \(m/z \ 201\) easily breaks into \(m/z \ 117\) by loss of \(\text{C}_4\text{H}_4\text{O}_2\) (neutral mass 84 amu) or vice versa generating a weak peak at \(m/z \ 83\) from the loss at 118 amu. The \(m/z \ 113\) fragment can be produced by consecutive loss of a pyruvic acid monomer (88 amu) and water (18 amu) directly from the oxo-C\(_8\) product. This pyruvic acid loss can
also be observed at $m/z$ 87. Finally, the oxo-C₈ product can fragment into the two halves $m/z$ 147 and neutral 72 amu or $m/z$ 71 and neutral 148 amu.

**Figure 3.S1.** UHPLC-MS extracted ion chromatograms (EIC) for impurities in pyruvic acid reagent. Top panel: EIC at $m/z$ 157.01 for zymonic acid (red dashed line) before and (gray dashed line) after vacuum distillation. Bottom panel: EIC at $m/z$ 175.02 for parapyruvic acid (blue dashed line) before and (gray dashed line) after vacuum distillation.
Figure 3.S2. UHPLC (black line) UV chromatogram and ESI(-)/MS EIC for 100.9 mM pyruvic acid photolyzed to a 19.3% conversion. EICs at (gray line) m/z 87.01 for pyruvic acid; (red line) m/z 175.06 for oxo-C_7 product eluting at 0.65 min, (blue line) m/z 177.04 for 2,3-dimethyltartaric acid (DMTA), (green line) m/z 219.05 for oxo-C_8 product, and (purple) m/z 137.02 for salicylic acid internal standard.

Figure 3.S3. Time series of (gray circle) pyruvic acid loss, (red star) oxo-C_7 product, (blue square) DMTA, and (green triangle) oxo-C_8 product for the experiment in D_2O at pD = 1.0 and 298 K. Initial slopes were used for the calculation of KIE reported in Table 3.1.
Figure 3.S4. High resolution MS/MS fragmentation patterns for (A) pyruvic acid (m/z 87); (B) oxo-C$_7$ (m/z 175); and (C) DMTA (m/z 177) in a solution of 100 mM pyruvic acid after photolysis to 20 % conversion.

Figure 3.S5. 150 MHz $^{13}$C NMR spectrum of 97.9 mM $^{13}$C labeled PA at pD = 1.0 for a 9 min photolysis corresponding to a 50.2% conversion.
Figure 3.S6. UHPLC-MS chromatograms from hydrazones of (top panel) $^{13}$C labeled PA ($m/z$ 270.05) and (bottom panel) oxo-C$_7$ product ($m/z$ 362.11) from an experiment with $[^{13}$C PA] = 115.2 mM photolyzed during (red) 0, (blue) 1, (green) 3, (purple) 5, and (gray) 10 min. Top panel inset: EIC for $m/z$ 271.05. Bottom panel inset: Trace production of $^{13}$C acetaldehyde ($m/z$ 225.05) after smoothing with a Gaussian function.

Figure 3.S7. 400 MHz $^1$H NMR spectrum of 99.9 mM PA photolyzed to 20.0 % conversion (blue) before and (red) after spike addition of lactic acid to a final concentration of 2.24 mM. The inset shows an expansion of the –CH$_3$ region for the doublet where lactic acid does not overlap well with the signal of the photoproducts.
Figure 3.S8. Dependence of the photolytic rate of pyruvic acid, \( k_{hv} \times [PA]_0 \), on the photon absorption rate, \( I_a \), during direct photolysis of aqueous solutions (\( \lambda \geq 305 \text{ nm} \)) at pH = 1.0 and 298 K. Incident photon rate \( I_0 = 1.14 \times 10^{-5} \text{ M s}^{-1} \).

Figure 3.S9. Initial rates of (gray circle) pyruvic acid loss and formation of (red star) the oxo-C\(_7\) product, (blue square) 2,3-dimethyltartaric acid, (green triangle) the oxo-C\(_8\) product, and (purple diamond) the sum for the three products at variable initial pyruvic acid concentration. Experimental conditions are the same reported in Figure 3.S7.
<table>
<thead>
<tr>
<th>Wavenumber (cm(^{-1}))</th>
<th>Photolyzed Oxo-C(_7) Product</th>
<th>Acetoin</th>
<th>Strength</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1033</td>
<td>1055-870</td>
<td>-</td>
<td>s</td>
<td>C-O-C symmetric stretch</td>
</tr>
<tr>
<td>1056</td>
<td>1125-1000</td>
<td>1124</td>
<td>s</td>
<td>C-O stretch, secondary alcohol</td>
</tr>
<tr>
<td>1085</td>
<td>1300-1100, 1120</td>
<td>-</td>
<td>s</td>
<td>C-O-C, asymmetric stretch</td>
</tr>
<tr>
<td>1316</td>
<td>1320-1210</td>
<td>-</td>
<td>m</td>
<td>C-O stretch, carboxylic acid</td>
</tr>
<tr>
<td>1265</td>
<td>1300-1100</td>
<td>1266</td>
<td>m</td>
<td>carbonyl bend</td>
</tr>
<tr>
<td>Indist.</td>
<td>1375</td>
<td>1372</td>
<td>s</td>
<td>methyl bend</td>
</tr>
<tr>
<td>Indist.</td>
<td>1715</td>
<td>1736</td>
<td>s</td>
<td>C=O stretch, ketone</td>
</tr>
<tr>
<td>Indist.</td>
<td>1800-1740</td>
<td>-</td>
<td>s</td>
<td>C=O stretch, carboxylic acid</td>
</tr>
<tr>
<td>Indist.</td>
<td>2872</td>
<td>2872</td>
<td>s</td>
<td>methyl C-H symmetric stretch</td>
</tr>
<tr>
<td>2967</td>
<td>2962</td>
<td>2987</td>
<td>s</td>
<td>methyl C-H asymmetric stretch</td>
</tr>
<tr>
<td>-</td>
<td>3550-3500</td>
<td>-</td>
<td>m</td>
<td>carboxylic acid OH stretch</td>
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<tr>
<td>-</td>
<td>3650-3590</td>
<td>3530</td>
<td>m</td>
<td>O-H stretch</td>
</tr>
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Table 3.S2. Assignment of the 150 MHz $^{13}$C NMR spectrum of 97.88 mM fully labeled $^{13}$C PA at pH = 1.0 photolyzed for 9 min for a 50.2% conversion.

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<tr>
<th>Chemical shift (ppm)</th>
<th>Assignmenta (molecule, functionality)</th>
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<tbody>
<tr>
<td>220.18</td>
<td>oxo-C$_7$, CH$_3$C(0)CH(CH$_3$)O-</td>
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<tr>
<td>201.00</td>
<td>pyruvic acid, CH$_3$C(O)COOH</td>
</tr>
<tr>
<td>179.96</td>
<td>oxo-C$_7$, -C(OH)(CH$_3$)COOH</td>
</tr>
<tr>
<td>179.14</td>
<td>2,3-dimethyltartraric acid, -C(OH)(CH$_3$)COOH</td>
</tr>
<tr>
<td>177.82</td>
<td>gem-diol of pyruvic acid, CH$_3$C(OH)$_2$COOH</td>
</tr>
<tr>
<td>167.39</td>
<td>pyruvic acid, CH$_3$C(O)COOH</td>
</tr>
<tr>
<td>127.36</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>95.39</td>
<td>gem-diol of pyruvic acid, CH$_3$C(OH)$_2$COOH</td>
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<tr>
<td>86.40</td>
<td>oxo-C$_7$, CH$_3$C(O)CH(CH$_3$)O-</td>
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<tr>
<td>84.34</td>
<td>2,3-dimethyltartraric acid, -C(OH)(CH$_3$)COOH</td>
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<td>81.43</td>
<td>oxo-C$_7$, -C(OH)(CH$_3$)COOH</td>
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<td>29.36</td>
<td>oxo-C$_7$, CH$_3$C(O)CH(CH$_3$)O-</td>
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<td>28.40</td>
<td>pyruvic acid, CH$_3$C(O)COOH</td>
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<td>23.19</td>
<td>oxo-C$_7$, CH$_3$C(O)CH(CH$_3$)O-</td>
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<td>22.94</td>
<td>oxo-C$_7$, -C(OH)(CH$_3$)COOH</td>
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<tr>
<td>21.77</td>
<td>2,3-dimethyltartraric acid, -C(OH)(CH$_3$)COOH</td>
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<tr>
<td>20.94</td>
<td>DSS, (CH$_3$)$_3$-Si-CH$_2$-CH$_2$-CH$_2$-SO$_3^-$</td>
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<td>17.49</td>
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<td>0.000</td>
<td>DSS, (CH$_3$)$_3$-Si-CH$_2$-CH$_2$-CH$_2$-SO$_3^-$</td>
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Table 3.S3. Assignment of the 600 MHz $^1$H NMR spectrum of 322 mM photolyzed pyruvic acid for a 20.3 % Conversion.

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<th>Chemical shift (ppm)</th>
<th>Multiplicity</th>
<th>Assignment&lt;sup&gt;a&lt;/sup&gt; (molecule, functionality, diastereomer&lt;sup&gt;b&lt;/sup&gt;)</th>
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<tr>
<td>4.420</td>
<td>q</td>
<td>oxo-C&lt;sub&gt;7&lt;/sub&gt;, O-CH-C(O)CH&lt;sub&gt;3&lt;/sub&gt;, A and B</td>
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<tr>
<td>2.463</td>
<td>s</td>
<td>pyruvic acid, CH&lt;sub&gt;3&lt;/sub&gt;C=O</td>
</tr>
<tr>
<td>2.910</td>
<td>t</td>
<td>DSS, -CH&lt;sub&gt;2&lt;/sub&gt;-SO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.322</td>
<td>s</td>
<td>oxo-C&lt;sub&gt;7&lt;/sub&gt;, O-CH-C(O)CH&lt;sub&gt;3&lt;/sub&gt;, A</td>
</tr>
<tr>
<td>2.309</td>
<td>s</td>
<td>oxo-C&lt;sub&gt;7&lt;/sub&gt;, O-CH-C(O)CH&lt;sub&gt;3&lt;/sub&gt;, B</td>
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<tr>
<td>2.271</td>
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<td>C&lt;sub&gt;8&lt;/sub&gt;, CH&lt;sub&gt;3&lt;/sub&gt;C(O)C-, A</td>
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<tr>
<td>2.217</td>
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<td>C&lt;sub&gt;8&lt;/sub&gt;, CH&lt;sub&gt;3&lt;/sub&gt;C(O)C-, B</td>
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<tr>
<td>2.086</td>
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<tr>
<td>1.686</td>
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<td>DSS, (CH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;3&lt;/sub&gt;-Si-CH&lt;sub&gt;2&lt;/sub&gt;-CH&lt;sub&gt;2&lt;/sub&gt;-</td>
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<tr>
<td>1.581</td>
<td>s</td>
<td>pyruvic acid gem-diol, CH&lt;sub&gt;3&lt;/sub&gt;C(OH)&lt;sub&gt;2&lt;/sub&gt;</td>
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<tr>
<td>1.551</td>
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<td>oxo-C&lt;sub&gt;7&lt;/sub&gt;, O-CH(CH&lt;sub&gt;3&lt;/sub&gt;)-C=O, A</td>
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<td>1.533</td>
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<td>2,3-dimethyltartric acid, HO-C(CH&lt;sub&gt;3&lt;/sub&gt;)-C(CH&lt;sub&gt;3&lt;/sub&gt;)-OH, A</td>
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<tr>
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<tr>
<td>1.499</td>
<td>s</td>
<td>2,3-dimethyltartric acid, HO-C(CH&lt;sub&gt;3&lt;/sub&gt;)-C(CH&lt;sub&gt;3&lt;/sub&gt;)-OH, B</td>
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<tr>
<td>1.472</td>
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<tr>
<td>1.451</td>
<td>s, broad</td>
<td>C&lt;sub&gt;8&lt;/sub&gt;, HOOC-C(OH)-CH&lt;sub&gt;3&lt;/sub&gt;, B and CH&lt;sub&gt;3&lt;/sub&gt;-C(O)-C-CH&lt;sub&gt;3&lt;/sub&gt;, A</td>
</tr>
<tr>
<td>1.435</td>
<td>s</td>
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</tr>
<tr>
<td>1.354</td>
<td>s</td>
<td>oxo-C7, HOOC-C(OH)-CH&lt;sub&gt;3&lt;/sub&gt;, A and B</td>
</tr>
<tr>
<td>0.624</td>
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</tr>
<tr>
<td>0.000</td>
<td>s</td>
<td>DSS, (CH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;3&lt;/sub&gt;-Si-</td>
</tr>
</tbody>
</table>

<sup>a</sup>Structures are given in Scheme 3.2 of the main paper. <sup>b</sup>Two diastereomers (labeled A and B) exist for each product.
3.7.2 Supporting Information For: Reply to “Comment ‘On the Debate over the Aqueous Photolysis of Pyruvic acid’”

Background on Pyruvic Acid Photochemistry in Water and Ice.

Eugene and Guzman (E&G)\(^2\) studied the photochemistry of aqueous pyruvic acid (PA) considering the combination of a body of work\(^5, 21, 68-70, 76, 77, 152\) that Vaida and coworkers (V&C)\(^154\) have partially ignored and misrepresented. The reaction mechanism in these PA photochemistry studies in water and ice\(^152\) not only identified the photoproducts but also integrated knowledge gained by 1) studying the reaction intermediates generated concomitantly to photodecarboxylation at cryogenic temperature,\(^68\) 2) measuring the kinetics of the reaction both in the condensed phase and the evolution of gases at variable temperature,\(^5, 68-70\) 3) exploring the dynamic nature of the hydration equilibrium at variable temperature in water and ice,\(^21\) 4) determining the dependence of the quantum yield on the initial [PA]_0,\(^2, 5\) 5) investigating the effect of variable concentration of radical scavengers such a 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) and dissolved O\(_2\),\(^2, 5, 69\) 6) measuring solvent kinetic isotopic effect (KIE)\(^2\) as well as using isotopic labels,\(^2, 5, 68\) and 7) contrasting the optical absorptivity and molecular composition of the mixtures of identifiable aliphatic polyfunctional oligomers obtained during photobleaching and thermochromism cycles.\(^76, 77\)

In opposition to the need from V&C to create a divergent mechanism,\(^154\) E&G have clearly indicated that: “For practical purposes, there is no difference if the (indistinguishable) photoinduced bimolecular process represents a hydrogen atom transfer, an electron transfer, or proton-coupled electron transfer (PCET)”.\(^2\) In other words, the same reactive species, ketyl (K’) and acetyl (Y’) radicals, are generated in all
cases after the photogeneration of a triplet excited state of PA.\textsuperscript{2} The primary decarboxylation reaction that generates $Y^\cdot$ from an acyloxy radical (Scheme 3.2) propels the process in an irreversible direction.\textsuperscript{68} The only way to form new C-C or C-O bonds to achieve products of higher molecular weight is by recombination of the prevalent photogenerated radicals from different solvent cages (otherwise the original molecules would be regenerated).\textsuperscript{68}

**Figure 3.S10.** (A) Ion chromatogram with conductivity detection for the region of elution of lactic acid for a control with 99.22 mM pyruvic acid at pH 1.0 photolyzed at $\lambda \geq 305$ nm for a 21\% reagent conversion under continuous sparging with N\textsubscript{2}(g) (red) before and (blue) after spiking to [lactic acid]\textsubscript{final} = 14.9 $\mu$M. The anions of all species were assigned with available standards and/or using the $m/z$ values obtained by tandem mass spectrometry. (B) Extracted ion chromatogram for lactic acid ($m/z$ - 89) (red) before and (blue) after spike addition of lactic acid for the same samples in panel A. More details of this method are available in Ref. \textsuperscript{2}. These results supplement those from Ref. \textsuperscript{70} that had already demonstrated the absence of acetoin during UHPLC-MS analysis of photolysis products treated with 2,4-dinitrophenylhydrazine.

Different recombination reactions among the produced radicals have been considered by Guzman et al. and E\&G,\textsuperscript{2, 5, 68, 69} including the recombination of $Y^\cdot$ (and its hydrate) and $K^\cdot$. However, the reaction $Y^\cdot + K^\cdot$ was experimentally discarded because $\alpha$-acetolactic acid standard was confirmed to be absent in the mixture of photoproducts. The $\alpha$-acetolactic acid standard also remained stable, i.e. did not decompose to produce CO$_2$, ...
under the same conditions (in the dark below 4 °C) that caused evolution of CO₂ from the postphotolysis mixture. ², ⁵, ⁶⁹ E&G have acknowledged the key radicals (K’ and Y’) participate in the same mechanism in water and ice, ² which proceeds to form 2,3-dimethyltartaric acid (DMTA) and 2-hydroxy-2-((3-oxobutan-2-yl)oxy)propanoic acid (oxo-C⁷ product). ⁵, ⁶⁸, ¹⁵² The unique oxo-C⁷ product results from a β–ketocarboxylic acid decarboxylation of the very unstable primary photoproduct, 2-(1-carboxy-1-hydroxyethoxy)-2-methyl-3-oxobutanoic acid (oxo-C⁸ product) with a rate constant $k = 1.21 \times 10^{-3}$ s⁻¹. ⁵, ⁶⁸, ⁶⁹
Chapter 4: Photochemistry of aqueous pyruvic acid: The roles of dissolved O₂ and pH in the production of reactive oxygen species

Alexis J. Eugene and Marcelo I. Guzman

To be submitted for publication.
4.1 Abstract

The sunlight-induced photochemistry of the organic chromophore pyruvic acid (PA) in water generates ketyl and acetyl radicals that contribute to the processing of atmospheric aerosols. The photochemical mechanism is highly sensitive to dissolved oxygen content among other environmental conditions. Thus, herein we investigate the photolysis ($\lambda \geq 305$ nm) of 10-200 mM PA in water covering the relevant ranges 1) $0 \leq [O_2(aq)] \leq 1.3$ mM, 2) $1.0 \leq \text{pH} \leq 4.5$, and 3) equivalent tropospheric photon fluxes between 1 and 10 suns. The rapid consumption of dissolved oxygen, $[O_2(aq)]$, by the intermediate photolytic radicals is monitored in real time with a dissolved oxygen electrode. The atmospheric oxidant singlet molecular oxygen ($^{1}\text{O}_2^*$) is trapped with furfuryl alcohol (FFA), an abundant molecule from biomass burning, during irradiation in the presence of $O_2(aq)$. Thus, a steady state level of $2 \times 10^{-12} \leq [^{1}\text{O}_2^*] \leq 9 \times 10^{-12}$ M is determined. The time series of organic photoproducts is monitored by ion chromatography-mass spectrometry (IC-MS) and shows that 2,3-dimethyltartaric acid (DMTA), 2-(3-oxobutan-2-yloxy)-2-hydroxypropanoic acid (oxo-C$_7$ product) and 2-(1-carboxy-1-hydroxyethoxy)-2-methyl-3-oxobutanoic acid (oxo-C$_8$ product) are formed under all conditions investigated. The rates of product formation and reactant decay at variable pH perfectly overlap with the predicted sigmoid curve for the undissociated fraction of PA. The matching inflection points at $\text{pH} = pK_{a,PA} = 2.39$ for the sigmoid curves indicate all reaction rates (except for acetic acid that is only formed in the presence of $O_2(aq)$) depend on the availability of pyruvic acid rather than its conjugate base. The production rate of DMTA and the oxocarboxylic acid products (oxo-C$_7$ + oxo-C$_8$) scale linearly for an increasing photon flux. However, the branching ratio of the products shifts away from
the simple bimolecular radical recombination that favors DMTA formation toward
multistep radical chemistry and formation of the more complex oxo products.

4.2 Introduction

Pyruvic acid (PA, $pK_a = 2.39$)\textsuperscript{136} is an abundant component of tropospheric aerosols,\textsuperscript{81, 82, 165} and one of few chromophores capable of undergoing photochemical reactions in water.\textsuperscript{2, 6, 70, 166-168} It is produced during the atmospheric photooxidative processing of biogenic and anthropogenic emissions.\textsuperscript{38, 169} If formed in the gas phase, PA preferentially partitions into the aqueous phase with a large Henry’s law constant ($K_H = 3.1 \text{ mol kg}^{-1} \text{ Pa}^{-1}$),\textsuperscript{170} and a reactive uptake coefficient by water of $\gamma_{PA} = 0.06$.\textsuperscript{74} During this uptake, the interfacial accommodation process for gaseous PA onto the surface of water implies the orientation of the $–COOH$, $C=O$, and $–CH_3$ groups is plane parallel to the interface.\textsuperscript{74, 171}

In addition, the $C=O$ group of PA undergoes cooperative reversible hydration (with equilibrium constant $K_{Hyd} = 1.83$) into 2,2-dihydroxypropanoic acid, a UV transparent gem-diol.\textsuperscript{5} Early studies of the photochemistry of PA reported that for a fraction of pyruvate larger than 0.99 (at pH 6.1), the reactant decays at least an order of magnitude slower than for the undissociated acid.\textsuperscript{63} A more recent study reported qualitative observations on the effect of pH during photolysis consistent with the earlier studies.\textsuperscript{71} The suppression of reaction in high pH solutions was attributed to a lack of carboxyl hydrogen available for abstraction, even though carboxyl hydrogens are rarely targets of abstraction and despite the fact that methyl hydrogens are still readily available at high pH.\textsuperscript{71}

Contrarily, pyruvate’s larger absorption than PA\textsuperscript{172} (reflecting less gem-diol in equilibrium) should have suggested a greater photoreactivity for a pH that exceeded the
pK$_a$ value by more than 2 units. The unpredictable effect of pH on photoreactivity changes of aqueous PA$^{63, 172}$ demands a better understanding of the reaction under speciation conditions dominated by the acid or the conjugate base. Therefore, we provide a quantitative study of the effect of pH on the decay of pyruvic acid and on the production of each photoproduct identified in the literature$^2$ using methods that eliminate overlap of each major analyte. We also provide an alternative reasoning for the reduction in reactivity at high pH.

In addition, the processing of PA in atmospheric waters by sunlight’s photons$^2, 5, 168$ and by hydroxyl radicals (HO•) from the photolysis of H$_2$O$_2$$^{38}$ has been reported. However, the effect of the in situ generated oxidizer singlet molecular oxygen (1O$_2$), the excited state of molecular oxygen, during the photochemistry of aqueous PA remains unexplored. In atmospheric waters 1O$_2$ can be supplied either from the gas phase after partitioning or from photochemical reactions within the aqueous particles.$^{173}$ The photochemical formation of 1O$_2$ requires energy transfer from a triplet exited state of an organic molecule to ground state oxygen. In water, under air saturated conditions and under sunlight, the photoexcited triplet state of PA (3PA•), should form a significant amount of 1O$_2$. Past measurements in atmospheric waters during the fall season have bracketed the range of [1O$_2$(aq)] = 2.7 × 10$^{-14}$ -1.1 × 10$^{-12}$ M,$^{174}$ with an average in winter of 2.6 × 10$^{-13}$ M.$^{173}$

Nevertheless, there is no data regarding 1O$_2$ photoformation and its effect in irradiated waters where PA is mixed with other markers of organic pollution. While 1O$_2$ is typically a minor fate for refractory compounds in atmospheric waters, electron rich species such as furans (i.e., furfuryl alcohol) emitted during biomass burning$^{47, 175}$ can be
especially susceptible to attack by this oxidizer. Therefore, this work tackles three major objectives needed to advance the understanding of the photochemistry of PA in atmospheric waters by 1) measuring the rate of formation of the previously identified photoproducts under variable pH and light intensity, 2) studying the effect of dissolved oxygen ([O_2(aq)]) in the photoproduction of radical intermediates, and 3) investigating the formation of \(^1\text{O}_2\) using furfuryl alcohol (a marker of biomass burning emissions) under relevant tropospheric conditions. The results show that the photoreactivity of PA in water closely follows the fraction of pyruvic acid in solution, indicating a key role of the acidic hydrogen in the mechanism. This work also explores the importance of monitoring and controlling the [O_2(aq)] during reactions to study processes with real atmospheric relevance. The high production of intermediate radicals and oxidizing \(^1\text{O}_2\) quantified here suggests an important role for PA as source of atmospheric oxidants in aqueous aerosol. Finally, reactions at variable light intensities allow for a more accurate determination of the quantum yield of PA photolysis.

4.3 Experimental

4.3.1 Preparation of Experiments

Each reaction was performed in duplicate. PA (Sigma-Aldrich, 98.5%, distilled under vacuum) in ultrapure water (18.2 MΩ cm\(^{-1}\), Elga Purelab Flex, Veolia) was prepared immediately before each photolysis. The choice of working concentrations was described in detail previously; briefly, it was estimated that acidic, urban aerosols may contain 5-200 mM PA\(^2\). The pH of solutions was adjusted by adding hydrochloric acid (EMD, 37.7%) or sodium hydroxide (Amresco, 99.0%) and measured using a calibrated pH electrode (Orion, Thermo Scientific). A 200 mL aliquot of the pH adjusted solution was
transferred to a 220 mL quartz photochemical reactor equipped with a jacket for temperature control (described previously),\textsuperscript{3} where it was sparged in the dark with air, O\textsubscript{2}, N\textsubscript{2} or a custom O\textsubscript{2}/N\textsubscript{2} mix through a Teflon tube (ID = 1.6 mm) or a 25 \( \mu \)m glass frit for 30 min prior to photolysis. For experiments with variable O\textsubscript{2} mixing ratios, mixing was achieved by combining streams of pure N\textsubscript{2} and O\textsubscript{2} via a gas proportioner (Cole-Parmer) utilizing a total gas flow of 100 mL min\(^{-1}\).

Monitoring of \([O_2(aq)]\) during the reactions was provided by a polarographic oxygen probe (Thermo Orion, 081010MD) calibrated at maximum saturation in an air-calibration sleeve and at 0% saturation in a saturated sodium sulfite/CoCl\(_2\) solution.

\textbf{4.3.2 Photochemical Conditions}

The solutions were irradiated with a high pressure 1 kW Xe-Hg lamp filtered through (1) water, (2) a cutoff filter at \( \lambda \geq 305 \) nm, and (3) only for selected experiments (as indicated in the corresponding caption to figures) neutral density filters to attenuate the photon flux of the lamp to 67.19, 32.45, 21.50, or 10.56% of the total. Chemical actinometry using phenylglyoxylic acid\textsuperscript{89} was used to measure the effective photon flux, \( I_0 \), of the full lamp and at each of the attenuated intensities in the absorption region 305 \( \leq \lambda \leq 420 \) nm. The effective photon fluxes reported were obtained after correcting the actinometric measurement by comparing the convoluted spectra of phenylglyoxylic acid with the lamp \textit{versus} PA with the lamp. The effective photon fluxes used in this work covered a range of \( 1.52 (\pm 0.13) \times 10^{-6} \leq I_0 \leq 1.13 (0.03) \times 10^{-5} \) Einstein L\(^{-1}\) s\(^{-1}\). The quantum yields reported correspond to the ratio of the rate of loss of PA or formation of products to the absorbed photon flux, \( I_a \), which is calculated from Equation 4.1:
where $I_0$ is the incident effective photon flux measured by actinometry; $\varepsilon$ is the molar absorptivity of PA at $\lambda_{\text{max}} = 321$ nm, 11.3 M$^{-1}$ cm$^{-1}$; $l$ is the path length of irradiation, 4.50 cm for this reactor; and the initial PA concentration is given by $[\text{PA}]_0$.

The relationship established by Equation 4.1 stipulates that light absorption and therefore the photolysis rate constant of this reaction varies linearly with $[\text{PA}]_0$ only below $\sim$1 mM for $l = 4.5$ cm$^2$ (originally explained by Guzman et al. for $l = 1$ cm to be $[\text{PA}]_0 < 4$ mM). The previous important concept$^{2,5}$ has been recently ignored by Mekic et al.$^{176}$ resulting in misleading conclusions about PA photochemistry based exclusively on inadequate UV-visible spectroscopy observations performed with impure PA. However, for $1 < [\text{PA}]_0 < 300$ mM, the photolysis rate constant must decrease as the rate of photon absorption drops. Therefore, PA loss approaches a maximum for the case of high enough solution absorbance that $I_0 = I_a$. During irradiation, the solutions were maintained at 25 °C and magnetically stirred while being continuously sparged with the desired gas. Liquid samples (5 mL) were removed from the reactor at regular intervals and analyzed immediately.

4.3.3 Analysis of Products

Organic products were quantified by IC-MS using a Dionex ICS-2000 ion chromatograph (IonPack AS11-HC , 2 mm column) equipped with a conductivity detector and an electrospray ionization (ESI) mass spectrometer (Thermo Scientific MSQ Plus) operating in the negative mode as described previously.$^2$ Samples were diluted 200 times in ultrapure water before injection onto the IC column. Quantification was performed using

$$I_a = I_0 \left(1 - e^{-2.303 \varepsilon l [\text{PA}]_0}\right) \quad (4.1)$$
commercially available standards for pyruvate (JT Baker, 99.4%) and acetate (EM science, 99.0%). For products that were unavailable, qNMR was used to calibrate the relative response factor in the MS as described previously. All reaction rates reported correspond to initial rates, where the decay of PA or product formation is linear unless otherwise specified.

4.3.4 Quantification of $^1$O$_2$

Furfuryl alcohol (Alfa Aesar, 99.1%, FFA), an abundant biomass burning molecule emitted during biomass burning was used in experiments measuring singlet oxygen ($^1$O$_2$) production. A spike of FFA was added to the solution of PA immediately before photolysis ([FFA]$_{\text{final}}$ = 1.2 mM). The decay of FFA was followed by HPLC (Agilent 1100) using an Agilent XDB-C18 column (5 μm, 4.6 × 150 mm) with UV detection at $\lambda$ = 218 nm. Samples were injected (25 μL) undiluted into the column. A flow rate of 1 mL min$^{-1}$ mixing solvent A made of 10 mM formate buffer (sodium formate, Acros, 99.9%; formic acid, Fisher Optima LC/MS, 99.5%) at pH 3 with solvent B made of acetonitrile (EMD Millipore LC/MS, 99.99%) was employed. Gradient elution allowed the furfuryl alcohol to be separated from PA and the photolysis products by using initially 5% solvent B for 3 min, then ramping to 25% solvent B over 5 min and holding at 25% solvent B for 1 min. The steady-state singlet oxygen concentration, [1O$_2$_ss], was calculated by dividing the experimental rate constant determined in this work by the recommended value for $k_{^1O_2+FFA} = 1.08 \times 10^8$ M$^{-1}$ s$^{-1}$, which is corrected for temperature and salt content. The experimental rate constant was obtained from the slope of a linear plot of $Ln([FFA]/[FFA]_0)$ vs. time. An example is shown in the Supporting Information (Figure 4.S1).
4.3.5 Monitoring of hydroxyl radicals (HO')

The production of HO' was discarded while monitoring photoreactions spiked to [benzoic acid]$_{\text{final}}$ = 0.98 mM or [terephthalic acid]$_{\text{final}}$ = 0.50 mM. Benzoic and terephthalic acids (both purchased from Acros, ≥ 99.0%) are known to trap HO' forming o-, m-, and p-hydroxybenzoic acids$^{173, 178}$ and 2-hydroxyterephthalic acid,$^{179}$ respectively. Each unreacted trap was separated from its hydroxylated product(s) by HPLC using the same column and gradient conditions described above for FFA. The UV detector allowed monitoring the decay of terephthalic acid at $\lambda$ = 243 nm or benzoic acid at $\lambda$ = 218 nm. No HO' production was observed using either probe with limits of detection (LOD) for the steady state concentration of hydroxyl radical, [HO']$_{ss}$, LOD = 1.3 $\times$ 10$^{-20}$ M for benzoic acid and LOD = 1.4 $\times$ 10$^{-20}$ M for terephthalic acid. Thus, it was possible to disregard the production of HO' as a contributing process during the photolysis of aqueous PA. Further experimental details can be found in the Supporting Information.

4.4 Results and Discussion

4.4.1 Time Series of PA Loss and Product Formation

Figure 4.1 shows the ion chromatography separation (red line) before and (blue line) after 1 h photolysis ($\lambda$ ≥ 305 nm) of PA at pH 1.0 for the full photon flux under continuous air sparging, which ensures the reaction reported proceeds in the presence of continuously replenished dissolved oxygen. The data in Figure 4.1B consist of extracted ion chromatograms in the negative ionization mode for the main photoproducts at mass-to-charge ratios ($m/z$) 177.04, 175.06, and 219.05 for 2,3-dimethyltartaric acid (DMTA), 2-(3-oxobutan-2-yloxy)-2-hydroxypropanoic acid (named oxo-C$_7$ product), and 2-(1-carboxy-1-hydroxyethoxy)-2-methyl-3-oxobutanoic acid (named oxo-C$_8$ product),
respectively. The structures of these products are shown in Scheme 4.1. The two peaks observable for each product are attributed to separable pairs of diastereomers that are the result of the photolysis proceeding through a radical mechanism. In agreement with our previous reports, under all sets of conditions used in this work, the same products are observed, albeit in different ratios. In addition, a small peak for acetic acid is observed in Figure 5.1 at m/z 59.01.

![Figure 4.1](image-url)

**Figure 4.1.** (A) Ion chromatogram with total conductivity detection of 100.0 mM pyruvic acid (PA) at pH 1.0 (red) before and (blue) after 1 h photolysis (λ ≥ 305 nm) under continuous air sparging at 25 °C for an effective photon flux $I_a = 1.13 \times 10^{-5}$ Einstein L$^{-1}$ s$^{-1}$. (B) IC-ESI(−)/MS extracted ion chromatograms for the same experiment in panel A for PA (m/z 87.01), DMTA (m/z 177.04), the oxo-C$_7$ product (m/z 175.06), the oxo-C$_8$ product (m/z 219.05), and acetic acid (m/z 59.01).
Scheme 4.1. Aqueous photochemistry of pyruvic acid (PA) in the presence of dissolved oxygen, $O_2(aq)$. Adapted from reference 2.
Figure 4.2 shows the typical kinetics for a photoreaction under air saturated conditions. The first-order decay of PA fits an exponential function with a rate constant, 
\[ k_{PA} = 7.43 \times 10^{-5} \text{ s}^{-1} \] and a coefficient of correlation \( r^2 = 0.995 \). The concentrations of (in order of abundance) DMTA, the sum for the oxo-C\(_7\) and oxo-C\(_8\) products, and acetic acid increase exponentially according to the equation:

\[
[\text{product}] = [\text{product}]_\infty \left(1 - e^{-kt} \right)
\]  
(4.2)

where the constant \([\text{product}]_\infty\) representing the asymptotic upper limit that would be reached as the time \( t \rightarrow \infty \), and \( k_f \) is the formation rate constant (both given in Table 4.1).

**Figure 4.2.** Kinetics of (gray circle) PA decay and formation of (blue square) DMTA, (gold diamond) acetic acid, (red star) the oxo-C\(_7\) product, and (green triangle) the oxo-C\(_8\) product for the same experiment in Figure 4.1.

The \( r^2 \) for all nonlinear fittings in Figure 4.2 are larger than 0.993. Based on the stoichiometric conversion factors of 2, 1, and 3 molecules of PA needed to produce DMTA, acetic acid, and the oxo-C\(_7\) + oxo-C\(_8\) products, respectively, the mass balance
after 1 h of irradiation is completely interpreted by the results of Figure 4.1. In addition, based on the stoichiometry of each product to the number of PA molecules consumed, the predicted [product]∞ in Table 4.1 can explain up to 95.7% of the mass balance as $t \to \infty$, which demonstrates an excellent understanding of the photoreaction based on these products.

<p>| Table 4.1. Exponential Growth Parameters for Products in Figure 4.2 |
|---------------------------------|-----------------|</p>
<table>
<thead>
<tr>
<th>[Product]∞ (mM)</th>
<th>$k_f$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMTA</td>
<td>20.4</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>6.31</td>
</tr>
<tr>
<td>Oxo-C$_7$ + oxo-C$_8$ products</td>
<td>16.2</td>
</tr>
</tbody>
</table>

Past work (see Chapter 3) using a reactor with a shorter path length has demonstrated the exponential behavior described above for Figure 4.2. Therefore, the linear appearance of the trends in Figure 4.2 is only a consequence of determining most concentration points during the early decay of PA to obtain the first order kinetics. For the oxygen saturated conditions corresponding to Figure 4.2, a high [O$_2$(aq)] = 200 μM remains during the course of the whole photoreaction, which constitutes 77% of the initial level for the air saturated solution. In consequence, the kinetics of the reaction are truly controlled by the initial [PA]$_0$ in the presence of O$_2$(aq), providing valuable information to mimic the process under realistic atmospheric conditions. This work
improves our previous findings \(^2\) (obtained for a remaining \([\text{O}_2(aq)] = 10.65 \ \mu\text{M}\) and shows a higher production of DMTA than the \text{oxo-C}_7 + \text{oxo-C}_8 \) products in the presence of \([\text{O}_2(aq)]_{\text{final}} = 200 \ \mu\text{M}\) (Figure 4.2). Similarly, the valuable \(k_f\) parameters reported for DMTA and the \text{oxo-C}_7 + \text{oxo-C}_8 \) products (Table 4.1) are an order of magnitude smaller than in our previous report, \(^2\) which is consistent with the scavenging effect oxygen has on the generated radicals.

### 4.4.2 Effect of [PA]_0 under Limited [O2(aq)]

Figure 4.3 shows the decay of \([\text{O}_2(aq)]\) in experiments with \(10.0 \leq [\text{PA}]_0 \leq 200.0 \ \text{mM}\). The results in Figure 4.3 correspond to limited mass transfer of \(\text{O}_2(g)\) to the solution, which differs from the optimized conditions in Figure 4.2. Remarkably, the experiment in Figure 4.2 using a frit creating gas bubbles with a diameter of 25 \(\mu\text{m}\) enabled us to achieve \textit{ca.} complete \(\text{O}_2(aq)\) saturation levels throughout the irradiation period. However, the bubbles created in the experiment of Figure 4.3 were 1.6 mm in diameter causing the complete depletion of \(\text{O}_2(aq)\) after \(\sim 6\) and \(60\) min irradiation of \(200.0\) and \(10.0\) \text{mM PA}, respectively. For higher [PA]_0 the consumption of \(\text{O}_2(aq)\) is accelerated until it levels off indicating depletion conditions in Figure 4.3. Instead, at lower [PA]_0, there is a delay for the first-order decay to reach complete \(\text{O}_2(aq)\) depletion. The initial \(\text{O}_2(aq)\) decay rates (\(R_{-O_2}\)) were obtained from the slopes of the traces in Figure 4.3 in the initial linear range where the reaction is effectively zero order in \(\text{O}_2(aq)\) for each [PA]_0 and plotted in Figure 4.4. The clear exponential rise to maximum correlation between \(R_{-O_2}\) and [PA]_0 in Figure 4.4 predicts the theoretical maximum rate of \(\text{O}_2(aq)\) consumption by ketyl and acetyl radicals as \([\text{PA}]_0 \rightarrow \infty\) (graphically the asymptote) to be \(R_{-O_2,\text{max}} = 43.12 \ \mu\text{M} \ \text{min}^{-1}\).
Figure 4.3. Dissolved oxygen consumption during the photolysis ($\lambda \geq 305$ nm, $I_o = 1.13 \times 10^{-5}$ Einstein L$^{-1}$ s$^{-1}$) for $10 \leq [PA]_0 \leq 200$ mM (as color coded in the figure) under continuous air sparging of 30 mL min$^{-1}$ at pH 1.0 and 25 °C.

Figure 4.4. Initial rate of dissolved oxygen decay, $R_{O_2}$, at variable initial $[PA]_0$ during irradiation of solutions at pH 1.0 under continuous air sparging at a rate of 30 mL min$^{-1}$.

This rate is important to modeling the photolytic $O_2(aq)$ depletion that can occur in aqueous tropospheric aerosols. To do this we can calculate the overall rate constant for the combined reaction of both of the photogenerated radicals from PA with $O_2(aq)$. The reactions involving oxygen that are considered for this estimate are based on the mechanism published in our previous work, shown in Scheme 4.1.²
Upon irradiation of PA (reaction R1) a singlet excited state is produced that undergoes intersystem crossing (ISC) forming the triplet $^3\text{PA}^\ast$, which is thermally lost, regenerating ground state PA (reaction R2), or reacts with ground state PA (reaction R3) via proton coupled electron transfer (PCET) to yield two intermediate radical species, a ketyl radical ($\text{K}^\ast$) and an acyloxy radical ($\text{X}^\ast$). The acyloxy radical $\text{X}^\ast$ decarboxylates quickly and irreversibly to form the acetyl radical, $\text{Y}^\ast$, which most likely gets hydrated.

Bimolecular reactions R5a and R7a indicate how $\text{K}^\ast$ and $\text{Y}^\ast$ are lost in the presence of $\text{O}_2(\text{aq})$ forming their respective peroxyl radicals. Reactions R1-R9 were discussed in detail previously. Reaction R10 is a new pathway not previously discussed, but the generation of singlet oxygen during PA photolysis is demonstrated later in this work.

Reaction R5 and R7 follow the typical bimolecular rate laws shown below:

$$\frac{-d[\text{O}_2]}{dt} = k_{R5}[\text{Y}^\ast][\text{O}_2] \quad (4.3)$$

$$\frac{-d[\text{O}_2]}{dt} = k_{R7}[\text{K}^\ast][\text{O}_2] \quad (4.4)$$

For the limit $[\text{PA}]_0 \to \infty$, the continuous photogeneration of large $[\text{K}^\ast]$ and $[\text{Y}^\ast]$, which are much larger than the remaining $[\text{O}_2(\text{aq})]$, can be included into the pseudo-first order rate constants $k'_{R5} = k_{R5}[\text{Y}^\ast]$ and $k'_{R7} = k_{R7}[\text{K}^\ast]$. Under such a condition, for the maximum rate, $R_{-\text{O}_2,max}$, under initial available $[\text{O}_2(\text{aq})] = 0.260 \text{ mM}$ at $25 \text{ °C}$ and 1 atm (assuming no additional source of $\text{O}_2$) the overall rate constant for the reaction of photogenerated radicals ($\text{R}^\ast = \text{K}^\ast + \text{Y}^\ast$) with $\text{O}_2(\text{aq})$ is estimated to be $k_{\text{R}^\ast+\text{O}_2} = 9.95 \text{ s}^{-1}$. The value of $k_{\text{R}^\ast+\text{O}_2}$ can be used to estimate a lifetime $\tau_{\text{O}_2} = 0.10 \text{ s}$ for $\text{O}_2$ in irradiated solutions with high $[\text{PA}]$. However, under real environmental conditions, dissolved $\text{O}_2$ should be replenished in aqueous particles and films continuously. The depth of
penetration of O$_2$ molecules into these water bodies during the 0.1 s time period can be estimated using the typical diffusion length equation,

$$ t \approx \frac{x^2}{2D} $$

where $t$ is time, $x$ is the distance travelled by molecules and $D$ is the diffusion coefficient of O$_2$ in water, $D_{O_2} = 2.20 \times 10^{-5}$ cm$^2$ s$^{-1}$. Thus, O$_2$ molecules entering aqueous particles through the interface with air can travel at most $6.63 \times 10^{-6}$ m during the 0.1 s when the local oxygen is being consumed by PA radicals. Consequently, aqueous films thicker than 6.6 μm or spherical droplets with diameters larger than 13.3 μm that are also high in [PA] may undergo oxygen depletion under solar irradiation so fast that they are oxygen free environments until PA is exhausted.

Figure 4.5 shows how the rate of oxygen consumption, $R_{O_2}$, for 20 mM PA solutions changes with increased air sparging flow through the solution. All other conditions are identical to those in Figures 4.3 and 4.4 to allow for comparisons. All air flows above the initially tested 30 mL min$^{-1}$ result in solutions where O$_2$(aq) is never completely consumed during the 60 min irradiation period i.e. the [O$_2$(aq)] levels off at a nonzero value after the initial decay. This plot indicates that using the 1.6 mm Teflon tube for continuous air sparging is most effective at the highest air flows, as the mass transfer of oxygen to the solution, $K_La$, for these flows is much higher. However, the bubbling of the solution became too violent above 0.9 L min$^{-1}$ to increase further while the $R_{O_2}$ still had not reached zero, i.e. the point at which mass transfer of O$_2$(g) to the solution equals or exceeds the O$_2$(aq) consumption by the organic radicals generated by PA photolysis.
Figure 4.5. Rate of dissolved oxygen decay, $R_{\text{O}_2}$, at variable air flows during irradiation of 20 mM solutions of PA at pH 1 with continuous air sparging (all $\text{O}_2(aq)$ consumed during reaction).

Consequently, the $K_{L\alpha}$ was increased further by changing the gas used from premixed air to onsite mixing of pure $\text{O}_2(g)$ and $\text{N}_2(g)$ streams, which allowed for variable adjustment of the $\text{O}_2(g)$ mixing ratio in the sparging gas, and therefore increased the maximum $[\text{O}_2(aq)]$ possible in the solution to 1240 $\mu$M at saturation. Figure 4.6 demonstrates the rates of oxygen consumption, $R_{\text{O}_2}$, at these varying compositions of sparging gas for 20 mM PA solutions utilizing a total gas flow of 100 mL$^{-1}$. As discussed earlier for air, for 20 mM solutions with flows greater than 30 mL min$^{-1}$, while $\text{O}_2(aq)$ decays, it is not completely consumed during the 60 min irradiations used here. The relevant conclusion from Figure 4.6 is that even at 100% $\text{O}_2(g)$, these conditions do not force $R_{\text{O}_2}$ to zero as would be ideal for studying this reaction in excess $\text{O}_2(aq)$ which simplifies kinetic analysis. As Figures 4.5 and 4.6 demonstrate, increasing the flow or fraction of $\text{O}_2(g)$ is not an efficient way to increase $K_{L\alpha}$. Therefore, subsequent experiments in a true excess $\text{O}_2(aq)$ environment were achieved by increasing the
interfacial area available for $O_2(g)$ to diffuse across, which increases the efficiency of mass transfer for the reactor.

**Figure 4.6.** Rate of dissolved oxygen decay vs (bottom x-axis) dissolved oxygen concentration and (top x-axis) $O_2$ in $N_2$ gas phase mixing ratio during irradiation of 20 mM solutions of PA at pH 1 with continuous air sparging at 100 mL min$^{-1}$ (all $O_2(aq)$ consumed during reaction).

### 4.4.3 Experiments in excess $O_2(aq)$

After characterizing the limited $K_{La}$ conditions in our original set up with a 1.6 mm ID sparger, a new 25 $\mu$m gas sparger was installed for subsequent experiments. This coarse frit allowed for bubbles of gas with a larger surface area to volume ratio than the previous larger diameter sparger, enhancing $O_2(g)$ molecules’ access to the gas-liquid interface. Complete $O_2(aq)$ saturation throughout the 1h irradiation period even for 100 mM PA solutions was therefore achieved i.e. no $O_2(aq)$ decay was observed during the reactions and instead a constant high $O_2(aq)$ was observed during the full reaction. Now, under kinetically simplified, true $O_2(aq)$ excess, the effects of other parameters, namely pH and
The reaction were able to be explored. Additionally, the significance of the reaction of PA radicals with O$_2$(aq) became even more apparent than previously reported.$^2,^7$

**4.4.4 Effect of pH in excess O$_2$(aq)**

Figure 4.7 shows how the rates of PA loss and DMTA and oxo-C$_7$ production closely follow the speciation curve for pyruvate/PA based on the literature pKa of 2.39 for PA.$^{136}$ Taking the second derivative of each sigmoid shown in Figure 4.7 (for PA, DMTA, oxo-C$_7$) yields an average inflection point of pH = 2.424 ± 0.007, very close to the reported pKa value for PA. Acetic acid does not follow this trend and is instead independent of pH. The oxo-C$_8$ product trend drops with increasing pH but the concentrations are small, so an inflection point was not able to be determined from the data. These results indicate that the reactive species is PA rather than pyruvate and that H$^+$ plays a key role in the reaction. This finding is consistent with the results of kinetic isotope effect (KIE) experiments using D$_2$O as the solvent,$^2$ where the acidic hydrogen is essentially replaced with deuterium, which indicated large primary KIEs for all products resulting from the acidic proton being involved in the key step of each product’s formation mechanism. The importance of the acidic proton to the mechanism suggests that the formation of the initial radical species K and X/Y in steps R3a-c of Scheme 4.1 does not proceed by hydrogen abstraction, and instead involves the transfer of H$^+$, likely between the hydrogen bonded dimer of the excited state PA and a ground state PA, as shown in Scheme 4.1.$^{181}$ This conclusion is also supported by simple bond strength comparisons (H–C 96-105 kcal mol$^{-1}$, H–O 110-119 kcal mol$^{-1}$)$^{93}$ that indicate if hydrogen abstraction were to occur, one of the methyl hydrogens would be abstracted preferentially over the acidic hydrogen of PA or one of the hydroxyl hydrogens from the gem-diol of PA. If the
carboxyl hydrogen was transferred directly from the hydrogen donor to $[^3]{\text{PA}}^*$, theoretical gas phase calculations for the similar case of formic acid carboxyl hydrogen transfer to HO' show that a proton-coupled electron transfer mechanism involving a 5 member ring transition state is still favored over traditional hydrogen abstraction.\textsuperscript{182} In this mechanism, the electron transferred would come from the C=O of the carboxyl group, rather than with the proton transferred (i.e. from the O-H or C-H bond).

Figure 4.7. Rate of decay for (gray circle) PA and rate of production for (blue square) DMTA; (red star) oxo-C$_7$; (gold diamond) acetic acid; and (green triangle) oxo-C$_8$ during irradiation of 100 mM solutions of PA at variable pH with continuous air sparging so [O$_2$(aq)] never reaches zero. Dashed lines are sigmoid fittings to experimental data. The solid black line is the speciation curve for PA based on the reported pKa of 2.39.\textsuperscript{136}

4.4.5 Effect of $I_a$ in excess O$_2$(aq)

Figure 4.8 demonstrates that the reaction rates scale linearly with light intensity. Because quantum yields ($\Phi$) are calculated from rate of formation or decay divided by photon flux, we can report the $\Phi$ for each species as the slope of each linear regression in Figure 4.8, with the error coming from the regression calculation. These $\Phi$ are reported in Table 4.2. The $\Phi$ should be the same when compared across studies performed under the same
conditions and can be included in atmospheric models to help improve understanding of aqueous processing of organic molecules.

**Figure 4.8.** Rate of decay for (gray circle) PA and rate of production for (blue square) DMTA; (red star) oxo-C_{7}; (gold diamond) acetic acid; and (green triangle) oxo-C_{8} during irradiation at variable absorbed light intensities ($I_a$) of 100 mM solutions of PA at pH 1 with continuous air sparging so [O$_2$(aq)] never reaches zero.

**Table 4.2.** Quantum Yields ($\Phi$) at pH 1 and 25°C for photolysis of 100 mM pyruvic acid (PA) solutions. $\Phi$ are calculated from the slopes in Figure 4.8.

<table>
<thead>
<tr>
<th>Species</th>
<th>$\Phi$ (%) in excess O$_2$(aq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA</td>
<td>58.45 ± 2.53</td>
</tr>
<tr>
<td>DMTA</td>
<td>17.73 ± 1.07</td>
</tr>
<tr>
<td>Oxo-C$_7$</td>
<td>7.64 ± 0.44</td>
</tr>
<tr>
<td>Oxo-C$_8$</td>
<td>0.15 ± 0.069</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>3.24 ± 1.49</td>
</tr>
</tbody>
</table>
Based on the proposed mechanism, two PA molecules will be needed to form DMTA, three will be required for oxo-C\textsubscript{7} and oxo-C\textsubscript{8}, and one molecule for acetic acid. A weighted sum of the respective products’ quantum yields in Table 4.2 results in a total value of 62.07 ± 2.93, which is within error of the corresponding overall Φ measured by loss of PA, indicating that these four products (any carbon lost to CO\textsubscript{2} is already accounted for by including products from the other half of the PA molecule lost) account for the vast majority, if not all, of the PA lost under excess O\textsubscript{2}(aq).

![Figure 4.9](image)

**Figure 4.9.** Branching ratio calculated as the rate of DMTA (R\textsubscript{DMTA}) production over the sum of the production rates of the two oxo products (R\textsubscript{oxoC7 + oxoC8}) at variable absorbed light intensity (I\textsubscript{a}) from the data in Figure 4.8.

Even though the reaction rates scale linearly with photon flux, the distribution of products observed shifts toward the higher complexity species, oxo-C\textsubscript{7} and oxo-C\textsubscript{8}. Figure 4.9 shows how the product formation rate ratio changes with the number of photons absorbed by the solution. The oxo branch of the mechanism becomes more favorable at higher light intensity, behavior that can be explained by the increased
conversion of PA at higher light intensities and that the oxo products require a total of three PA molecules, while DMTA requires only two. Upon removing the influence of the required number of PA molecules, i.e. expressing the rates in Figure 4.9 in terms of how many PA molecules are lost (3 for oxo products and 2 for DMTA), the trend persists to a smaller extent (Supporting Information, Figure 4.S2). Even when the influence of the number of PA molecules needed is removed, the ratio $R_{\text{DMTA}}/R_{\text{oxoC7+oxoC8}}$ levels out at ~1.5 at high photon fluxes, indicating the rate constant of the reaction that forms DMTA should be approximately 1.5 times larger than the overall rate constant for the reactions forming oxo-C$_7$ and oxo-C$_8$. The rate constant for combination of two K radicals to yield DMTA was reported previously as $2 \times 10^9$ M$^{-1}$s$^{-1}$, so a reasonable estimate of the combined rate constant for formation of the oxo-C$_8$ and oxo-C$_7$ products is $1.3 \times 10^9$ M$^{-1}$s$^{-1}$.

4.4.6 Significance of reactions involving O$_2$(aq)

In our previous work we reported that as much as 90% of the ketyl radicals produced are recycled back into PA by reacting with O$_2$(aq) in laboratory studies.$^2$ However, in mixed aqueous aerosols, this is potentially a large amount of RO$_2^*$ type radicals, which have been identified as central intermediates in aqueous aerosol oxidations,$^{31}$ able to undergo reactions with other species in the aerosol. In addition to forming the R$^*$ and RO$_2^*$ radicals that are so important to atmospheric chemistry, PA irradiated in the presence of O$_2$(aq) also has the potential to form $^1$O$_2^*$ from reaction with the almost exclusively triplet state photoexcited PA molecules. Figure 4.10 shows how irradiation of solutions of PA across the concentration range estimated to be present in atmospheric aerosol can produce significant steady state concentrations of singlet oxygen. The values measured
here vary with PA concentration in the range $[^1\text{O}_2]_{ss} = 2.05 \times 10^{-12} \text{ M}$ to $8.62 \times 10^{-12} \text{ M}$, while studies of singlet oxygen production from irradiated dissolved organic matter (DOM) collected from fog samples produced on the order of $10^{-13} \text{ M}$ for winter solstice levels of photons. The increase of $[^1\text{O}_2]_{ss}$ with $[\text{PA}]_0$ follows an exponential rise to maximum, with an upper limit of $9.01 \times 10^{-12} \text{ M}$ possible under this set of conditions. This large $[^1\text{O}_2]_{ss}$ indicates that PA in aerosols can be a significant source of atmospheric oxidants on par with natural organic matter. This result suggests that solar irradiation of aerosols containing PA will enhance the destruction of the aromatic products of biomass burning in aerosol which are particularly susceptible to reaction with $^1\text{O}_2$.47

Figure 4.10. Steady state singlet oxygen production, $[^1\text{O}_2]_{ss}$, during 1 h of air saturated photolysis of PA solutions at pH 1 under continuous air sparging with 25 μm sparger at 25 °C for an effective photon flux $I_a = 1.13 \times 10^{-5} \text{ Einstein L}^{-1} \text{ s}^{-1} (\lambda \geq 305 \text{ nm})$.

4.5 Conclusions

This work demonstrates the increasingly understood importance of performing laboratory experiments under adequate oxygenation conditions for atmospheric photochemical processes. We have shown that pyruvic acid, rather than pyruvate, is the photoactive
species required to produce the oligomeric products identified previously,²,⁵ namely 2,3-dimethyl tartaric acid, and the oxo-C⁷ and oxo-C⁸ products, which provides additional indication that the initial reactive step proceeds through a proton coupled electron transfer, rather than a hydrogen abstraction.

We have also shown that concentrations of PA 5 mM and above are capable of generating reactive oxygen species up to an order of magnitude higher even than irradiated field samples of natural fog waters. Furthermore, this work shows that PA is so reactive toward molecular oxygen that some coarse mode aerosols may be oxygen-free environments which could allow for more diverse radical chemistry, but could also be mitigated by dilution effects. Also any aerosols rich in PA will have enhanced oxidation capacity due to the photogeneration of \(^{1}\text{O}_2^\bullet\) and \(\text{RO}_2^\bullet\) radicals by aqueous PA photolysis.

4.6 Acknowledgements
M.I.G. thanks the National Science foundation for research funding under NSF CAREER award CHE-1255290. A.J.E. acknowledges support by the NASA Earth and Space Science Fellowship (NESSF) Program.
4.7 Supporting Information For Photochemistry of Aqueous Pyruvic Acid: The Roles of Dissolved O₂ and pH in the Production of Reactive Oxygen Species

Additional Experimental Section

Furfuryl alcohol (FFA) was used as a probe for singlet oxygen detection in select experiments. In these experiments, the concentration of FFA in each sample was monitored over time (\(t\)) by HPLC to give [FFA]ₜ values. The previous values were dividing by the initial concentration of FFA in the reaction, [FFA]₀, and the natural log of the ratio [FFA]ₜ/[FFA]₀ was plotted against time to get the linear form of the pseudo-first order decay kinetics, as displayed in Figure 4.S1. The slope of the plot is equivalent to the experimental rate constant of singlet oxygen production, \(k_{exp}\) here, which can be divided by the recommended value for the rate constant, \(k_{1O₂+FFA} = 1.08 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}\) (corrected for temperature and salt content),¹⁷⁷ to give the steady state production of singlet oxygen, \([¹⁰₂]_{ss}\).

\[\text{Figure 4.S1. Decay of furfuryl alcohol (FFA) during the photolysis of 100.0 mM pyruvic acid at pH 1.0. The slope is the experimental rate constant for FFA + ¹⁰₂, } k_{exp}.\]
**Figure 4.S2.** Product branching ratios in excess O$_2$(aq) calculated as the rate of PA lost to 2,3-dimethyltartaric acid (RPA$_{\text{DMTA}}$) production over the sum of the PA lost to the production of the two oxo products (RPA$_{\text{oxo}}$) at variable absorbed light intensity ($I_a$) for the data in Figure 4.8 of the main paper.

**Figure 4.S3.** (Red) Benzoic acid and (blue) terephthalic acid HO$^*$ probes during 1 h of air saturated photolysis of 100 mM pyruvic acid solutions at pH 1 under continuous air sparging with 25 μm sparger at 25 °C for an effective photon flux $I_a = 1.13 \times 10^{-5}$ Einstein L$^{-1}$ s$^{-1}$ ($\lambda \geq 305$ nm).
Chapter 5: Enhanced Acidity of Acetic and Pyruvic Acids on the Surface of Water

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![Scheme 5.1. Graphical abstract.](image)

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5.1 Abstract

Understanding the acid-base behavior of carboxylic acids on aqueous interfaces is a fundamental issue in Nature. Surface processes involving carboxylic acids such as acetic and pyruvic acids play roles in 1) the transport of nutrients through cell membranes, 2) the cycling of metabolites relevant to the origin of life, and 3) the photooxidative processing of biogenic and anthropogenic emissions in aerosols and atmospheric waters. Here we report that 50% of gaseous acetic acid and pyruvic acid molecules transfer a proton to the surface of water at pH 2.8 and 1.8 units lower than their respective acidity constants $pK_a = 4.6$ and 2.4 in bulk water. These findings provide key insights on the relative Bronsted acidities of common carboxylic acids versus interfacial water. In addition, the work estimates the reactive uptake coefficient of gaseous pyruvic acid by water to be $\gamma_{PA} = 0.06$. This work is useful to interpret the interfacial behavior of pyruvic acid under low water activity conditions typically found in haze aerosols, clouds, and fog waters.

5.2 Introduction

The study of dissociation constants ($K_a$) at interfaces has been the goal of recent experimental and theoretical work.$^{171, 184}$ Surface sensitive measurements by online electrospray ionization mass spectrometry (OESI-MS) for hexanoic acid and theoretical calculations combined with molecular dynamics simulations for pyruvic acid show a drop in their $pK_a = -\log K_a$ on the surface of water.$^{171, 184}$ OESI-MS reveals signals for the fast collision of gaseous organic acids on the surface of water, sampling $pK_a$ values of the nanometric interface layer that are independent of the behavior of bulk molecules.$^{184}$ A major challenge for this kind of experiment is to quantify the shift in $pK_a$ upon adsorption.
of the gaseous carboxylic acid on the surface of water. OESI-MS should not be confused with the traditional configuration of electrospray ionization mass spectrometry (ESI-MS) that only samples bulk phase composition. Infused solutions into an ESI-MS configuration show p\(K_a\) values of hexanoic and octanoic acid in bulk water that agree well with long-established titration techniques.\(^{185}\) Similarly, surface tension titrations of millimolar concentrations of octanoic acid display an identical p\(K_a\) to the accepted bulk value.\(^{186}\)

Understanding the behavior of carboxylic acids such as acetic and pyruvic acids on the surface of water is a fundamental chemical problem with biochemical, prebiotic chemistry, and environmental implications. For example, the biochemical uptake of pyruvic acid by cells is mediated by a transporter bringing pyruvate across the membrane.\(^{187}\) Also of interest are prebiotic chemical cycles that use pyruvic acid as a central metabolite in interfacial reactions related to the origin of life.\(^{188-191}\) In addition, pyruvic acid is abundant in tropospheric aerosols,\(^{25, 81, 82}\) due to the atmospheric photooxidative processing of biogenic and anthropogenic emissions.\(^{31, 38, 169}\) As a chromophore, pyruvic acid is one of the few carboxylic acids in the atmosphere that can undergo solar photochemistry in the aqueous phase.\(^{1, 2, 6, 70, 157, 168}\) Thus, the transfer of gaseous pyruvic acid to the aqueous phase, with a large Henry’s law constant (\(K_H = 3.1\) mol kg\(^{-1}\) Pa\(^{-1}\)),\(^{170}\) is of particular atmospheric interest. The C=O group of pyruvic acid (p\(K_a\) = 2.39 in bulk water)\(^{136}\) undergoes cooperative reversible hydration (with equilibrium constant \(K_{\text{Hyd}} = 1.83\)) into 2,2-dihydroxypropanoic acid, a UV transparent gem-diol.\(^{21}\)
The complexity of considering the orientation of pyruvic acid within the accommodation process on the surface of water and the effect of pH on photoreactivity changes demand a better understanding of the acid-base properties that pyruvic acid senses and exerts at the air-water interface. In addition, to the best of our knowledge there is no literature available reporting the reactive uptake coefficient of gaseous pyruvic acid by water ($\gamma_{PA}$). Thus, this work uses a customized OESI-MS setup to study the acid dissociation constants of acetic and pyruvic acids. The Bronsted basicity of the air-water interface has been characterized by OESI-MS in a different setup. Herein, we contrast the p$K_a$ of acetic or pyruvic acids in water by ESI-MS with those measured by OESI-MS on the surface of pH-adjusted microdroplets impinged by gas phase molecules of the acids. Using reliable parameters for acetic acid and present measurements allows the estimation of $\gamma_{PA}$.

5.3 Materials and Methods

5.3.1 Sample Preparation for Experiments in Water

Aqueous solutions were prepared from pyruvic acid (Sigma-Aldrich, 98.5%, distilled under vacuum) and acetic acid (glacial, $\geq$99.7%, Acros) with formula masses of 88.06 and 60.05 Da, respectively. All solutions were prepared in ultrapure water (Elga Purelab flex, 18.2 MΩ cm$^{-1}$). Sodium hydroxide (NaOH, Acros, 0.1 N) and hydrochloric acid (HCl, EMD, 12.1 M) were used to prepare stock solutions for pH adjustment. The dropwise addition of HCl and NaOH solutions was performed under continuous stirring with burets (Kimble KIMAX, Class A) and measured with a calibrated pH meter (Mettler Toledo). Throughout, the pH values reported herein are the pH values measured in the bulk solutions prior to infusion into the nebulizing chamber. Since pH represents
chemical potential, all phases in equilibrium, viz., interfacial and bulk water, have the same pH.

5.3.2 Sample Preparation for Experiments on the Surface of Water

A 1.43 M pyruvic acid solution in water held at 25.0 °C in a wash bottle for gases and immersed in a chiller bath (Thermo Scientific A25) was sparged with a 0.10 L min⁻¹ flow of N₂(g). The gas carrying pyruvic acid was then mixed with a 0.10 L min⁻¹ flow of N₂(g) using a custom built gas proportioner. After dilution, the total flow of 0.20 L min⁻¹ was transported through a stainless steel tube to the aerosolization chamber. Similarly, a 1.00 M acetic acid solution in water or cyclohexanol (J.T. Baker, 100%) was held in the wash bottle for gases at 17.0 °C and sparged with a 0.020 L min⁻¹ flow of N₂(g). The gas carrying acetic acid was then diluted with a 2.150 L min⁻¹ flow of N₂(g) using a gas proportioner. A common inlet flowmeter was used to divert only 0.20 L min⁻¹ of the diluted acetic acid into the aerosolization chamber.

5.3.3 Instrumental Conditions

The conditions for ESI-MS and OESI-MS measurements were set to a nebulizer voltage of -2.2 kV, a cone voltage of 40 V, and a nebulizer pressure of 70 psi N₂(g). The only difference was the drying gas temperature of 350 °C in ESI-MS and 50 °C in OESI-MS. All reported titration curves were built by plotting normalized I₅⁹ and I₈⁷ values for the anions CH₃COO⁻ and CH₃COCOO⁻ of acetic and pyruvic acids, respectively, versus the measured bulk pH of the solution from which the droplets were formed. The largest registered ion count in each set of experiments was arbitrarily assigned unity value and used to normalize all other Iₘ/z values. The mass spectrum of each sample was acquired at fixed time intervals (e.g., time ≥1 min).
5.3.4 ESI-MS

Solutions were pumped at 50 µL min$^{-1}$ into the pneumatically assisted ESI interface coupled to a single quadrupole MS (Thermo Scientific MS Q Plus) described in detail before.$^{85}$ For experiments “in” water, the solutions of acetic and pyruvic acids at the adjusted pH were directly infused for single ion monitoring (SIM) in the negative ionization mode registering the ion count ($I_{m/z}$) at the mass-to-charge ratios ($m/z$) 87 and 59, respectively.

5.3.5 OESI-MS

For experiments “on” the surface of water, the 0.20 L min$^{-1}$ flow of N$_2$(g) carrying the carboxylic acid was directed via an electrically grounded stainless steel tube to collide with the generated microdroplets.$^{8,192}$ For the production of microdroplets, solutions were pumped at 50 µL min$^{-1}$ into the same electrospray probe of the MSQ Plus instrument mentioned above. The gas-phase molecules impinging the microdroplets during a contact time $\tau_c > 1$ µs generate signals that sense the interface of water.$^{8,192}$ In this setup the exhaust of the gas mixture was positioned 32 mm away from the entrance cone and 12 mm from the needle tip carrying the solution. Furthermore, a final 61 times dilution with the N$_2$(g) nebulizing gas (12.0 L min$^{-1}$) occurred in this chamber.$^{192}$ The fact that the same products are identified after offline analysis of surface reactions lasting a few hours and in situ studies under a few microseconds of contact time validates the OESI-MS setup for inspecting reactions at the air–water interface.$^{8,9}$

The temperature-dependent vapor pressure of the acids$^{193}$ was used to calculate the final mixing ratios of 2.00 and 1.40 ppmv for acetic and pyruvic acids, respectively. These final mixing ratios are the ones encountered by the surface of continuously
generated aqueous microdroplets of adjusted pH. The gas-phase molecules of acetic or pyruvic acids accommodate on the surface of the microdroplets prepared at fixed pH values, which were measured with the calibrated pH meter. Deprotonation proceeds only during this encounter with the first generation of microdroplets that conserve the measured bulk pH. *A posteriori* of deprotonation and with the help of the desolvation gas, the cascade breakup of the particles forming ions proceeds, which are detected by the mass spectrometer within 1 ms in the negative ionization mode.

### 5.4 Results and Discussion

#### 5.4.1 Experiments “in” Water Solution

Figure 5.1 displays the titration curve for acetic acid in water determined by ESI-MS in a typical experiment. Considering the Henderson-Hasselbalch equation, $I_{59}$ values are directly proportional ($\propto$) to the measured dissociation fraction of acetate in Figure 5.1:

$$\alpha_{\text{CH}_3\text{COO}^-} = \frac{[\text{CH}_3\text{COO}^-]}{[\text{CH}_3\text{COO}^-] + [\text{CH}_3\text{COOH}]} \propto I_{59} \tag{5.1}$$

The dissociation fraction of acetate (and the representative normalized $I_{59}$ value) span from $\alpha_{\text{CH}_3\text{COO}^-} = 0$ for the bulk solution with only undissociated acid up to $\alpha_{\text{CH}_3\text{COO}^-} = 1$ when only the conjugate base is present. The inflection point of the characteristic sigmoid curve fitting the dataset in Figure 5.1 marks the $pK_a$, which indicates at which bulk acid strength the concentrations of the acid and base forms are equal. More specifically, the inflection point is located at $I_{59} \approx \alpha_{\text{CH}_3\text{COO}^-} = 0.5$, where $p\text{H} = pK_a = 4.55$, and the asymptote tends to 1.
Figure 5.1. (A) ESI-MS titration curve for 100 µM acetic acid in water. ○) The normalized ion count for acetate anion at m/z 59, \(I_{59}\), as a function of pH fitted with a (red line) sigmoid curve with coefficient of correlation \(r^2 = 0.990\). (B) (Blue line) First derivative of the sigmoid curve in (A), where the maximum corresponds to the \(pK_a\).

The titration curve for pyruvic acid in water obtained by ESI-MS is presented in Figure 5.2. The normalized \(I_{87}\) values are also directly proportional to the measured dissociation fractions of pyruvate at variable pH in Figure 5.2:

\[
\alpha_{\text{CH}_3\text{COCOO}^-} = \frac{[\text{CH}_3\text{COCOO}^-]}{([\text{CH}_3\text{COCOO}^-] + [\text{CH}_3\text{COCOOH}])} \propto I_{87} \quad (5.2)
\]

As explained above for the case of acetate, the normalized dissociation fraction of pyruvate is also limited to the range \(0 \leq \alpha_{\text{CH}_3\text{COCOO}^-} \leq 1\). The inflection point for pyruvic acid in water is located at \(I_{87} \cong \alpha_{\text{CH}_3\text{COCOO}^-} = 0.5\), where pH = \(pK_a = 2.43\) (Figure 5.2).

The nonlinear fittings in Figures 5.1 and 5.2 with correlations \(r^2 \geq 0.98\) allow the determination of the \(pK_a\) values by reproducing the titration curves of acetic and pyruvic acids in bulk solutions with < 5.0% error relative to respective literature values of 4.75 and 2.39.136 Any deviations from literature \(pK_a\) values possibly due to electric fields at the surface of water,194 are therefore negligible. and can be disregarded. This statement is
corroborated by the fact that both gas-phase bases (like trimethylamine) and acids are protonated and deprotonated on the surface of water of pH < 4. Electric fields do not perturb the charge balance of the double layer, or otherwise they should have had opposite effects on the titration of acids and bases on the surface of water.\textsuperscript{184,185}

**Figure 5.2.** (A) ESI-MS titration curve for 100 $\mu$M pyruvic acid in water. (○) The normalized ion count for pyruvate anion at $m/z$ 87, $I_{87}$, as a function of pH fitted with a (red line) sigmoid curve with coefficient of correlation $r^2 = 0.988$. (B) (Blue line) First derivative of the sigmoid curve in (A), where the maximum corresponds to the p$K_a$.

Therefore, this procedure is valid for determining p$K_a$ values of low molecular weight carboxylic acids, in agreement with previous observations made for $n$-hexanoic and $n$-octanoic acids with a different ESI-MS setup.\textsuperscript{185} Thus, the thoughtful determination of p$K_a$ values implies that standard Equations 5.3 and 5.4 also govern the deprotonation behavior registered for acetic and pyruvic acids, respectively:\textsuperscript{185}

\begin{equation}
\alpha_{\text{CH}_3\text{COO}^-} = \frac{1}{1 + 10^{m_{\text{CH}_3\text{COOH}}(pK_a^{\text{CH}_3\text{COO}^-} - \text{pH})}} \tag{5.3}
\end{equation}

\begin{equation}
\alpha_{\text{CH}_3\text{COO}^-} = \frac{1}{1 + 10^{m_{\text{CH}_3\text{COO}^-}(pK_a^{\text{CH}_3\text{COO}^-} - \text{pH})}} \tag{5.4}
\end{equation}
where $m_{CH_3COOH}$ and $m_{CH_3COCOOH}$ are the slopes of the respective sigmoid curves that in traditional titration methods take unity value, and the dissociation fractions clearly depend on the difference ($pK_a - pH$) rather than only on pH. Therefore, the observed titration curves reflect functions in which neither $pK_a$ nor pH have practically shifted relative to their bulk values. Thus, it can be deduced that the pH of the experiments in Figures 5.1 and 5.2 cannot be discriminated from that in bulk solution.

5.4.2 Experiments “on” the Surface of Water

Figure 5.3 shows the results for a typical OESI-MS experiment detecting acetate generated from the dissociation of gaseous acetic acid deposited on the surface of water adjusted to variable pH with HCl and NaOH. In this case acetic acid is carried out by a flow of N$_2$(g) from an aqueous solution before it collides on the surface of the aqueous microdroplets.

**Figure 5.3.** (A) OESI-MS titration curve for acetic acid (from a solution in water) deposited on the surface of water for microdroplets exposed to 2.00 ppmv ($\approx 4.55 \times 10^{13}$ molecules cm$^{-3}$ at the experimental conditions) for a contact time $\tau_c > 1$ $\mu$s. (○) The normalized ion count for acetate anion at $m/z$ 59, $I_{59}$, as a function of pH fitted with a (red line) sigmoid curve with coefficient of correlation $r^2 = 0.986$. (B) (Blue line) First derivative of the sigmoid curve in (A), where the maximum corresponds to the $pK_a$. 

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The inflection point for the sigmoid fitting of Figure 5.3A, matching the maxima for its first derivative in Figure 5.3B indicates a $pK_a = 1.78$ for gaseous acetic acid deposited on the surface of water.

Figure 5.4 displays the OESI-MS titration curve for gaseous acetic acid sparged on the surface of water after it was carried out from a solution in cyclohexanol by a flow of $N_2(g)$. The use of cyclohexanol discards that water vapor carried with the stream of carboxylic acid has any effect on the measured $pK_a$. The experiment in Figure 5.4 shows a $pK_a = 1.71$, which is only 3.9% different from that determined using water as the starting solvent (Figure 5.3). The key finding here is that the measured $pK_a$ for CH$_3$COOH($g$) deposited on the surface of water (Figures 5.3 and 5.4) drops 2.75($\pm$0.05) pH units relative to CH$_3$COOH($aq$) in bulk water (Figure 5.1).

Figure 5.4. (A) OESI-MS titration curve for acetic acid (from a solution in cyclohexanol) deposited on the surface of water for microdroplets exposed to 2.00 ppmv ($\equiv 4.55 \times 10^{13}$ molecules cm$^{-3}$ at the experimental conditions) for a contact time $\tau_c > 1$ $\mu$s. ($\circ$) The normalized ion count for acetate anion at $m/z$ 59, $I_{59}$, as a function of pH fitted with a (red line) sigmoid curve with coefficient of correlation $r^2 = 0.987$. (B) (Blue line) First derivative of the sigmoid curve in (A), where the maximum corresponds to the $pK_a$. 

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By contrasting the results in Figure 5.1 with those in Figures 5.3 and 5.4, it is evident that CH₃COOH(g) on the air side of the interface behaves as a 590 times stronger Bronsted acid than CH₃COOH(aq) on the water side of the interface under the same bulk pH. In addition, these results confirm that the detection of I₅⁹ corresponds to acetate ions that have not yet dissolved in the bulk of the microdroplets, but that are produced from the air side of the interface. Otherwise, there should be no difference in the pKₐ values of the titrations in Figures 5.1 with those in Figures 5.3 and 5.4.

The experiments in Figures 5.3 and 5.4 employed a high mixing ratio of 2.00 ppmv CH₃COOH(g), which is larger than the maximum 300 ppbv (≡ 7.39 × 10¹² molecules cm⁻³) of organic acid needed to reach the plateau for Langmuir adsorption in this kind of system. Therefore, the available water surface of generated microdroplets is fully covered by impinging acetic acid molecules. Under these conditions, it is clear that gaseous acetic acid does not transfer its proton to neutral water molecules directly, but to the interfacial dangling basic groups, HO⁻, which limit the amount of CH₃COO⁻ ions generated and detected. The surface density of HO⁻ toward the air side of the aqueous microdroplet interface, S_HO⁻_interface, available to interact with CH₃COOH(g) molecules can be estimated from the product of two terms, the frequency of collisions provided by the kinetics theory of gases and τ_c ≥ 1 μs:

\[
S_{HO^-_{interface}} = \frac{\gamma_{AA} \nu_{AA} n}{4} \times \tau_c
\]

(5.5)

where the reactive uptake coefficient for acetic acid is \(\gamma_{AA} = 0.05\),¹⁹⁶ the mean thermal velocity of gaseous acetic acid at 298 K is \(\nu_{AA} = 3.52 \times 10^4\) cm s⁻¹, and for 300 ppbv
acetic acid the number density is \( n = 7.39 \times 10^{12} \) molecules cm\(^{-3}\). By substitution of the previous values in Equation 5.5, an estimate \( S_{\text{HO}^-_{\text{interface}}} = 3.25 \times 10^9 \) HO\(^-\) cm\(^{-2}\) is obtained for the experiments in Figures 5.3 and 5.4, which is equivalent to a surface charge density \( q = 0.52 \) nC cm\(^{-2}\). The reported \( S_{\text{HO}^-_{\text{interface}}} \) and its associated \( q \) value in our aqueous microdroplet setup are in excellent agreement with those reported from experiments with hexanoic acid sparged on the surface of a water jet during 10 µs contact time.\(^{184}\) Previous work has demonstrated that anions can populate the interface of water with air at depths that are inversely proportional to the square of their size, as represented by the ionic radius, even for dissimilar concentrations affecting the sublayers.\(^{85, 197, 198}\) Thus, the fact that \( q \) is \( 2 \times 10^3 \) times smaller than observed from \( \zeta \)-potential measurements in the electrophoresis of bubbles and oil droplets in water\(^{195, 199}\) is logically explained as reflecting experiments that have tested the basicity of water at different depths.\(^{184}\)

When a gas stream of pyruvic acid, generated by a flow of \( \text{N}_2(\text{g}) \) through a solution in water, encounters the interface of the pH-adjusted aqueous microdroplets, pyruvate is immediately formed by dissociation (Figure 5.5). The sigmoid fitting of Figure 5.5A for gaseous pyruvic acid deposited on the surface of water at variable pH has an inflection point at \( pK_a = 0.65 \) matching the maxima for its first derivative in Figure 5.5B. By comparison, the measured \( pK_a \) for \( \text{CH}_3\text{COCOOH(\text{g})} \) deposited on the surface of water (Figures 5.5) drops 1.78 pH units relative to \( \text{CH}_3\text{COCOOH(\text{aq})} \) in bulk water (Figure 5.2). Thus, the air side of the interface senses \( \text{CH}_3\text{COCOOH(\text{g})} \) to be 55 times stronger as a Bronsted acid than \( \text{CH}_3\text{COCOOH(\text{aq})} \) on the water side of the interface.
Figure 5.5. (A) OESI-MS titration curve for pyruvic acid (from a solution in water) deposited on the surface of water for microdroplets exposed to 1.40 ppmv (= 3.28 × 10^{13} molecules cm^{-3} at the experimental conditions) for a contact time τ_c > 1 μs. (○) The normalized ion count for acetate anion at m/z 87, I_{87}, as a function of pH fitted with a (red line) sigmoid curve with coefficient of correlation r^2 = 0.967. (B) (Blue line) First derivative of the sigmoid curve in (A), where the maximum corresponds to the pK_a.

The high [CH_3COCOOH(g)] = 3.28 × 10^{13} molecules cm^{-3} in the experiment of Figure 5.5 also exceeds the expected plateau for Langmuir adsorption of low molecular weight carboxylic acids. A reasonable estimate for the reactive uptake coefficient of gaseous pyruvic acid by water of γ_{PA} = 0.06 can be retrieved after rearranging Equation 5.5 for this molecule as follows:

\[
γ_{PA} = \frac{4 S_{\text{HO}^-_{\text{interface}}}}{n_{PA} \tau_c}
\] (5.6)

where the mean thermal velocity used is that of CH_3COCOOH(g) at 298 K, v_{PA} = 2.91 × 10^4 cm s^{-1}, n = 7.39 × 10^{12} molecules cm^{-3} \cite{184}, τ_c = 1 × 10^{-6} s for this system, \cite{192} and the value S_{\text{HO}^-_{\text{interface}}} = 3.25 × 10^9 HO^- cm^{-2} is obtained above using acetic acid.

It is important to highlight that before this study only one experimental report had tackled the investigation of pK_a shifts by observing a transfer of protons initiated from the
air side of the air-water interface.\textsuperscript{184} It should be emphasized that Bronsted acidity and basicity are relative concepts that describe the extent of proton transfer of acids and bases with the local solvent. Herein, we demonstrate that the surface of water is more basic than the bulk, meaning that it is a better proton acceptor than bulk water. Vibrational sum frequency spectroscopy (VSFS) experiments on interfaces of aqueous solutions of acetic acid (of unreported pH) revealed the sole presence of undissociated acid,\textsuperscript{200} even at the lowest acid concentrations, which exceeded those used in our experiments by a factor of 3. VSFS experiments were apparently unable to detect acetate,\textsuperscript{200} possibly due to limitations on the alignment of moieties at the interface.\textsuperscript{201} Furthermore, we wish to point out the absence of VSFS experiments on acetate solutions.\textsuperscript{200} We consider that VSFS data are moot regarding the extent of dissociation of acetic acid on the surface of its aqueous solutions.\textsuperscript{200} In short, acidity is a property that depends on the medium, and is defined by the local equilibrium constant for the process $\text{AH} + \text{H}_2\text{O} \rightleftharpoons \text{A}^- + \text{H}_3\text{O}^+$. This equilibrium may have different $pK_a$ values at the surface vs in the bulk because the acid and water may have different acid-base properties due to the differential hydration of reactants and products, as discussed in the next section.

5.4.3 Dependence of the $pK_a$ shifts on the Selective Hydration of Acid/Base Pairs

The $pK_a$ shift of 2.75 units from the bulk phase to the surface of water for acetic acid agrees well with observations for hexanoic and octanoic acids.\textsuperscript{184, 185} Similarly to simple monocarboxylic acids, the impinging gaseous acetic acid molecule has its $-\text{COOH}$ group oriented in an upright position with respect to the surface.\textsuperscript{202} In other words, when gaseous acetic acid reaches the local plane of the interface, its $-\text{COOH}$ group points to
the aqueous core with the plane established by the –COOH group with a C$_{sp^2}$ oriented perpendicularly to the surface plane. Simultaneously, the hydrophobic –CH$_3$ of acetic acid is repelled into the air, aligning the axis defined by the C-C bond close to the surface normal (with a tilt ≤ 15° from the surface normal).$^{202}$ A similar assumption could be made for the corresponding –COO$^-$ group$^{203}$ from quickly formed acetate after proton loss of the acid form that has accommodated at the interface.

Additional water molecules can contribute to hydrate the –COO$^-$ group formed at the interface, changing the amphiphilic balance of the molecule.$^{204}$ On this basis, photoelectron spectroscopy and VSFS measurements have proposed an enrichment of acetic acid relative to acetate occurs at the interface.$^{200, 204}$ However, we interpret the previous spectroscopy studies of acetic acid solutions to have sampled the interior layer of the interface of acetic acid solutions. Instead, in our work the external layers of the interface are directly sampled as gaseous acetic acid collides with water, showing that the acid has a larger capacity to transfer its proton to surface water (that behaves as a base) than in the bulk. Therefore, acetic acid behaves as a stronger acid at the interface and the ratio [–COO$^-$]/[–COOH] sensed from the air side of the air-water interface is larger than in the bulk. In other words, the interfacial ratio [–COO$^-$]/[–COOH] cannot be predicted by bulk pK$_a$ values.

For pyruvic acid a smaller pK$_a$ shift of 1.78 units is observed, which is in agreement with the 1.84 pK$_a$ units shift computed at the quartz-water interface using a combination of ab initio molecular dynamics simulations.$^{171}$ While the likelihood of natural hydrophobic quartz surfaces is low (covered with siloxane Si-O-Si bridges), Parashar et al. modeled the most common hydrophilic quartz surface with –OH groups from the
Because the orientation of pyruvic acid at the air-water interface remains unexplored, the quartz-water model serves here as a first-order approximation for visualizing the behavior of the hydrophilic –OH groups from the terminal silanols in contact with water. Related VSFS measurements show that the interfacial potential at the quartz-water and air-water interfaces for the surface adsorption of lysozyme is controlled by the protonation/deprotonation of the protein’s groups. While at the air-water interface some lysozyme residues are organized at the hydrophobic air surface, major changes are only observed on the hydrophilic quartz-water interface for pH > 8.0, when a net negative charge is developed. Therefore, such an extrapolation of the interfaces provides an initial model to interpret the mechanism by which gaseous pyruvic acid interacts with the surface of water under the experimental pH conditions explored in Figure 5.5.

At variance with the perpendicular geometry for acetic acid, the –COOH group of pyruvic acid is pictured to be practically plane parallel to the surface of water (as shown in the graphical abstract, Scheme 5.1). Remarkably, radial distribution functions for pyruvic acid and pyruvate with the oxygen and hydrogen atoms of water, respectively, suggest the acid remains localized with the C=O accommodated on the surface of water while getting hydrated. The previous configuration constrains the hydrophobic –CH₃ group of the acid to be in contact with the surface of water. Therefore, the different surface propensity of pyruvic acid relative to acetic acid also affects the interfacial abundance of each acid/base pair. Pyruvate also plays an important role favoring the dissociation of pyruvic acid at the interface. On the surface, pyruvate not only forms hydrogen bonds with water from the first monolayer but as it gets buried deeper than the
neutral molecule, it also gains additional stabilization by H bonds with the contiguous layer of water.\textsuperscript{171} Thus, the enhanced acidity of pyruvic acid at the interface is ascribed to the differential microsolvation of the acid vs its conjugated base. The p$K_a$ shift is associated with the molecular orientation of pyruvic acid/pyruvate at the air-water interface as well as the local structure and proton-accepting properties of interfacial water.\textsuperscript{2}

The results presented in the previous section for gaseous acetic and pyruvic acids follow the thermodynamically favorable interfacial proton transfer behavior\textsuperscript{184} dictated by reactions R1 and R2 with dissociation constants $K_{a,R1} = 1.78 \times 10^{-2}$ and $K_{a,R2} = 2.24 \times 10^{-1}$, respectively:

\begin{align}
\text{CH}_3\text{COOH}(g) + \text{HO}_{\text{interface}}^-(g) & \rightleftharpoons \text{CH}_3\text{COO}^-(g) + \text{H}_2\text{O}(g) \\
\text{CH}_3\text{COCOOH}(g) + \text{HO}_{\text{interface}}^-(g) & \rightleftharpoons \text{CH}_3\text{COCOO}^-(g) + \text{H}_2\text{O}(g)
\end{align}

(R1) \hspace{2cm} (R2)

In the surface process studied here, increased dissociation constants are observed for both acids relative to bulk water. An alternative explanation to the smaller acidity shift exerted by pyruvic acid involves an assessment of the larger net water cluster containing the $\text{HO}_{\text{interface}}^-$ that participates in reaction R2 relative to R1. Comparing the results for both acids on the surface of water (Figure 5.5 vs Figures 5.3 and 5.4) indicates that the experimentally determined ratio $K_{a,R2}/K_{a,R1} = 12.44$:

\begin{equation}
\frac{K_{a,R2}}{K_{a,R1}} = \frac{[\text{CH}_3\text{COCOO}^-](1+\chi)[\text{H}_2\text{O}(g)]}{[\text{CH}_3\text{COCOOH}(g)][\text{HO}_{\text{interface}}^-(g)]} \times \frac{[\text{CH}_3\text{COOH}(g)][\text{HO}_{\text{interface}}^-(g)]}{[\text{CH}_3\text{COO}^-](g)[\text{H}_2\text{O}(g)]}
\end{equation}
where the factor \((1 + x)\) is included to account for the larger cluster. A simple numerical solution of Equation 5.7, \(12.44 = (1 + x)/1\), is obtained by assuming a constant level of \(\text{HO}^-_{\text{interface}}(g)\) at \(\text{pH} = \text{p}K_a\), when both ratios of dissociated to undissociated acid cancel out as they equal 1/2 each, and that other processes such as the reversible hydration of the carbonyl group of pyruvic acid, the formation of stabilizing hydrogen bonds with the acid/base pair, etc., demand \(x\) \(\text{H}_2\text{O}(g)\) molecules more for reaction R2 than acetic acid requires in reaction R1. Thus, close interactions with water clusters are formed by gaseous pyruvic acid and the freshly formed pyruvate during and after proton transfer to \(\text{HO}^-_{\text{interface}}\). These water clusters are about \(x = 11\) \(\text{H}_2\text{O}(g)\) molecules larger than for acetic acid/acetate.

### 5.5 Conclusions

The different \(\text{p}K_a\) values for acetic and pyruvic acids on the surface of water relative to the bulk point out the ability of interfacial water molecules to accept protons at lower pH. The variable \(\text{p}K_a\) shifts for each acid from the bulk to the surface of water is due primarily to the different local equilibrium constants and interfacial accommodation and secondarily to changes in the surface hydration of each acid/base pair. Pyruvic acid and pyruvate, by having a carbonyl group, may interact with a larger number of interfacial water molecules than acetic acid. Future work should aim to optimize the minimum energy orientation of pyruvic acid and pyruvate at the air-water interface also needed to evaluate the difference in the free energy of hydration of both acids at the interface.
Calculations should estimate hydration energies and entropies to be matched with our experimental pK_a measurements.

This work has explored for the first time the transfer of protons to the air-water interface by acetic acid and pyruvic acid. A value of 0.06 has been estimated for the reactive uptake coefficient of gaseous pyruvic acid by water. The new understanding gained by contrasting the role of carboxylic acids “in” water and “on” the surface of water is of major atmospheric relevance.\textsuperscript{206} Under environmentally relevant conditions of atmospheric particles with pH < 5, both acetic and pyruvic acids spontaneously transfer protons to the interfacial dangling basic groups, $\text{HO}^-_{\text{interface}}$, of water. Remarkably, the size of the water cluster involved in the previous uptake is larger than for acetic acid. The previous observation implies that the accommodation of pyruvic acid at the air-water interface must involve the participation of a carbonyl moiety (which undergoes reversible hydration) in addition to the −COOH group. For example, as the pH of the air-water interface drops when any carboxylic acid yields its proton, the molecules of pyruvic acid available in these nanoscopic layers can become more photoreactive as the undissociated fraction grows. Therefore, the photochemical formation of reactive ketyl and acetyl radicals from the $\lambda > 295$ nm photolysis of pyruvic acid is favored,\textsuperscript{2, 168} contributing to the environmental processing of lipids,\textsuperscript{6} and as a potential source of complex secondary organic aerosol generation.\textsuperscript{2}
5.6 Acknowledgements

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Chapter 6: Dissertation Conclusions

The formation of organic aerosol and SOA from aqueous phase oxidation reactions in atmospheric water is underpredicted in current atmospheric models, very likely because there are unknown sources of SOA formation. This vastly affects the uncertainties of estimates of atmospheric aerosol loading and hence the effect that organic aerosols have on the energy balance of Earth’s climate. In Chapter 2, it was shown that aqueous GA irradiation under atmospheric conditions was able to produce glyoxal, a reactive dicarbonyl species responsible for the production of as much as 14% of summertime SOA over the southeast United States alone. This corresponds to an additional 0.8 μg m⁻³ of SOA contributed to the local atmosphere from glyoxal. The UV-visible and fluorescence study of glyoxylic acid photoproducts indicated that irradiation-dark aging cycles that imitate day/night changes allowed for destruction and then regeneration of solar actinic absorbing compounds, a behavior which was also previously observed for PA. This indicates that real atmospheric aerosols made up of these kinds of species will likely have large fluctuations in their optical properties, influencing their contributions to the total radiative forcing of organic aerosols (RFari). The results of Chapter 2 indicated that the presence of electrolytes and air reduces the thermochromism recovered during dark aging. The overall trend across all four processing stages shows a shrinking absorption profile with increasing number of light-dark diurnal mimicking cycles, an indication that these types of aerosols will absorb less and less incoming radiation while they age, contributing a net cooling effect on the local environment.

The quantum yield of GA photodecomposition (ΦGA = 1.78% for pH 1, with background electrolytes: [Na⁺] = 468 mM; [Cl⁻] = 545 mM; [SO₄²⁻] = 28.2 mM under air
saturated conditions) is now available for inclusion in atmospheric models, and can help to improve current estimates of atmospheric SOA loadings. The work in this dissertation allows for a comparison of the importance of direct photolysis as a glyoxylic acid fate with indirect oxidation by HO’. The comparison indicates that direct photolysis is, while not the primary fate, important enough to be included in models. The lifetimes of GA against the two possible aqueous phase processes are 11 days for direct photolysis vs 5 days for reaction with HO’. Assuming these are the two dominant aqueous phase processes experienced by GA, which they should be, then about 4.99 Tg GA per year\(^{43}\) are lost to direct photolysis, yielding glyoxal, formic acid, oxalic acid, tartaric acid, CO and CO\(_2\). The molar yield of glyoxal at early reaction times is about 40% of the GA consumed, which represents the lower limit of glyoxal that can be produced from GA under these conditions because it is at steady state. Therefore an estimated 1.99 Tg GA is converted to glyoxal through aqueous phase processing each year yielding at least 1.56 Tg of glyoxal per year or 3.5% of the total 45 Tg estimated to be emitted per year.\(^{131}\) A recent study on SOA formation from glyoxal determined that about 5 \(\mu\text{g m}^{-3}\) SOA could be generated by processing of glyoxal at a level of 50 mM in the aqueous phase,\(^{208}\) indicating that the glyoxal generated from GA photolysis ([glyoxal]\(_{ss}\) = 2 mM) may contribute more than 0.2 \(\mu\text{g m}^{-3}\) to the global yearly SOA budget, equivalent to 3.5% of the summertime SOA observed over the southeastern United States.\(^{207}\)

By comparison, the quantum yield measured for PA in chapter 3 verified that direct photolysis was the dominant aqueous fate, and that it is able to compete significantly with gas phase photolysis because of the ease with which gaseous pyruvic acid partitions into atmospheric water. The large partitioning is in part explained by the enhanced acidity of
carboxylic acids at the air-water interface of aerosols that was demonstrated in chapter 5. This enhanced acidity describes a shift in equilibrium that provides more of the highly water soluble carboxylate anion to the interface, increasing the likelihood of carboxylic acids dissolving into the bulk phase of atmospheric water to undergo subsequent chemical reactions. Given the relative lifetimes of PA toward direct photolysis and HO\textsuperscript{•} oxidation in both the gas and aqueous phases discussed in chapter 3, direct aqueous photolysis is likely responsible for at least 34\% (based on the rate constants for each process) of the PA destroyed in the atmosphere each year or 0.292 Tg or of PA per year, second only to gas phase photolysis. The quantum yields measured in excess oxygen in chapter 4 show that each PA molecule lost contributes to an oxidized organic product that will remain in the particle phase to contribute to SOA. Using the relative product yields of the direct photolysis mechanism indicated by the quantum yields in table 5.2, the total SOA burden of direct aqueous photolysis from PA is estimated to be 0.257 Tg per year.

This SOA production is not as high as what is expected from GA, mostly due to the lower amounts of PA found in the atmosphere, but the high reactivity of the radicals photogenerated from PA toward dissolved oxygen and, ostensibly, other organic compounds that could be present in atmospheric aerosols suggests that PA may act as an intermediate or promotor or direct oxidant in various atmospheric chemical cycles with a central role similar to its role in biochemical cycles. Indeed, recent studies have already shown that the high steady state radical concentrations produced by irradiation of PA is sufficient to enhance MRI sensitivity,\textsuperscript{209} and to promote reactivity of other organic compounds, including GA (see Appendix A).\textsuperscript{210} PA photolysis was also found to be a source of singlet oxygen missing from current models, with a singlet oxygen production.
an order of magnitude higher (ca. $10^{-12}$ M vs. $10^{-13}$ M) than that of irradiated natural organic matter extracted from fog water. Overall, this work has contributed to a better understanding of aqueous SOA formation pathways and the interfacial environment responsible for the dissolution of aqueous SOA precursors.
Appendix A. Cross Photoreaction of Glyoxylic and Pyruvic Acids in Model Aqueous Aerosol\(^\dagger\)

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**Scheme A.1.** Graphical abstract

\(^\dagger\)Further permissions related to the material excerpted should be directed to the American Chemical Society.

**Abstract**

Aerosols of variable composition, size, and shape are associated with public health concerns as well as with light-particle interactions that play a role in the energy balance of the atmosphere. Photochemical reactions of 2-oxocarboxylic acids in the aqueous phase are now known to contribute to the total secondary organic aerosol (SOA) budget. This work explores the cross reaction of glyoxylic (GA) and pyruvic (PA) acids in water, the two most abundant 2-oxocarboxylic acids in the atmosphere, under solar irradiation and dark thermal aging steps. During irradiation, pyruvic and glyoxylic acids are excited and initiate proton-coupled electron transfer or hydrogen abstraction and \(\alpha\)-cleavage reactions, respectively. The time series of photoproducts is studied by ion chromatography (IC) with conductivity and electrospray ionization (ESI) mass spectrometry (MS) detection, direct ESI-MS analysis in the negative ion mode, and
nuclear magnetic resonance spectroscopy (NMR). The use of one-dimensional ($^1$H and $^{13}$C NMR) and two-dimensional NMR techniques includes gradient correlation spectroscopy (gCOSY) and heteronuclear single quantum correlation (HSQC). The aging of photoproducts in the dark is monitored by UV–visible spectroscopy. The periodicity in the time domain of the optical properties is explained in terms of chromophores that undergo alternating thermochromism and photobleaching between nighttime and daytime cycles, respectively. A reaction mechanism for the cross reaction of GA and PA explaining the generation of trimers with general formulas $C_5H_8O_5$ (148 Da), $C_6H_{10}O_5$ (162 Da), and $C_5H_8O_6$ (164 Da) is provided based on all experimental observations.

**Introduction**

Photochemical reactions in the aqueous phase can contribute significantly to the total secondary organic aerosol (SOA) budget. Simple 2-oxocarboxylic acid such as glyoxylic acid (GA) and pyruvic acid (PA) can photogenerate radicals in the aqueous phase, where inorganic electrolytes such as ammonium and sulfate can participate in thermal processes. GA ($pK_a = 3.13$) and PA ($pK_a = 2.39$) are widely available in aqueous SOA due to the photooxidative processing of isoprene and aromatics forming them. GA and PA are the two most abundant 2-oxocarboxylic acids in organic aerosols identified at different sites. The net rates of GA and PA generation in atmospheric waters are $2.95 \times 10^{10}$ mol year$^{-1}$ = $0.71$ (±0.21) TgC year$^{-1}$ and $1.80 \times 10^9$ mol year$^{-1}$ = $64.7$ (±16.7) GgC year$^{-1}$, respectively. The partitioning of GA and PA into the particle phase can be as high 88% and 67%, respectively.
Our previous work has explained the chemical fate of GA and PA during self-photoreactions. However, the cross photoinduced reaction of GA and PA and the possible associated mechanism for this process has remained unexplored and is tackled here for the first time. This laboratory work reports how sunlight can trigger the cross photoreaction of GA and PA in atmospheric water mimics. Then, thermal reactions of the photoproducts are monitored during dark periods. Photoproducts are identified and monitored during the reaction by 1) ion chromatography (IC) with dual conductivity and electrospray (ESI) mass spectrometric (MS) detection, 2) direct ESI-MS analysis in the negative ion mode, 3) nuclear magnetic resonance spectroscopy (NMR) analysis including one-dimensional (\(^1\)H and \(^{13}\)C NMR), and two-dimensional gradient correlation spectroscopy (gCOSY) and heteronuclear single quantum correlation (HSQC) experiments.

Materials and Experimental Methods

Preparation of Experiments and Controls. Mixtures of PA (Sigma-Aldrich, 99.1%) and GA (Sigma-Aldrich, 51.7 wt. % in H\(_2\)O) were prepared daily in ultrapure water (Elga Purelab flex, 18.2 MΩ cm\(^{-1}\)). The mixture of 30 to 50 mM PA and 250 to 300 mM GA was doped with inorganic electrolytes including [Na\(^+\)] = 468 mM, [Cl\(^-\)] = 545 mM and [SO\(_4^{2-}\)] = 28.2 mM prepared from NaCl (Fisher, 100.3%), Na\(_2\)SO\(_4\) (Fisher, 100.1%) and HCl (EMD, 37.7%). Additional environmental considerations explaining all the previous concentrations are provided in the Supporting Information. The mixture was adjusted to pH 1.0 with [HCl] = 2.0 M, and 180.0 mL were transferred to a customized fused silica photoreactor of 220.0 mL capacity. The reactor was sealed and ethanol (Ricca, 69.6% v/v) was recirculated through a jacket (Thermo Scientific, A25 bath circulator) to keep
the temperature at 298 K. Solutions underwent continuous sparging at a flow rate of 100 mL min$^{-1}$ with 1 atm air (Scott-Gross, UHP) starting 30 min before photolysis for $[O_2(aq)]_0 = 0.26$ mM. The control experiments in Table A.1 were designed to study the effect of inorganic electrolytes and the concentration of $O_2(g)$ on the cross reaction of GA and PA, and included: Control A under 1 atm $N_2(g)$ (Scott-Gross, UHP), control B under 1 atm $O_2(g)$ (Scott-Gross, UHP) for $[O_2(aq)]_0 = 1.24$ mM, control C without electrolytes, control D without electrolytes under 1 atm $N_2(g)$, control E without electrolytes under 1 atm $O_2(g)$, and control F without light.

Table A.2. Experiments and Controls to Investigate the Cross Reaction of GA and PA

<table>
<thead>
<tr>
<th>Conditions</th>
<th>$h\nu$</th>
<th>Electrolytes</th>
<th>Atmosphere</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Air</td>
</tr>
<tr>
<td>Experiment</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
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<td>✓</td>
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</tr>
<tr>
<td>Control B</td>
<td>✓</td>
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<tr>
<td>Control C</td>
<td>✓</td>
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<td>Control D</td>
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<tr>
<td>Control F</td>
<td></td>
<td>✓</td>
<td>✓</td>
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</tbody>
</table>

Table key: $h\nu$ indicates the presence of light. Electrolytes include $Na^+$, $Cl^-$ and $SO_4^{2-}$. 
Photochemical Experiments and Thermal Treatment of Photolyzed Samples. The photochemical setup employed includes a 1 kW high pressure Xe-Hg lamp (Newport). A water filter was used (Newport) to remove unwanted infrared radiation, and a cutoff filter (Oriel) at $\lambda \geq 305$ nm provided actinic radiation in the solar window. Potassium ferrioxalate (Alfa Aesar, 98.9%) was used as an actinometer to measure the actinic flux ($1.06 \times 10^{-5}$ Einstein L$^{-1}$ s$^{-1}$ for $\lambda \geq 305$). During the first stage of processing (Stage I in Scheme A.2), samples of the experiment and controls (Table A.1) were irradiated for 6 h and 5.0 mL aliquots were withdrawn from the reactor at 0, 0.5, 1, 2, 3, 4, 5, and 6 h. A 30% of the aliquot was immediately frozen at –20 °C and stored in the dark for later analyses by IC-ESI-MS, direct ESI-MS, and NMR spectroscopy. The analysis of freshly photolyzed samples (before freezing) by these techniques verified that none of the products reported could result from the freezing process. The remaining 3.5 mL of sample was transferred to a Suprasil cuvette (Starna Cells, 10-mm optical path length) to monitor its aging (Stage II in Scheme A.2) in the dark by UV-visible spectroscopy for 15 h at 298 K followed by 9 h at 323 K. The same analyses performed on the irradiated samples were also applied to the aged samples after dark periods.

About 130.0 mL of the remaining photolyzed sample was stored in the dark for 24 h and then rephotolyzed (Stage III in Scheme A.2) under the same conditions previously described. The later rephotolyzed samples underwent a second aging process in the dark monitored by UV-visible spectroscopy (Stage IV in Scheme A.1). The conversion of individual samples GA and PA have been previously reported in the same setup.\textsuperscript{2, 3, 70, 168}
**Scheme A.2.** Representation of the processing of glyoxylic acid (GA) and pyruvic acid (PA) mixtures. Stages I and III are both 6 h photolysis periods. Stages II and IV each refer to (dark) thermal aging during 15 h at 298 K followed by 9 h processing at 323 K. Adapted from ref. 3.

**Analysis of Products.** Samples were analyzed by 1) UV-visible spectroscopy, 2) IC-MS also equipped with a conductivity detector, 3) direct infusion electrospray mass spectroscopy (ESI-MS) in the negative ionization mode, and 4) a combination of nuclear magnetic resonance spectroscopy (NMR) experiments including one dimensional (1D) $^1$H and $^{13}$C NMR, and two dimensional (2D) gradient correlation spectroscopy (gCOSY) and heteronuclear single quantum correlation (HSQC). The uncertainty associated with UV-visible spectroscopy, IC-MS, and ESI-MS measurements, based on the propagation of systematic errors,$^{213}$ was lower than 1.0%. The presence or absence of carbonyl groups in the cross products was verified by UHPLC-MS analysis of samples derivatized with 2,4-dinitrophenylhydrazine (DNPH).

UV-visible spectra (190 ≤ λ ≤ 750 nm) of initial and irradiated samples were recorded immediately after sampling with an Evolution 220 scanning spectrophotometer (Thermo Scientific) equipped with a temperature control multicell holder (Thermo Scientific, SPE.
8 W). In addition, spectra were recorded during the aging processing every 30 min. Actual initial concentrations of GA and PA in the mixture were solved from total absorbance using Beer’s Law and the calculated molar absorption coefficient of pure GA \((\varepsilon_{GA} = 11.6 \text{ M}^{-1} \text{ cm}^{-1} \text{ at } \lambda = 240 \text{ nm})^{118}\) and PA \((\varepsilon_{PA} = 11.3 \text{ M}^{-1} \text{ cm}^{-1} \text{ at } \lambda = 320 \text{ nm})^{11}\) standard solutions in water. Multiple wavelengths \((\lambda = 260, 270, 280, 290, 300, \text{ and } 320 \text{ nm})\) were chosen to calculate the molar absorption coefficient of each standard and their relative contribution to the total absorbance in the mixture. The molar absorption coefficients were calculated for PA \((\varepsilon_{260} = 26.7 \text{ M}^{-1} \text{ cm}^{-1}, \varepsilon_{270} = 7.50 \text{ M}^{-1} \text{ cm}^{-1}, \varepsilon_{280} = 5.00 \text{ M}^{-1} \text{ cm}^{-1}, \varepsilon_{290} = 5.70 \text{ M}^{-1} \text{ cm}^{-1}, \varepsilon_{300} = 7.60 \text{ M}^{-1} \text{ cm}^{-1}, \text{ and } \varepsilon_{320} = 11.3 \text{ M}^{-1} \text{ cm}^{-1})\) and GA \((\varepsilon_{260} = 2.60 \text{ M}^{-1} \text{ cm}^{-1}, \varepsilon_{270} = 2.50 \text{ M}^{-1} \text{ cm}^{-1}, \varepsilon_{280} = 2.20 \text{ M}^{-1} \text{ cm}^{-1}, \varepsilon_{290} = 1.70 \text{ M}^{-1} \text{ cm}^{-1}, \varepsilon_{300} = 1.40 \text{ M}^{-1} \text{ cm}^{-1}, \text{ and } \varepsilon_{320} = 1.00 \text{ M}^{-1} \text{ cm}^{-1})\) using Beer’s Law equation:

\[
A = \varepsilon b [X]
\]

(A.1)

for a path length \(b = 1.0 \text{ cm}\), and the measured absorbance of individual standard solutions \((X = \text{GA or PA})\) of \([\text{GA}] = 90.3 \text{ mM}\) and \([\text{PA}] = 28.8 \text{ mM}\). Microsoft Excel Solver was used to solve the actual initial concentrations for GA and PA in the experimental mixture, which were typically \(ca. [\text{PA}]_0 = 27.9 \text{ mM}\) and \([\text{GA}]_0 = 235.6 \text{ mM}\). More details about this calculation can be found in the Supporting Information.

Samples for ion chromatography were diluted 500 times with ultrapure water before injection with an autosampler (Dionex AS) in an IC (Dionex ICS-2000) provided with an IonPack AS11-HC (2 mm) analytical column and a conductivity detector. An ESI probe interfaced the output of the conductivity detector to a mass spectrometer (Thermo
Scientific, MSQ Plus). The same chromatographic and mass spectrometer conditions previously established by Zhou and Guzman\textsuperscript{87} were used in this work. Briefly, the initial 1 mM potassium hydroxide concentration at a flow of 0.38 mL min\textsuperscript{-1} was increased following three linear gradient steps (1.4 mM min\textsuperscript{-1} up to 15 mM, 1.5 mM min\textsuperscript{-1} up to 30 mM, and 3.0 mM min\textsuperscript{-1} up to 60 mM).\textsuperscript{87} For direct infusion analysis, samples were diluted 1000 times with ultrapure water and injected in the ESI-MS, which was operated in negative ion mode at 70 psi nitrogen nebulizing gas, 450 °C, 2.4 kV needle voltage, and 40 V cone voltage, unless otherwise indicated in the text.

NMR (Varian INOVA 400) spectra at room temperature were recorded using a 5-mm NMR tube (Wilmad) with 540 μL of sample spiked with 60 μL D\textsubscript{2}O (Cambridge Isotope, 99.9 %), for field-frequency lock doped with 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS, Sigma-Aldrich, 97 %) internal standard. A water suppression enhanced through T\textsubscript{1} effects (WET) solvent suppression method was applied using VnmrJ 3.2 software to eliminate more than 99.3% of the H\textsubscript{2}O signal. Further details of the NMR methods can be found in the Supporting Information.

Results and Discussion

Optical Properties. Figure A.1 shows how UV-visible spectra for a mixture with 235.6 mM GA and 27.9 mM PA in the presence of electrolytes under 1 atm air (experiment in Table A.1) vary during the four stages described in Scheme A.2. Before irradiation a peak at \( \lambda = 321 \) nm from PA is observed together with a shoulder at \( \lambda = 276 \) nm due to GA. After 6 h of irradiation, the loss of C=O groups in the UV spectrum is registered due to photobleaching during stage I (Scheme A.2). For the following 24 h of thermal aging in stage II, an absorbance increase is observed reflecting thermochromism.\textsuperscript{76} For the
second irradiation period, which simulates a second daytime cycle (stage III in Scheme A.2), photobleaching produces a practically identical spectrum to the one observed at the end of stage I. Similarly, a second nighttime cycle developing thermochromism in dark stage IV results in strong absorption that recovers to the level developed during stage II.

Figure A.1. UV-visible absorption spectra (pink solid line) before photolysis, (red short dashed line) postphotolysis at the end of stage I; (black dashed line) followed by 15 h of thermal aging at 298 K and 9 h at 323 K in stage II, (blue dash-dot-dot line) after a second 6 h photolysis in stage III, and (green dash-dot line) after a second thermal aging in stage IV. Experimental conditions: Mixture of [GA] = 235.6 mM and [PA] = 27.9 mM, [Na\textsuperscript{+}] = 545 mM, [SO\textsubscript{4}\textsuperscript{2-}] = 28.2 mM, and [Cl\textsuperscript{-}] = 468 mM, at pH 1.0 under 1 atm air.

The optical properties of the mixture of products change during the four processing stages reflecting the presence of alcohol, aldehyde, and ether groups.\textsuperscript{76} During nighttime under mild atmospheric conditions, these functional groups react with low activation energy barriers to produce chromophoric species. For example, the self-condensation of aldehydes,\textsuperscript{86,91} the formation of alkenes from alcohols,\textsuperscript{93} and the esterification of acids,\textsuperscript{214} are viable atmospheric reactions. Particularly, unsaturated hydrocarbons can undergo
facilitated hydration in the presence of light, which explains the photobleaching registered in the UV-visible spectra.

Figure A.2 shows the normalized areas integrated under the UV-visible absorption spectra in the range $295 \leq \lambda \leq 500$ nm for each stage in Scheme A.2 for an experiment and all controls in Table A.1. For normalization, all integrated areas were divided by its initial area before irradiation. Control F in the dark is not included in Figure A.2 because it continued unchanged for the four stages. The largest change in Figure A.2 corresponds to the comparison of stages I and III under irradiation displaying photobleaching versus dark (aging) stages II and IV corresponding to thermochromism. The presence of $[O_2(aq)]$ and electrolytes affects each stage differently.

**Figure A.2.** Normalized area ($\lambda_{\text{min}} = 295 \text{ nm}$ and $\lambda_{\text{max}} = 500 \text{ nm}$) under the UV-visible absorption spectra for (red ●) an experiment, (blue ▲) control A in N$_2$, (pink ▼) control B in O$_2$, (violet ■) control C without electrolytes, (orange ♦) control D without electrolytes in N$_2$, and (black ▲) control E without electrolytes in O$_2$. Experimental conditions defined in Figure A.1 and controls given in Table A.1.

Some minor changes are observed at the end of stage I (Figure A.2), and thermochromism becomes pronounced during stage II. After stage II, for increasing
[O₂\textit{(aq)}] in the presence of electrolytes (control A < experiment < control B in Table A.1) thermochromism decreases. The areas at the end of stage II increase in the order O₂(g) < air < N₂(g): 1.30 < 1.44 < 2.17, which is 3.5 (±0.1) larger than observed for the self-reaction of GA in the presence of electrolytes. The same interpretation is applicable to the subset of experiments in the absence of electrolytes (controls C, D, and E). Electrolytes affect the development of thermochromism for stage II as demonstrated by contrasting the subsets in air (experiment vs control C), in N₂(g) (controls A vs D), and in O₂(g) (controls B vs E). The sample with no electrolytes displays greater thermochromism in air, which comparatively increases in N₂(g) but drops in O₂(g). In stage III a second photobleaching cycle starts, while during stage IV, a reversal in the magnitude of the areas is observed for the pairs 1) experiments and control C, 2) controls A and D, and controls B and E. However, the previous absorption enhancement due to the presence of electrolytes is much less pronounced for the latter case in 1 atm O₂(g).

In summary, the presence of O₂ is detrimental for the formation of chromophores. Electrolytes play a significant different role enhancing thermochromism in stage IV and diminishing it in stage II. The previous observation implies that the formation of complex polyfunctional CₓHᵧO𝒛 molecules observed at the end of stages I and II contribute to the production of less oxygenated species as shown by the relatively lower number of peaks in the 65-85 ppm region of the $^{13}$C NMR Spectra in Figure A.S1 (Supporting Information) compared to stages III and IV.

These new CₓHᵧO𝒛 species created from the mix of ~28 mM PA and ~236 mM GA undergo thermochromism through interactions with electrolytes after stage III. For comparison, the analysis of two cycles of photobleaching and thermochromism for the
processing of 26.0 mM PA (no GA) for controls G-M listed in Table A.S1 is available in Figure A.S2. The effect of electrolytes ([Na\(^+\)] = 545 mM, [Cl\(^-\)] = 468 mM, and [SO\(_4^{2-}\)] = 28.2 mM, excluding NH\(_4^+\)) in Figure A.S2 agrees well with previous observations (for higher 80 mM solutions of PA in 2 M ammonium bisulfate)\(^76\) for the enhancement of thermochromism for PA alone during stages II and IV. Thus, thermochromism increases (Figures A.2) with higher ionic strength (from inert electrolytes) and temperature, which promotes the dehydration of hydroxyl functional groups in alcohols >CH-C(OH)< to form olefin groups >C=C<.\(^76\)

The hydration of unsaturated carbons is facilitated through photoinduced reactions in water.\(^76\) GA behaves as a typical species that can alter the reactivity of PA by cross-reacting with it, which affects the composition of the complex organic matter created. As a result, the new organic matter from the cross-reaction possesses a higher O/C ratio of 0.83-1.20 compared to 0.71-1.00 for the photoproducts of PA in the absence of GA. To sum up, the optical properties of the mixture along the different stages in Scheme A.2 are affected by multiple functional groups in the photolyzed and thermally aged samples. The complex chemical composition of the products should enable light interactions (absorption and scattering) in the presence of electrolytes.\(^76\) The presence of electrolytes and dissolved O\(_2\) do affect the chemical reactivity of the organic species upon irradiation and thermal aging.

**Identification of Products.** The cross reaction products for the photolysis of GA and PA mixtures are analyzed by 1) IC-ESI-MS, and 2) direct infusion ESI-MS, excellent techniques for species possessing –COOH groups. The information from direct infusion ESI-MS reveals the mass-to-charge ratio (m/z) for all anionic products present in the
sample. IC-ESI-MS also provides $m/z$ values, but after chromatographic separation of the mixture, which eliminates possible artifacts caused by infusing a mixture of products directly. The data below for both methods reports a similar composition of product for the experiment and control C, suggesting that the presence of inorganic electrolytes does not determine the structure of products.

Figure A.3 shows chromatograms with conductivity and ESI-MS detection in the negative ion mode for the same experiment in Figure A.1 before and after 6 h photolysis. In Figure A.3A before irradiation, peaks 1 through 4 eluting at 6.624, 10.674, 11.900 and 19.487 min in the conductivity detector are assigned by their $m/z$ values and with matching standards to pyruvate ($C_3H_3O_3^-$, $m/z$ 87), glyoxylate ($C_2H_1O_3^-$, $m/z$ 73), chloride ($Cl^-$), and bisulfate ($HSO_4^-$, $m/z$ 97), respectively.

Upon 6 h of photolysis, new peaks 6, 7, and 8 elute in Figure A.3B, and correspond to the products from the self-photoreaction of PA, 2-(3-oxobut-2-yl)oxy)-2-hydroxypropionate ($C_7H_{11}O_5^-$, $m/z$ 175) and two diastereomers of 2,3-dimethyltartrate ($C_6H_9O_6^-$, $m/z$ 177). Peaks 9 and 10 are assigned to a trace of tartrate ($C_7H_{11}O_5^-$, $m/z$ 149, a self-reaction product of GA) eluting at 19.086 min, and to a trace of oxalate ($C_2H_1O_4^-$, $m/z$ 89, at 20.937 min), as observed in the photolysis of GA alone. Three additional new peaks labeled 5, 6, and 8 appear in the conductivity detector due exclusively to the cross-reaction of GA with PA. However, some conductivity peaks correspond to overlapping species based on the distinctive $m/z$ values extracted with the help of the MS detector. For example, peak 6 contains anions at $m/z$ 147, 161, and 175, while peak 8 includes the anions at $m/z$ 177 and 163, which can be resolved as extracted ions. However, because the species at $m/z$ 163 is absent in experiments with pure PA or
GA,2,3,70,168 this species must be a cross photolysis product of GA and PA. The extracted ion count at m/z 175 and 177 shows that there are one and two peaks in the chromatogram (Figure A.3B) for each of them, respectively.

**Figure A.3.** Ion chromatogram with conductivity and ESI-MS (-) detection for the experiment in Figure A.1 (A) before and (B) after 6 h of irradiation. Key for peaks: (1) PA (m/z 87), (2) GA (m/z 73), (3) chloride, (4) sulfate (m/z 97) (5) C₅H₇O₅⁻ (m/z 161), (6) C₅H₇O₆⁻ (m/z 177), a shoulder of C₆H₉O₅⁻ (m/z 161), and 2-(3-oxobutan-2-yloxy)-2-hydroxypropionic acid (C₇H₁₁O₅⁻, m/z 175), (7) a diastereomer of 2,3-dimethyltartaric acid (C₆H₉O₆⁻, m/z 177), (8) a second diastereomer of 2,3-dimethyltartaric acid and C₅H₇O₆⁻ (m/z 163), (9) tartaric acid (m/z 149), and (10) oxalic acid (m/z 89).

The peak eluting at 5.796 min for m/z 175 in Figure A.3B is assigned to 2-(3-oxobutan-2-yloxy)-2-hydroxypropionate or simply an oxo-C₇ product of PA,5 whose area grows at longer irradiation times. The other major photoproducts with a formula mass of 178 Da originate from the self-reaction of PA, and elute closely at 17.927 and 18.538 min.
in the conductivity detector (peaks 7 and 8 in Figure A.3B). Based on previous work for
the self-photoreaction of pyruvic acid, the peaks at 17.927 and 18.538 min are assigned
to two diastereomers (2R,3R and 2R,3S) of 2,3-dimethyltartaric acid (Figure A.S4,
Supporting Information) produced from the combination of ketyl radicals of PA, K⁺. In
conclusion, the new species for peaks 5, 6 and 8 with molecular mass of 162, 148 and
164 Da, respectively, result from the cross-photoreaction of GA and PA. The new
C₅H₈O₅ (148 Da) molecule eluting in peak 5 with m/z 147 is proposed to be 2-hydroxy-2-
(2-oxopropoxy)acetic acid (oxo-C₅ cross-product) or an isomer. Coeluting with the
previous is a similar C₆H₁₀O₅ (162 Da) molecule contributing to peak 6 with m/z 161
tentatively assigned to 2-hydroxy-2-((1-oxopropan-2-yl)oxy)propanoic acid (oxo-C₆
cross-product), which at its tail overlaps with 2-hydroxy-2-((3-oxobut-2-yl)oxy)propanoic acid (176 Da) (oxo-C₇ product of PA). Finally, the new C₅H₈O₆ (164
Da) molecule is proposed to be 2,3-dihydroxy-2-methylsuccinic acid or an isomer, and
elutes with peak 8 overlapping 2,3-dimethyltartaric acid (178 Da). Therefore, the new
species in peaks 5, 6 and 8 should have similar structures to the coeluting species listed
above.

Figure A.4 shows direct infusion ESI-MS spectra reflecting composition changes
before and after 1, 2, 4, and 6 h of irradiation for the experiment with GA and PA. No
products heavier than 250 Da are observed during irradiation. The cross-reaction products
with general formulas C₅H₈O₅ (148 Da), C₆H₁₀O₅ (162 Da), and C₅H₈O₆ (164 Da) are
observed after irradiation for 1 h.
Figure A.4. ESI-MS(-) spectra for the experiment in Figure A.1 during stage I (Scheme A.2) (A) before photolysis, and after (B) 1, (C) 2, (D) 4, and (E) 6 h of irradiation.

The peaks in Figure A.4 with m/z 127 and 129, which would correspond to compounds with formula mass of 128 and 130 Da, are actually ascribed to clusters of the most abundant (99.67%) gem-diol form of GA (K$_{hyd} = [\text{gem-diol form of GA}]/[\text{keto form of GA}] = 3.0 \times 10^2$ at 298 K)\(^{20}\) with chloride. The area under each MS peak indicates that the abundance ratio of the anions $B_{127} = 0.74$ and $B_{129} = 0.26$ is related by the natural abundance of $^{35}\text{Cl}$ to $^{37}\text{Cl}$.\(^{216}\) (Figure A.S3A, Supporting Information). A similar isotopic signature for one chlorine in the cluster: $B_{127}/B_{129} = 0.74/0.26$ is observed for the photolysis of 252.1 mM GA with electrolytes in air\(^3\) (Figure A.S3B, Supporting Information). Because neither of the peaks at m/z 127 and 129 is detected in the IC-ESI-MS chromatograms, they are interpreted as ESI clusters and not actual products.
The change in the ion count for direct infusion ESI-MS and IC-MS for GA, PA, and the photoproducts during the initial photolysis stage is shown in Figure A.5A and A.5B, respectively. GA \((m/z\ 73)\) and PA \((m/z\ 87)\) decay in the time series of Figure A.5A due to photolysis in stage I, both first order reactions in the concentration of each reactant.\(^{217}\)

The species that grow with irradiation time are those identified above with a mass of 162, 164, and 178 Da. Figure A.5B shows how the areas under chromatographically separated extracted ion peaks change for reactants and photoproducts. The mass spectrometer allows the identification of species that could coelute at the same retention time by distinguishing their different \(m/z\) values. The species eluting at 4.8 min \((m/z\ 161)\), 5.9 min \((m/z\ 175\ and \ 161)\), 18.0 min \((m/z\ 177)\), and 18.6 min \((m/z\ 177,\ 163)\) grow exponentially over time in Figure A.5B. However, the area for the peak at \(m/z\ 175\) reaches a steady level during the beginning stage of photolysis. A comparison to the results from PA photolyzed controls G to M (Table A.S1, Supporting Information) indicates that the efficiency for the production of 2-(3-oxobutan-2-yloxy)-2-hydroxypropionic acid \((m/z\ 175)\) is largely diminished by the presence of GA.

The use of NMR spectroscopy provides additional structural information to characterize the photolysis mixture. Figure A.6 displays the \(^{13}\text{C}\) NMR spectrum of a photolyzed mixture of GA and PA after 6 h irradiation (red trace) compared to the spectrum before photolysis (black trace). The signals at \(\delta = 0.000, 17.594, 21.701,\) and 56.964 ppm corresponded to DSS. There are three peaks that belong to the carbonyl form of PA at the following chemical shifts: 1) 28.496 ppm for the –\(\text{CH}_3\) carbon, 2) 166.377 ppm for the –\(\text{COOH}\) carbon, and 3) 200.01 ppm for the \(\text{C}=\text{O}\) carbon in PA. Similarly, there are other three peaks for the gem-diol form of PA, 2,2-dihydroxypropanoic acid
Figure A.5. (A) Ion count from direct infusion ESI-MS(-) and (B) areas under the extracted IC-MS chromatographic peaks of species during the beginning of photolysis (stage I) for the experiment in Figure A.1. Anions correspond to $m/z$ (black ▲) 73, (red ●) 87, (brown ×) 89, (dark yellow ▲) 147, (blue empty ▽ and filled ▼) 161, (pink empty ○ and filled ●) 163, (filled ■) 175, and (violet ♦ and ◊) 177.

(DHPA), at 1) 27.891 ppm for the –CH$_3$ carbon, 2) 177.686 ppm for the –COOH carbon, and 3) 95.412 ppm for the carbon with two hydroxyl groups, –C(OH)$_2$. For GA and its gem-diol, the –COOH groups are found at $\delta = 194.090$ and 175.900 ppm, respectively. The gem-diol (–C(OH)$_2$) group of glyoxylic acid is found at $\delta = 88.894$ ppm in Figure A.6, while the aldehyde in equilibrium is at $\delta = 175.886$. After 6 h of irradiation, the peaks for PA and DHPA at 27.891, 28.496, 95.412, 177.686, 194.090 and 200.001 ppm disappear. Simultaneously, the intensity drop of GA and PA, e.g., from their gem-diols in
equilibrium respectively at $\delta = 88.894, 95.412$ ppm indicate their loss. The red trace for the products in Figure A.6 reveals new signals in the region 155-185 ppm, which arise from carboxylic acids. In more detail, the peaks at 168.501 ppm for oxalic acid and 179.401 ppm for formic acid both from GA self-reaction, and 179.912 ppm for the oxo-C$_7$ product of PA self-reaction are accompanied by new cross-products signals at 165.891 and 179.806 ppm. The appearance of ether groups is evidenced by new peaks within the 40-80 ppm range$^{218}$ ($\delta = 70.278, 72.179, 77.397, 77.535, 79.489, 79.751$ ppm) that grow during the postirradiation period for the GA + PA mixture.

![Figure A.6](image-url) 100 MHz $^{13}$C NMR spectra for the experiment in Figure A.1 (bottom black line) before and (top red line) after 6 h photolysis. The inset shows the spectrum in the range 55-85 ppm. No signals appear within the two excluded spectral brackets.

Similar observations have been made for experiments with individual GA$^3$ or PA.$^2,^{168}$ Because GA is consumed over time during the cross photolysis reaction, as shown by the data in Figure A.3, the large intensity peak for a carbonyl at $\delta = 175.895$ ppm that
practically overlaps the carbonyl form of GA reflects the formation of aldehyde products. This observation is also confirmed by the results in Figure A.6 that display the production of aldehydes. In summary, this data provides additional evidence that the C$_5$- and C$_6$-carboxylic acids of mass 148, 162, and 164 Da possess ether and aldehyde groups. Additional information, including 1D $^1$H and $^{13}$C NMR, and 2D gCOSY and HSQC, is provided in the Supporting Information (Figures A.S5-S7).

Figure A.7 shows the ion count change over the different processing stages in Scheme A.2 for the experiment with GA and PA. Initial photoproducts can be consumed during the

![Figure A.7](image-url)

**Figure A.7.** ESI-MS(-) spectra of the experiment in Figure A.1 during stages I-IV in Scheme A.2 (A) before photolysis, and (B) after 6 h irradiation in stage I, (C) at the end of the dark aging process in stage II, (D) after 6 h photolysis in stage III of panel C, and (E) after the completion of the aging process in stage IV.
second photolysis (Stage III), because of their chromophoric carbonyl groups. The lack of major changes in the mass composition observed in Figure A.7 after Stage I indicates that persistent species capable of undergoing reversible thermochromism and photobleaching are formed. Additionally, the analysis of $^{13}$C NMR spectra in the range of $\delta = 65$-85 ppm, corresponding to saturated carbon (sp$^3$) arising from hydrocarbons with ether groups,$^{218}$ shows new peaks appear during stage III and some disappear during stage IV (Figure A.S1, Supporting Information). This versatile behavior could be provided by some complex products capable of decomposing and recombining under sunlight irradiation, which have functional groups that can react in the dark with low activation energy.

Figure A.8 is presented to explore whether higher mass products are generated during different stages of processing. Data in red columns (Figure A.8) shows how the total ion count integrated in the interval 50-500 Da varies over all stages. Integrated ion count, $I_{IC}$, is calculated from

$$I_{IC} = \sum_{50}^{500} I_i \quad (A.3)$$

where $I_i$ is the ion count for individual peaks of mass $i$. The blue columns in Figure A.8 show the change in average mass, $\bar{M}$, calculated from

$$\bar{M} = \frac{\sum_{50}^{500} \frac{m_i I_i}{I_i}}{\sum_{50}^{500} I_i} \quad (A.4)$$
where \( m_i \) stands for the mass of species \( i \). It is clear that only for the transition from stage III to IV \( \bar{M} \) decreases while IIC increases, which matches earlier observations for the decomposition of PA photoproducts during the aging process.\(^{76}\) No heavier mass than 250 Da formed in the cross photoreaction of PA and GA. The change of \( \bar{M} \) is small after the first photolysis and reflects that the loss of total organic carbon during the whole process is due mainly to decarboxylation of PA with a quantum yield \( \Phi_{\text{CO}_2}^{\text{PA}} = 0.78 \) in water,\(^{69}\) rather than to the much less efficient process for GA with \( \Phi_{\text{CO}_2}^{\text{GA}} = 0.002.\(^3\)

![Graph](image.png)

**Figure A.8.** Integrated ion count and average mass in the ESI-MS for the experiment in Figure A.1 (0) before photolysis and after the four stages in Scheme A.2.

**Mechanism of the Cross Photoreaction of GA and PA.** From the learning disclosed in the discussion above and based on individual photolyses of GA\(^3\) and PA,\(^{168}\) a mechanism for the cross reaction of GA and PA via radical chemistry is proposed in Scheme A.3. Comparing the stronger absorption of pyruvic acid than glyoxylic acid in the actinic region and that in the hydration equilibria for the concentrations in Figure A.1 there are 12 more carbonyl molecules of pyruvic acid \((K_{\text{Hyd}} = 1.83)^{21}\) than glyoxylic acid.
it is clear that the preferential absorption of pyruvic acid initiates the reaction. Thus, first, PA is activated by light ($\lambda > 300$ nm) to produce singlet excited state $^{1}\text{PA}^*$ (reaction R1a in Scheme A.3), which undergoes intersystem crossing to a triplet $^{3}\text{PA}^*$ (reaction R1b) that has a lifetime of $\sim 0.5$ $\mu$s. The thermal loss of $^{3}\text{PA}^*$ can occur by reaction R2.

Scheme A.3. Proposed Mechanism for the Cross Reaction of Aqueous GA and PA.

Then, $^{3}\text{PA}^*$ can react via overall reaction R3a + R3b with the ground state of GA by proton-coupled electron transfer (PCET) to produce two possible triplet radical-ion pairs, $^{3}\text{RP}_1$ and $^{3}\text{RP}_2$, which are analogous to those observed in frozen pyruvic acid solutions.
After protonation via reactions R5a and R5b and deprotonation via reactions R6a and R6b, the radical anions and radical cations respectively form the pairs of ketyl (the lactic acid radical K’ and the glycolic acid radical Y’) and oxyl (X’ and Z’) radicals. The same radicals should result from coordinated hydrogen atom transfer and electron transfer as an alternative to PCET. The major total organic carbon loss of the entire process is related to decarboxylation, which occurs in a few picoseconds and originates partially in reactions R7a and R7b from the oxyl radicals Z’ and X’ that form formyl and acetyl radicals H’ and Q’, respectively. All these new radicals can combine with neutral molecules available, such as PA, DHPA, 2-hydroxyacrylic acid (the enol of PA), GA, and 2,2-dihydroxyacetic acid. The previous reactions of radicals with neutral molecules (e.g., R8a and R8b) form secondary radicals, which combine with formyl (reaction R9) and acetyl (reactions R10a and R10b) radicals. Subsequently proceeds the formation of polyfunctional carboxylic acid termed oxo-C7 cross-intermediate, oxo-C8 intermediate of PA, and oxo-C6 cross-intermediate. The resulting carboxylic acids can undergo decarboxylation contributing to the loss of total organic carbon by reactions R11a, R11b, and R11c to respectively produce an oxo-C6 cross-product, an oxo-C7 product of PA, and an oxo-C5 cross-product. Decarboxylation rate constants for this kind of reactions are in the order of $1 \times 10^{-3}$ s$^{-1}$ at 298 K.$^{69}$ The formation of the oxo-C5 cross-product at $m/z$ 147 and the oxo-C6 cross-product at $m/z$ 161 originate from the pathways described above, based on the evidence of detecting their precursor (intermediates) as anions at $m/z$ 191 and $m/z$ 205 (Figure A.3), respectively, before decarboxylation. The product 2,3-dihydroxy-2-methylsuccinic acid with the molecular mass of 164 Da is formed from the combination of two ketyl radicals, K’ and Y’ in reaction R12, which should proceed with an approximate rate
constant of $\sim 1.2 \times 10^9 \, \text{M}^{-1} \, \text{s}^{-1}$. In addition, reactions R13 and R14 in Scheme A.3 show how the detected self-reaction products of PA (2,3-dimethyl tartaric acid) and GA (tartaric acid) are formed, respectively.

This work also considers an alternative mechanism involving H-abstraction from DHPA by preformed radicals (Scheme A.S1, Supporting Information). However, Scheme A.S1 is discarded based on the negative results for the detection of the hydrazones of 2-hydroxypropanal (MW 74.079) and acetoin (MW 88.106) that should have developed peaks at $m/z$ 253 and 267 in the UHPLC-ESI-MS chromatogram of photolysis samples derivatized with DNPH. Instead, the UHPLC-MS analysis in the negative ionization mode shows the hydrazones with $m/z$ 341 and 327 in Figure A.S8 (Supporting Information) have C=O moieties, and also confirms the product of neutral mass 164 Da from reaction R12 (Scheme A.3) does not have a carbonyl. Subtracting the DNPH fragment contributing 180 Da from the identified peaks with $m/z$ 341 and 327 indicates these peaks correspond to the oxo-C$_6$ cross-product and oxo-C$_5$ cross-product with neutral masses of 162 and 148 Da, respectively. Finally, other possibilities for the recombination of radicals and their addition to neutral molecules beyond Scheme A.3 are considered in Schemes A.S2-A.S6 of the Supporting Information, where isomeric products such as peroxides are provided.

**Conclusions and Atmospheric Implications**

This work has studied the cross photoreaction of GA and PA under simulated solar irradiation and the thermal processing of the photolyzed reaction mixture in the dark, also exploring the effects of inorganic electrolytes and $[\text{O}_2(\text{aq})]$. The lifetimes of aqueous GA and PA in the lower troposphere against direct photolysis at $40^\circ$ latitude are 11 d and 22
min, respectively.\textsuperscript{3, 168} The mechanism proposed via radicals of dicarbonyl species in water provides plausible pathways for the generation of complex organic matter. The cross reactivity of GA and PA produces species with higher O/C ratios (0.83-1.2) than observed for the self-reaction of PA (0.71-1). Thus, GA increases the formation of more oxygenated species and expands the reactivity channels of PA in atmospheric aerosols. PA behaves as a photosensitizer that induced the reactivity of GA forming new species. Therefore, similar processes should be operative in tropospheric waters to produce aqueous SOA of higher structural complexity than the precursors.

Structural information gained from direct infusion ESI-MS, IC-MS, and NMR spectroscopy, monitoring of optical property changes, and previous photolysis studies for GA and PA serve as the basis for the proposed mechanism (Scheme A.3) for the cross photoreaction in water.\textsuperscript{2, 3, 5, 21, 68-70, 85, 168} The mechanism involves the direct participation of radicals and explains the production of the C\textsubscript{5}- and C\textsubscript{6}-carboxylic acid cross-products with carbonyl and ether functional groups. In addition, other possibilities for the mechanism are depicted in Schemes A.S1-A.S6 (Supporting Information). The new species with molecular formulae C\textsubscript{5}H\textsubscript{8}O\textsubscript{5} (148 Da), C\textsubscript{6}H\textsubscript{10}O\textsubscript{5} (162 Da), and C\textsubscript{5}H\textsubscript{8}O\textsubscript{6} (164 Da) are enhanced in the presence of inorganic electrolytes, and are formed very favorably in air vs. in N\textsubscript{2}(g). Notably, the cross reaction disfavors the production of the oxo-C\textsubscript{7} product, which drops \textasciitilde34 times compared to the self-reaction. However, the generation of 2,3-dimethyltartaric acid, another self-reaction product of PA, is still important during the cross reaction. The production of a trace of tartaric acid, a dimer of GA self-reaction, is detected.
Reproducible photobleaching and thermochromism periods coincide with irradiation and thermal aging stages. The environmental implications of this observation relate the optical properties of complex oligomers with the effect of inorganic electrolytes and dissolved $O_2(aq)$ levels. Only an $O_2(g)$ saturated atmosphere has a detrimental effect on the development of thermochromism. However, the absorbance of products increases as the $[O_2(aq)]$ decreases following the order $O_2(g) < \text{air} < N_2(g)$. In this regard, the optical properties of organic aerosols are affected by their variable and complex particle size, structure, and chemical composition, which should be integrated with thermochromism and photobleaching parametrization factors into climate models. Accordingly, the variable direct and indirect effects of organic aerosols on the Earth’s energy balance could be better constrained. 219, 220

**Supporting Information:**

**Additional Environmental Considerations**

All samples for photolysis studies are prepared after assuming that the upper limit to the water content of aerosol droplets is determined by the deliquescence curve of an ammonium bisulfate solution at 50 % relative humidity (RH). For such conditions, the droplets in aerosols contain 0.6 g of $H_2O/g$ of $SO_4^{2-}$ or a pyruvic acid concentration $[PA] > 20$ mM under very acidic conditions. Similarly, the concentration of glyoxylic acid, $[GA]$, in arctic secondary organic aerosol (SOA) can be estimated, to be as high as 287 mM, for a molar ratio $[GA]/[PA] \sim 8$-14. Even higher concentration can be used in laboratory experiments that simulate a polluted environment, because the level of PA and GA in a city such as Tokyo can exceed by 30 and 3 times, respectively, those measured in the pristine Arctic aerosol. 15, 117
Additionally, experimental conditions of ionic strengths, temperature, and photon flux are chosen to simulate those encountered by nascent sea spray aerosols mixing with pollution at coastal regions. The model starting mixtures contain \( \text{ca. [PA]} = 28.0 \text{ mM} \) and \( \text{[GA]} = 236.0 \text{ mM} \), which are exposed to UV-visible light in the range of surface solar radiation. The variables under study include samples with and without the most abundant electrolytes in seawater (\([\text{Na}^+] = 468 \text{ mM}, \text{[Cl]} = 545 \text{ mM} \) and \( \text{[SO}_4^{2-} = 28.2 \text{ mM})^8 \) under a 1 atm \( \text{N}_2(g) \), 1 atm \( \text{O}_2(g) \), and 1 atm air. This work presents an advanced analysis that contrasts how the chemical composition and optical properties of model aqueous SOAs vary during photochemical and thermal processes.

**Calculation of Initial [GA] and [PA] in a Mixture via UV-visible Absorption Spectroscopy**

**Spectroscopy**

Actual initial concentrations of PA and GA in the mixture are determined by UV-visible absorption spectroscopy analysis using Beer’s Law (Equation A.S1)

\[
A = \varepsilon b[X]
\]  (A.S1)

where \( \varepsilon \) is the molar absorption coefficient, \( b \) is the optical path length, and \( [X] \) is the concentration of the component. Considering that the absorption bands of pure GA and pure PA significantly overlap in their mixture, a method to resolve their concentrations is introduced using a least square analysis. For this purpose, the individual spectra of standard \( \text{[PA]} = 28.8 \text{ mM} \) and \( \text{[GA]} = 90.3 \text{ mM} \) is recorded. The absorbance at wavelength \( \lambda = 260, 270, 280, 290, 300, \) and \( 320 \text{ nm} \) from each one of the standards, and their mixture \( (A_m, \text{the same sample in Figure A.1}) \) is extracted. Then, the molar absorption
coefficient of both PA and GA can be solved for each wavelength using Beer’s Law. Spectral data is imported to a spreadsheet in Microsoft Excel for the previous purpose, where Equation S2

\[ A = \varepsilon(X) \, b \, [X] + \varepsilon(Y) \, b \, [Y] \]  

(A.S2)
is employed to calculate the absorbance (\( A_{\text{cal}} \)) for the mixture of GA and PA at each wavelength from the estimated concentrations \([\text{GA}]_{\text{guess}}\) and \([\text{PA}]_{\text{guess}}\) (two estimated values). Then, using the Microsoft Excel Solver function, the least square conditions, which minimize the sum of squares of \((A_{\text{cal}} - A_{\text{m}})^2\) by iteratively varying the guess values \([\text{GA}]_{\text{guess}}\) and \([\text{PA}]_{\text{guess}}\), the solution to the concentrations in the mixture is provided. The actual initial concentrations in the mixture of a typical experiment are \([\text{GA}] = 235.6 \text{ mM and } [\text{PA}] = 27.9 \text{ mM}\).218

**Additional Experimental Methods for NMR Analysis**

For \(^1\text{H}\) NMR spectroscopy, data was collected at 298 K, with 32 K complex points, using a 90° pulse length (measured for each sample, \(\sim 14.5 \mu\text{s}\)). Thirty-two scans were acquired with a spectral width of 6.419 kHz, an acquisition time of 1.276 s, and a relaxation delay of 1.0 s. The water suppression enhanced through T1 effects (WET) method applied to all proton spectra was set up from the software VnmrJ 3.2 with a bandwidth of 100 MHz. The \(^1\text{H}\) NMR spectra for the same experiment with a mixture of GA and PA displayed in Figure A.6 is presented in the Supporting Information. The \(^1\text{H}\) NMR peaks in the spectra provide information of the hydrogen atoms located in different positions, particularly at \(\delta = 8.25, 5.38, 2.46\) and 1.58 ppm, correspond to GA, the gem-diol form of GA, the methyl groups of PA, and the methyl groups of the gem-diol form of PA, respectively. DSS at \(\delta = 0.00 \text{ ppm}\) is used as a reference.
The HSQC spectrum reporting the correlation of protons and carbons via single-bond correlation\textsuperscript{218} for the experiment with a mixture of GA and PA (as in Figure A.6) is also provided in this document.

For $^{13}$C NMR spectroscopy, data was collected at 298 K, with 64 K complex points, using a 90° pulse length (measured for each sample, $\sim$ 9.1 $\mu$s). About 24000 scans were acquired with a spectral width of 25.1731 kHz, an acquisition time of 1.302 s, and a relaxation delay of 1.0 s.

For 2D gCOSY and HSQC 2D NMR experiments, the methods were built from the default experiments in VnmrJ 3.2 with the same conditions for proton and carbon spectra described above. The same WET method was also applied to proton spectra in 2D NMR, which provides the chemical shift information from $^1$H and $^{13}$C NMR. 2D NMR also offers the through-bond correlation between couples of protons on adjacent carbons in gCOSY measurements, and the correlation between proton and carbon via single-bond in HSQC experiments.\textsuperscript{218} The same gCOSY method is applied to DSS internal standard (that also serves as a reference with $\delta = 0.00$ ppm), GA, PA, and the mixture of GA and PA under the conditions of the experiment before and after photolysis. The gCOSY spectra is color coded for DSS (grey), GA (green), PA (red), and the mixture of GA and PA (blue). From both combined spectra, no signals could be observed for the correlation of two adjacent carbon atoms both having hydrogen atoms except the ones from DSS. The only coupled proton correlations registered are from the methylene groups in DSS ($\delta = 0.62$, 1.77, and 2.92 ppm). Thus, the likelihood of observing $>$CH-CH$<$ groups in the product mixture is low. The signal intensity for the mixture of products at the cross point of 8.33 ppm, which corresponds to the range of aldehyde hydrogens, is considerably
larger than the spectrum before photolysis, pointing to the existence of photoproducts with aldehyde groups.

**Table A.S1.** Controls with PA.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Electrolytes</th>
<th>Atmosphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>$h\nu$ (2 h)</td>
<td>Air</td>
<td>N₂</td>
</tr>
<tr>
<td>Control G</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Control H</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Control I</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Control J</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Control K</td>
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<tr>
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<td></td>
</tr>
<tr>
<td>Control M</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

Key: $h\nu$ indicates the presence of light. Conditions: pH 1.0 and the electrolytes were $[\text{Na}^+] = 545$ mM, $[\text{SO}_4^{2-}] = 28.2$ mM, and $[\text{Cl}^-] = 468$ mM.
Figure A.S1. 100 MHz $^{13}$C NMR spectra for the region of ether groups in the experiment of Figure A.1 (black) before irradiation and after (red) stage I, (green) stage II, (pink) stage III and (blue) stage IV in Scheme A.2.

Figure A.S2. Normalized area under UV-visible absorption spectra ($\lambda_{\text{min}} = 295$ nm and $\lambda_{\text{max}} = 500$ nm) for controls of PA photolysis in Table S1 for the four stages in Scheme A.2. Key: (red •) control G for the photolysis of 26.0 mM PA with electrolytes in air at pH 1.0. (Blue ▲) control H in N$_2$, (pink ▼) control I in O$_2$, (violet ■) control J without electrolytes, (orange ◆) control K without electrolytes in N$_2$, and (black ▲) control L without electrolytes in O$_2$. Control M in the absence of light remained stable over the four stages and is not included.
Figure A.S3. Areas under ESI-MS peaks with $m/z$ 127 and 129 for samples before irradiation for (A) a mixture of GA and PA in the experiment of Figure A.1 and (B) a GA solution with electrolytes in air at pH 1.0.

![Graph A](image1)

![Graph B](image2)

Figure A.S4. Structures of the two diastereomers of 2,3-dimethyltartaric acid ($m/z$ 177).
Figure A.S5. 400 MHz $^1$H NMR spectra for the experiment with a mixture of GA and PA (as in Figure A.6) before (top black trace) and after 6 h photolysis (bottom red trace) recorded on a Varian Inova 400 instrument.

Figure A.S6. HSQC solvent (water) peak subtracted spectra of a mixture of 235.6 mM GA and 27.9 mM PA with electrolytes in air (experiment in Table 1) after 6 h photolysis (Stage I in Scheme 2), recorded on a Varian Inova 400 instrument.
Figure A.S7. gCOSY registered using WET suppression and water peak subtraction for (green) 235.6 mM GA and (red) 27.9 mM PA (blue) mixed solution for the experiment in Table 1 with (grey) DSS internal standard (A) before and (B) after 6 h photolysis.
UHPLC- MS Analysis of Hydrazones

For carbonyl analysis, 26 μL of the sample was diluted to 5 mL with water. To this solution, 5 mL of 25 mM 2,4-dinitrophenylhydrazine (DNPH, Sigma, HPLC grade, 99.6%) in acetonitrile (EMD Millipore, LC-MS grade, 99.99%) with 4% (v/v) sulfuric acid (Acros, 95.6%) was added. In the negative ionization mode DNPH adds a mass of 180 Da to the hydrazone of the originating monoanion. The mixture was inverted for mixing and allowed to incubate at room temperature for 1 h. After incubation, 750 μL of the derivatized mixture was diluted 1:1 with acetonitrile. This final dilution was analyzed by UHPLC-MS.

After derivatization, 25 μL of the samples containing the hydrazones were injected and separated on a Thermo Scientific Accela 1250 UHPLC equipped with a photodiode array detector set at 360 nm and an ESI-MS (negative mode) detector (MSQ Plus). The separation used a reversed phase C18 column (Agilent, Zorbax Eclipse Plus C18 RRHD, 2.1 × 100 mm, 1.8 μm) using a solvent gradient of (A) 5 mM formic acid (Fisher Optima LC-MS grade) and (B) 5 mM formic acid in acetonitrile at a flow rate of 400 μL min⁻¹. The initial conditions were 17% B for 5 min, which was then ramped to 60% over 9 min. Ionization conditions were set to cover the m/z range 40-740 and utilized a drying gas temperature of 400 °C, a nebulizer voltage of -2.4 kV, a cone voltage of -25 V, and a nebulizer pressure of 70 psi N₂(g).
The only hydrazones observed at $m/z$ 253 and 267 decrease over time and correspond to those of GA (MW 74) and PA (MW 88), respectively. The new peaks at $m/z$ 327 and 341 correspond to the cross-products with MW 148 and 162, respectively. No peaks are detected for the cross-product with MW 164 at $m/z$ 343.

**Figure A.S8.** UHPLC with (A) MS and (B) UV detection at 360 nm for hydrazones (blue) before and (red) after 6 h photolysis for the experiment in Figure A.1 of the paper.
Alternative Reaction Mechanisms Considered

Scheme A.S1 considers the possible H-abstraction from the gem-diol of PA from preformed radicals, in the mechanism of cross reaction of GA and PA. If this pathway would be of importance, the mechanism in Scheme A.S1 would predict the formation of intermediates and products with formula mass 136, 118, 150, 132, 88, and 74 Da. However, the final products acetoin (88 Da) and 2-hydroxypropanaldehyde (74 Da) possess a carbonyl that should have reacted with DNPH to be detected after chromatographic separation by UHPLC-ESI-MS. In addition, the underivatized sample did not display the anions at $m/z = 135, 117$ and 131 in the IC-MS chromatogram. Therefore, it can be concluded that the mechanism in Scheme A.S1 is not competing with the proposed mechanism in Scheme A.3.

Scheme A.S1. Considered Mechanism for the Cross Reaction of Aqueous GA and PA with Hydrogen Abstraction from the Gem-diol of PA.
Scheme A.S2. Considered Mechanism for the Cross Reaction of Aqueous GA and PA with Alternative Pathways to those Presented in Scheme A.3.
**Scheme A.S3.** Considered Reactions of Oxyl radical $K^+$ from PA in Scheme A.S2 with Neutral Molecules.
Scheme A.S4. Considered Reactions of Ketyl Radical K’ from PA in Scheme A.S2 with Neutral Molecules.
Scheme A.S5. Considered Reactions of Ketyl Radical Y’ from GA in Scheme A.S2 with Neutral Molecules.
Scheme A.S6. Considered Reactions of Oxyl Radical Y” from GA in Scheme A.S2 with Neutral Molecules.
References


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Professional Publications


