Interstellar Formation of Biorelevant Pyruvic Acid (CH₃COCOOH)

Prebiotic pyruvic acid is unlikely to have formed on the early earth, where liquid water was not present. Hence, it may have formed in space in ices containing acetaldehyde (CH₃CHO) and carbon dioxide (CO₂), whose reactions were triggered by energetic cosmic radiation. We demonstrate a key reaction pathway for pyruvic acid synthesis through non-equilibrium reactions in interstellar cold molecular clouds and star-forming regions, thus offering a unique entry point to abiotic organic synthesis in deep space.

N. Fabian Kleimeier, André K. Eckhardt, Peter R. Schreiner, Ralf I. Kaiser
prs@uni-giessen.de (P.R.S.)
ralfk@hawaii.edu (R.I.K.)

HIGHLIGHTS
Pyruvic acid, essential to life, forms in the absence of a biochemical machinery
Interstellar ices are breeding grounds for organic molecules
High-energy electrons initiate chemical reactions in interstellar ices
The results guide the radioastronomical identification of pyruvic acid in space
Interstellar Formation of Biorelevant Pyruvic Acid (CH$_3$COCOOH)

N. Fabian Kleimeier, 1,2,4 André K. Eckhardt, 3,4,5 Peter R. Schreiner, 3,* and Ralf I. Kaiser 1,2,6,*

SUMMARY

Pyruvic acid represents a key molecule in prebiotic chemistry to form metabolites and amino acids. Without liquid water on the early Earth, endogenous formation of pyruvic acid is unlikely, and an exogenous delivery constitutes an appealing alternative. However, despite the detection of more than 200 molecules in space, pyruvic acid is elusive. Here, we describe its formation by barrierless recombination of hydroxycarbonyl (HOCO$^+$) and acetyl (CH$_3$CO$^+$) radicals in ices of acetaldehyde (CH$_3$CHO) and carbon dioxide (CO$_2$) modeling interstellar conditions driven by cosmic rays. Exploiting isotopically labeled ices and photoionization reflectron time-of-flight mass spectrometry, the reaction products were selectively photoionized in the temperature-programmed desorption phase and isomers discriminated based on their ionization energies. This reveals a key reaction pathway for pyruvic acid synthesis through non-equilibrium reactions in interstellar cold molecular clouds and star-forming regions, thus offering a unique entry point to abiotic organic synthesis in deep space.

INTRODUCTION

Pyruvic acid (1, CH$_3$COCOOH) along with its deprotonated pyruvate anion (CH$_3$COCOO$^-$) represent critical molecules in modern biochemistry and plays a key role in contemporary chemical processes that occur within living organisms in order to maintain life (metabolism) (Scheme 1). Serving as the starting material for the Krebs cycle (citric acid or TCA cycle [tricarboxylic acid cycle]) and the pyruvate dehydrogenase complex, 1 links glycolysis with the TCA cycle. After decarboxylation of 1 and nicotinamide adenine dinucleotide (NAD$^+$) reduction, acetyl CoA (2) forms with cofactor A (CoA–SH, Scheme 1). Here, the energy is provided via carbon-carbon bond cleavage and the release of carbon dioxide (CO$_2$) in an oxidative decarboxylation of 1 as the very first step. With thiamine pyrophosphate (TPP, 3)—a thiamine (vitamin B$_1$) derivative with a N-heterocyclic carbene (NHC) as the active entity—the amino enol structure 4 forms, which is referred to as the Breslow intermediate. 1,2 The polarity of the formerly electrophilic carbonyl carbon atom is switched and now reveals nucleophilic character. Therefore, NHC organocatalysis and such Umpolung chemistry has found widespread applications in organic chemistry. 3–5 Only recently, Berkessel et al. characterized several hitherto elusive Breslow intermediates by NMR spectroscopy and X-ray crystallography. 6,7

In prebiotic chemistry, 1 may have served as a fundamental building block for key biological compounds. 8,9 Pyruvic acid can be reduced to lactic acid (CH$_3$CH(OH)COOH, 5) 10 or acts as a precursor for the amino acid alanine (6) formed in the reductive amination of 1. 11 Recently, Muchowska et al. reported a captivating chemical reaction network promoted by Fe(II), in which 1 and glyoxylate (HCOOCO$^-$) build up nine of the 11

The Bigger Picture

One of the key questions is how life could have emerged on early Earth and what chemicals and key reactions were involved. Terrestrial biomolecules, such as DNA, RNA, and peptides, formed from building blocks like nucleobases and amino acids. But where do these come from? Simple chemical building blocks could have formed on icy grains in space and may have survived comet impact on the early Earth. Pyruvic acid is widely accepted as a key prebiotic starting material, as it may have served as a fundamental building block for biorelevant molecules. This is underlined by the identification of pyruvic acid in carbonaceous meteorites. This study investigates the formation of pyruvic acid under interstellar conditions to encourage scientists in other fields to consider pyruvic acid as a potential interstellar molecule and include it in their radio-astronomical line searches. For chemists, the study will lead to a better understanding of the fundamental processes of abiotic syntheses of organic molecules.
intermediates of the TCA cycle. Adding hydroxylamine (NH$_2$OH) and metallic iron into the system produces four biological amino acids (6), glycine, aspartic acid, and glutamic acid. In this reaction network, both α-ketocarboxylic acids along with hydroxylamine serve as fundamental starting materials. Further, Powner et al. highlighted the critical role of 1 by synthesizing 2-phosphoenolpyruvate (PO$_4^{2-}$/C$_6$H$_7$COO$^-$, PEP)—the highest energy phosphate found in living organisms—from simple prebiotic nucleotide precursors: glycolaldehyde (HOCH$_2$CHO) and glyceraldehyde (HOCH$_2$CH(OH)CHO). Pyruvate synthesis may occur during the abiotic degradation of carbohydrates and their phosphates, by the oxidation of lactic acid in aqueous media, and in the presence of iron sulfides in very low yields of only 0.07% under hydrothermal vent conditions, e.g., at elevated temperatures (523 K) and pressures (200 MPa) or in the presence of other transition metals. However, these scenarios rely on very specific reaction conditions to produce labile components, such as 1, which might not have been widespread under prebiotic conditions on the early Earth.

In the gas phase, 1 thermally decarboxylates (Scheme 1) at elevated temperatures yielding carbon dioxide and methylhydroxycarbene (7, HO–C–CH$_3$); the latter undergoes isomerization via [1,2]H-tunneling to acetaldehyde (8, CH$_3$CHO), an observation that leads to the tunneling control reactivity paradigm. In general, hydroxycarbenes undergo barrierless carbonyl ene reactions with carbonyl compounds in the gas phase, yielding biorelevant molecules, including the carbohydrates glycolaldehyde and glyceraldehyde. Eckhardt et al. and Turner et al. recently demonstrated the formation of glyoxal acid (HCO(O)COOH) and even (di)phosphates (PO$_4^{3-}$/P$_2$O$_7^{4-}$) in ices modeling interstellar conditions, providing compelling evidence that essential biorelevant molecules, together with their precursors, might form in deep space. At least a fraction of these abiotically synthesized compounds might be incorporated into meteoritic parent bodies and could have survived successive meteorite or comet impact on the Earth, thus

Scheme 1. Interstellar Formation of Pyruvic Acid

Interstellar formation of pyruvic acid (1) in low-temperature ices containing acetaldehyde (8) and carbon dioxide by energetic processing through simulated galactic cosmic rays. Pyruvic acid serves as a precursor for critical biomolecules, including the amino acid alanine (6, bottom middle). In biochemistry, pyruvic acid is part of the citric acid cycle and forms acetyl CoA (2) in an enzymatic catalyzed reaction by pyruvate dehydrogenase (bottom right) with the Breslow intermediate 4 as the key intermediate (top right).
reinforcing the theory of an exogenous source of key prebiotic molecules on the Earth.\textsuperscript{27} This setting defines an appealing alternative to competing theories like the formation of biorelevant molecules in hydrothermal vents on the prebiotic Earth. Although pyruvic acid is prone to decarboxylation, especially in the presence of transition metals and at elevated temperatures, Cooper et al. identified pyruvic acid in carbonaceous meteorites at 15 nmol g\textsuperscript{−1}, indicating that even such potentially unstable compounds are able to survive the entrance of the meteorite into the atmosphere and participate in further prebiotic processes.\textsuperscript{28} Therefore, abiotic formation in the interstellar medium and delivery to the early Earth by meteorites constitutes another plausible source of prebiotic molecules on the Earth. This finding clearly underlines the importance of non-equilibrium processes in the fields of astro- and prebiotic chemistry.\textsuperscript{29,30}

RESULTS AND DISCUSSION

Ice Preparation

Here, we demonstrate the first abiotic synthesis of 1 under conditions mimicking extraterrestrial environments via the barrierless radical-radical reaction of the hydroxycarbonyl (HOCO\textsuperscript{−}, 9) and acetyl (CH\textsubscript{3}CO\textsuperscript{−}, 10) radicals (Scheme 1) by exposing low-temperature model ices to ionizing radiation with high-energy electrons simulating secondary electrons formed in the path of galactic cosmic ray (GCR) particles penetrating ices on interstellar grains in molecular clouds.\textsuperscript{31,32} Cold molecular clouds encompass the raw material of stars and planetary systems with nanometer-sized grain particles consisting of amorphous and polyaromatic carbon\textsuperscript{33} and olivine-type silicates\textsuperscript{34} ("interstellar dust") accumulating icy layers of mainly water (H\textsubscript{2}O), methanol (CH\textsubscript{3}OH), carbon dioxide (CO\textsubscript{2}), and carbon monoxide (CO) at temperatures as low as 10 K.\textsuperscript{35–37} These ices are chemically processed by the internal ultraviolet (UV) field\textsuperscript{38} and by energetic GCRs, leading to the synthesis of an array of organic molecules, such as the simplest sugar-related compound—glycolaldehyde,\textsuperscript{39} other aldehydes\textsuperscript{40} (e.g., Abelson,\textsuperscript{8} Bennett et al.,\textsuperscript{31,32}), ketones (e.g., acetone), and carboxylic acids\textsuperscript{41,42} (e.g., formic acid\textsuperscript{43} and acetic acid\textsuperscript{44}).\textsuperscript{35} When a molecular cloud transits into a star-forming region, matter is incorporated into circumstellar disks, which, in turn, contain the material out of which planets, planetoids, and comets may form. Therefore, 1, initially formed in interstellar ices, can be integrated into matter of solar systems eventually untangling the fundamental chemical reaction(s) of how and where in the universe the molecular precursors to the origins of life can arise.

In contrast to the complex mixture of ice constituents found in the interstellar medium, laboratory model ices typically only consist of binary or ternary mixtures of molecules detected in interstellar ices to facilitate the assignment of newly formed molecules based on mass-to-charge ratios, isotopic shifts, and ionization energies. Therefore, only a fraction of the molecules expected to form in interstellar ices are typically found in lab-based experiments. However, molecules formed in different individual simplified mixtures can be expected to form in interstellar ices, based on the availability of the reactants used.

The experiments were conceived to unravel the abiotic synthesis of 1 upon exposing polar model ices of 8 and carbon dioxide (CO\textsubscript{2}) to proxies of galactic cosmic rays in the form of high-energy electrons under conditions mimicking the lifetime of molecular clouds of up to a few million years.\textsuperscript{45} These anhydrous model ices were chosen to explore the proof-of-concept that 1 can be synthesized via interaction of interstellar ices with ionizing radiation. Accounting for data from the Spitzer space
telescope, ices containing carbon dioxide at levels of up to 37% relative to water were observed toward low- and high-mass stars, field stars, and Galactic center sources\(^4^6\)–\(^4^8\); \(^8^\) was only detected tentatively on grains\(^4^9\),\(^5^0\), but laboratory experiments verified that \(^8^\) forms easily on interstellar grains containing carbon monoxide (CO) and methane (CH\(_4\)).\(^3^1\),\(^4^0\) During the radiation exposure of \(^8^\) and carbon dioxide or \(^8^\) and carbon dioxide-\(^1^8^O_2\) (C\(^1^8^O_2\)) ices, multiple novel infrared (IR) absorption features emerged (Figures 1 and S1–S5; Table S1).

**IR Analysis**

These absorptions can be linked to four discrete molecules: carbon monoxide (CO, 2,131 cm\(^-1\)), carbon monoxide-\(^1^8^O\) (C\(^1^8^O\), 2,086 cm\(^-1\)), the acetyl radical (10, 1,840 cm\(^-1\), Figure 1), and methane (CH\(_4\), 1,304 cm\(^-1\)).\(^5^1\)–\(^5^3\) The assignment of 10 is further supported by the isotopic shift in a mixture of acetaldehyde-\(d_4\) (CD\(_3\)CDO) and carbon dioxide-\(^1^8^O_2\), where the acetyl-\(d_3\) radical is detected at 1,851 cm\(^-1\), which agrees well with matrix isolation studies of acetyl and previous studies on the irradiation of pure acetaldehyde ices.\(^5^1\),\(^5^3\) However, since electron irradiation can produce a significant array of new species and isomers with close vibrational absorptions, infrared spectroscopy is capable of determining only newly formed functional groups, but often does not allow further identification of individual complex organic molecules. In contrast to inert gas matrix isolation spectroscopy, no inert host gas was used in our experiments, since they aim to replicate interstellar ices; therefore, all observed infrared absorptions were typically very broad and not sharp, which made assignments of specific molecules extremely challenging. The features at 954 cm\(^-1\) and 1,052 cm\(^-1\) can be linked to CH\(_2\) wagging and C–O stretching vibrations, respectively. In addition, a broad absorption feature between 1,200 and 1,300 cm\(^-1\) was observed after irradiation that was probably connected to C–O–H deformation vibrations. Due to the low concentration, broad absorptions, and signal overlaps in the critical regions, we could not identify 1 unambiguously. However, since the formation of 10 from \(^8^\) released suprathermal hydrogen, which could easily overcome the barrier of addition to carbon dioxide to form 9,\(^5^4\) it is very likely that 9 formed in the ices. Unfortunately, in both isotopically labeled and the unlabeled
ices, the absorption features of 9 coincided with acetaldehyde absorption features at 1,796 cm$^{-1}$ and the acetyl feature at 1,833 cm$^{-1}$, respectively. Moreover, no absorptions associated with vinoxy radicals (2-oxoethyl, CH$_2$CHO, 1,542 cm$^{-1}$) were observed. This is in agreement with a recent study on radical-radical reactions in pure acetaldehyde ices, revealing that reaction products from vinoxy radicals did not form. Comparing the molecular structure of 1 with the radical building blocks 9 and 10 suggests that 1 could have formed in the ices via a barrierless radical-radical recombination (Scheme 1). However, an alternative analytical technique is required for the firm identification of 1.

**PI-ReTOF-MS Study**

This could be achieved by exploiting photoionization reflectron time-of-flight mass spectrometry (PI-ReToF-MS) during the temperature-programmed desorption (TPD) phase, in which, the irradiated ice was heated to 320 K. This approach allowed us to identify different isomers in the gas phase, based on their distinct ionization energies and desorption profiles, which is outlined below. In contrast to electron impact ionization, which can lead to strong fragmentation even of the parent molecule, photoionization is ideally fragmentation free, thereby facilitating the assignment of mass-to-charge ratios of specific isomers. The PI-ReToF-MS experiments were performed using ices of 8 and $^{18}$O isotopically labeled carbon dioxide (C$_{18}$O$_2$). This combination ensured a unique mass-to-charge ($m/z$) ratio for the reaction products investigated as the main reaction products from pure acetaldehyde ices subjected to ionizing radiation were diacetyl ($m/z = 86$), the acetaldehyde dimer ($m/z = 88$), and the protonated dimer ($m/z = 89$), all of which had a lower ionization energy than 1. With the isotopic labeling, however, the mass-to-charge ratio of 1-$^{18}$O$_2$ shifted to $m/z = 92$, at which no reaction products of 8 or C$_{18}$O$_2$ were detected at the photon energies used; at the same time, reaction products from pure acetaldehyde ices were not shifted by the isotopic labeling.

As adiabatic ionization energies of key complex organic molecules have not yet been determined experimentally, they had to be computed with high confidence. All molecular structures were optimized at B3LYP/cc-pVTZ level of theory in their electronic ground state and in their radical cationic form that resembles the most similar conformation. All electronic energies were improved by extrapolated high level ab initio coupled cluster CCSD(T)/CBS energies and were corrected by zero-point vibrational energies (ZPVEs). The difference of the ZPVE corrected radical cationic electronic energy and the ZPVE corrected energy of the studied molecule equaled the adiabatic ionization energy. As the difference of deuterated (or heavier) and non-deuterated isotopologs in the ZPVEs is generally marginal, we used the ZPVEs of non-deuterated isotopologs for IE calculations and assumed them to be the same for our experiments with heavier isotopologs. For a comparison and error analysis, the adiabatic ionization energies of selected molecules have been computed and compared with experimental values (Table S2). The comparison showed that the computed values were within an error regime of $-0.04$ eV to $+0.19$ eV to the experimentally determined ones. This estimated error and a reduction of ionization energies of 0.03 eV due to the electric field of the extractor plate of the time-of-flight spectrometer$^{26}$ were taken into account for the design of our following experimental studies.

In our PI-ReToF-MS studies, 1 and 2-hydroxyacrylic acid (CH$_2$(COH)COOH, 11) could be distinguished based on their experimental ($IE = 10.1$ eV for 1)$^{27}$ and computed adiabatic (CCSD(T)/CBS/B3LYP/cc-pVTZ +ZPVE) ionization energies (Table S3) by tuning the photon energy. Utilizing photons at 10.49 eV, all isomers could be photoionized. By tuning the photon energy down to 9.75 eV, 1 ($IE = 9.90–10.02$ eV) could
not be ionized, leaving only its enol isomer 11 to contribute to the ion signal at m/z = 92. Decreasing the photon energy further to 9.20 eV excluded all conformers of 2-hydroxyacrylic acid (IE = 9.28–9.84 eV). The temperature-dependent mass spectra over the range of m/z = 40–200 at each photon energy are shown in Figure 2. As 8 polymerized quickly upon electron irradiation, it desorbed from its sublimation onset at around 90 K up to a maximum temperature of 320 K and hence dominated the ion counts at 10.49 eV. The spectrum recorded at this energy has therefore been scaled to the height of the highest product peak to provide better visibility of the products formed. To exclude contributions from impurities in the ice, a TPD experiment was also conducted at 10.49 eV on unirradiated ice (Figures 3C and S6), revealing m/z = 117 as the only significant ion signal apart from the reactants. Figure 3A shows the corresponding TPD profiles of m/z = 92 recorded at different ionization energies. At 10.49 eV, a bimodal distribution peaking at 200 K and 235 K was clearly visible, suggesting the formation of both 1 and its enol isomer 11. This was confirmed when comparing the TPD profile recorded at 9.75 eV, which was below the ionization energy of 1. Evidently, the second sublimation event vanished at this photon energy. The early sublimation event only disappeared at 9.20 eV. These findings indicate that the first desorption event (175–225 K) was due to 11 and the second event (200–255 K) was linked to the desorption of 1. The desorption of 1 might have been delayed in comparison with 11 because of one free carbonyl group that might have been involved in an intermolecular hydrogen bonding network within the irradiated ice (see also Figure 3D). In 11, intramolecular hydrogen bonding (cf. relative energies in Table S3, anti-(Z,Z) conformer) was dominating the molecular structure.

The individual desorption profiles of 1 and 11 could be extracted from the data by scaling the data collected at 9.75 eV and subtracting them from the TPD profile recorded at 10.49 eV, as visualized in Figure 3B. The assignment of the second desorption event to 1 was further verified when comparing this difference spectrum to that of 1, as shown in Figure 3D. Pure pyruvic acid desorbed at 175–200 K (green curve), however, when co-deposited with acetaldehyde and C\(^{18}\)O\(_2\) and irradiated using the same parameters as in the experiment (black curve), a second, broad desorption event spanning from 200–235 K became evident. This event originated from 1 trapped inside the polymer matrix of 8, thereby delaying the desorption. This part of the TPD profile matched with that of the desorption event only seen at 10.49 eV (blue curve, Figure 3D) in our experiments, which further confirmed the synthesis of 1 in the ice. The divergence of the profiles at higher temperatures could be explained by 1 trapped in sites of the polymer matrix that were not accessible to the co-deposited pyruvic acid, whereas the absence of the first desorption event was due to the fact that 10 formed from irradiation was trapped in the polymer matrix. These effects were previously also seen for diacetyl forming in pure ices of 8.\(^{53}\)

**Conclusion**

The results of this study aid in our understanding of how 1 can form abiotically in acetaldehyde-containing interstellar ices upon interaction with ionizing radiation. The molecular structure of 1 suggests a barrierless radical-radical recombination of 9 and 10 as a key formation pathway, since 10 can clearly be identified by infrared spectroscopy and 9 is known to form easily via addition of suprathermal hydrogen to carbon dioxide. Due to overlap of the main absorptions of 1 with other constituents of the ice, its presence in the irradiated ice at 5 K could, however, not be spectroscopically confirmed, and it remains to be seen whether 1 forms upon irradiation at 5 K or during the warm-up phase when radicals become mobile in the ice and can therefore easily recombine. Exploiting TPD with tunable photoionization...
reflectron time-of-flight mass spectrometry and infrared spectroscopy, this study reveals a non-equilibrium pathway for the formation of \( \text{C_3H_4O_2} \) in interstellar ices. The non-equilibrium nature of this reaction is evident from the endoergic cleavage of the carbon-hydrogen bond in \( 8 \) leading to the formation of the acetyl radical \( 10 \).
requiring 3.9 eV. Further, the overall reaction leading to the synthesis of 1 from 8 and carbon dioxide is endoergic by 0.8 eV with the energy supplied by the energetic processing of the ice samples.

In cold molecular clouds, once 1 forms within interstellar ices and/or during the annealing phase of the ices when the new star forms (star-forming regions) and temperatures may reach up to 300 K, such as in Sagittarius B2, 1 can sublime to be incorporated into planetoids, asteroids, and comets. Detailed analyses of meteorites like the Murchison meteorite revealed that biorelevant molecules, such as amino acids embedded in meteoric matter, can survive harsh conditions in space as well as impact on the Earth. Even 1, which is a labile compound prone to decarboxylation, has been detected in meteoric samples. Therefore, organic molecules formed in molecular clouds could have been delivered to the early Earth, thus highlighting the possibility of an exogenous source of prebiotic molecules as a plausible additional pathway to the formation of biorelevant molecules besides their potential formation in hydrothermal vents on the prebiotic Earth. As 8 has been detected in molecular clouds, such as the Taurus molecular cloud (TMC-1) and Sagittarius B2 as well as toward hot cores like NGC 6334F, the presence of 1 in these environments seems conceivable. Therefore, the results of this study indicate that directed searches for 1 by the latest generation of telescopes, such as the Atacama large millimeter/submillimeter array (ALMA) toward these regions of space might be successful to further shed light on the origins of prebiotic molecules. This will lead to a better understanding of the fundamental processes of abiotic syntheses of organic molecules in space.
EXPERIMENTAL PROCEDURES

Resource Availability

Lead Contact

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Ralf I. Kaiser (ralfk@hawaii.edu).

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

The datasets generated during this study are available upon request.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.chempr.2020.10.003.

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AUTHOR CONTRIBUTIONS

R.I.K. designed the experiments. N.F.K. and A.K.E. performed all experiments and carried out all computations. N.F.K., A.K.E., P.R.S., and R.I.K. co-wrote the manuscript. N.F.K. and A.K.E. contributed equally to this work.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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