

Detection and formation scenario of citric acid, pyruvic acid, and other possible metabolism precursors in carbonaceous meteorites

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Carbonaceous meteorites deliver a variety of organic compounds to Earth that may have played a role in the origin and/or evolution of biochemical pathways. Some apparently ancient and critical metabolic processes require several compounds, some of which are relatively labile such as keto acids. Therefore, a prebiotic setting for any such individual process would have required either a continuous distant source for the entire suite of intact precursor molecules and/or an energetic and compact local synthesis, particularly of the more fragile members. To date, compounds such as pyruvic acid, oxaloacetic acid, citric acid, isocitric acid, and α -ketoglutaric acid (all members of the citric acid cycle) have not been identified in extraterrestrial sources or, as a group, as part of a “one pot” suite of compounds synthesized under plausibly prebiotic conditions. We have identified these compounds and others in carbonaceous meteorites and/or as low temperature (laboratory) reaction products of pyruvic acid. In meteorites, we observe many as part of three newly reported classes of compounds: keto acids (pyruvic acid and homologs), hydroxy tricarboxylic acids (citric acid and homologs), and tricarboxylic acids. Laboratory syntheses using ¹³C-labeled reactants demonstrate that one compound alone, pyruvic acid, can produce several (nonenzymatic) members of the citric acid cycle including oxaloacetic acid. The isotopic composition of some of the meteoritic keto acids points to interstellar or presolar origins, indicating that such compounds might also exist in other planetary systems.

pyruvate | citrate | Murchison meteorite | Alan Hills | interstellar nitriles

Soluble organic compounds detected in carbonaceous meteorites include amino acids, carboxylic acids, mono- and poly hydroxy carboxylic acids, and nitrogen heterocycles (1–3). However, absent from the list of meteoritic organic compounds are keto acids (e.g., pyruvic acid, acetoacetic acid) and citric acid and homologous compounds. Some of these compounds are critically important to biological processes such as glycolysis and the citric acid (or “tricarboxylic acid”) cycle—processes considered to be among the earliest in the history of life (4). Using gas chromatography-mass spectrometry (GC-MS) to analyze extracts of multiple carbonaceous meteorites including Murchison, Murray and Allan Hills (ALH) 83102, we have identified homologous series of keto acids, tricarboxylic acids, and hydroxy tricarboxylic acids (Fig. 1). Below, “identified” compounds refers to those whose GC retention times and mass spectra match those of laboratory standards. The vast majority of the compounds have been identified in several meteorite extracts and as multiple volatile derivatives: tert-butyl dimethylsilyl (tBDMS), trimethylsilyl (TMS), and isopropyl ester (ISP). Although drawn in the acid forms, all compounds are salts in the meteorites due to the generally neutral-alkaline conditions of aqueous alteration on the meteorite parent body (5, 6).

Results and Discussion

Meteoritic Keto Acids. As a suite, keto monoacids were ubiquitous in examined meteorites. Identified members are the straight-

chained three-carbon (3C) pyruvic acid through the eight-carbon (8C) 7-oxooctanoic acid and the branched 6C acid, 3-methyl-4-oxopentanoic acid (β -methyl levulinic acid), Fig. 1, Table S1. 2-methyl-4-oxopentanoic acid (α -methyl levulinic acid) is tentatively identified (i.e., identified by mass spectral interpretation only). As a group, these keto acids are relatively unusual in that the ketone carbon is located in a terminal-acetyl group rather than at the second carbon as in most of the more biologically familiar α -keto acids (e.g., α -keto butyric acid, etc.). Several additional tentatively identified keto monoacids (Fig. S14; not all are indicated) also appear to be terminal-acetyl acids based on the similarity of their mass spectra. The straight-chained keto acids are more abundant than their branched isomers: a qualitative similarity to the suite of dicarboxylic acids (7) and dicarboxylic acid amides (8) in Murchison. A keto dicarboxylic acid (and dimer of pyruvic acid), 4-hydroxy-4-methyl-2-ketoglutaric acid (parapyruvic acid), is identified based on GC-MS interpretation of pyruvate reaction products (see below). This compound forms readily and is abundant in pyruvate solutions: it is a long known pyruvate condensation product (9–12). To date, the most notable report of possible self-condensation products of organic compounds in meteorites involves the glycine peptide glycyl-glycine and its cyclic form, diketopiperazine. They were seen in small amounts in the Yamato-791198 and Murchison meteorites (13).

In the present samples α -ketoglutaric acid (2-ketoglutaric acid or 2-oxoglutaric acid) is also a likely constituent based on GC retention time and the presence of its major fragment ion ($M^+ - 57 = 431$ amu) (see Fig. S1B legend) however its total mass spectrum is less definite than other compounds—due either to low abundances in the samples or low analytical recoveries. Oxaloacetic acid (Fig. 2B) is possibly present: the GC retention time and major ion (417) of oxaloacetic acid (tBDMS derivative) matches a compound in ALH 83102, Murray, and Murchison but the meteoritic spectra are weaker than those of α -ketoglutaric acid: specific analysis for oxaloacetic acid (a labile compound) must be done. In a review of GC-MS data from several previous extracts we have observed at least small amounts of the majority of the keto acids in Fig. 1 (including pyruvic acid and acetoacetic acid) even though the analytical procedures (see *Materials and Methods*) were usually meant for general identifications and in many cases deleterious to some keto acids. Therefore, we cannot rule out the presence of other keto acids.

Pyruvic acid recovered from ALH 83102 corresponds to approximately 15 nmole/gram. The concentration of its larger

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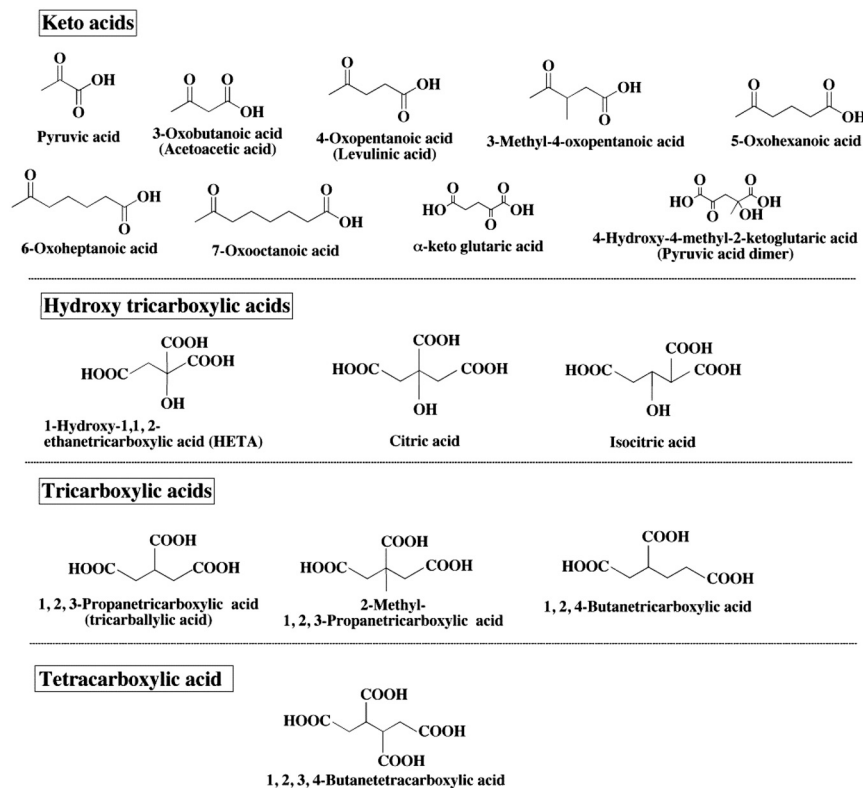


Fig. 1. Compounds identified in carbonaceous meteorites. Several more related compounds are tentatively identified (Fig. S1A).

homolog, levulinic acid, recovered from ALH 83102 and Murchison is approximately 45 and 5.4 nmole/gram, respectively (Table S1). In most homologous series of compounds in meteorites the lower molecular weight (MW) members are usually more abundant—the exception here in ALH 83102 may be due to the much greater reactivity (on the meteorite parent body) of pyruvate (see pyruvate reactions below). Reactivity may also account for the apparently low amount of oxaloacetic acid (if this compound is confirmed). In order to perform isotope measurements on some keto acids, the keto acid extract shown in Fig. S1A was further purified by ion chromatography (*Materials and Methods*) to isolate individual compounds (e.g., Fig. S2). We measured the $^{13}\text{C}/^{12}\text{C}$ values of levulinic acid, 5-oxo-hexanoic acid, and 6-oxo-heptanoic acid, and the D/H ratio of levulinic acid; all were from Murchison and were measured as their TMS derivatives. The resulting $\delta^{13}\text{C}$ values (‰) were +17.04, +15.24, and +12.94, respectively; the δD of levulinic acid was +425‰ (Table S1). All of these values are more positive than the typical range of Earth's organic compounds and point to low temperature extraterrestrial synthesis (2). The $\delta^{13}\text{C}$ values are comparable to values of meteoritic amino acids having the same carbon number. The δD value of levulinic acid is near the lower end of the range of values for meteoritic carboxylic acids (2).

Meteoritic Hydroxy Tricarboxylic Acids. Citric acid, the much less abundant, isocitric acid, and 1-hydroxy-1,1,2-ethanetricarboxylic acid are the identified hydroxy tricarboxylic acids (Fig. 1). While the GC chromatogram (Fig. S1A) best illustrates the abiotic distribution of these compounds, it is also somewhat anomalous in comparison to chromatograms of other extracts of Murchison, Murray, and ALH 83102. Observed is a 7C homolog that apparently has a higher concentration than citric acid (6C). In all other extracts, citric acid is the dominant member of the series: depending on the extract, some citric acid homologs are not seen at all. Overall, this group of compounds is relatively low in abundance:

the concentration of citric acid in one sample of Murchison is approximately 55 pmole/gram (Table S1).

Meteoritic Tri- and Tetracarboxylic Acids. Identified tricarboxylic acids are shown in Fig. 1. 1, 2, 3-propanetricarboxylic acid (tricarballic acid) at approximately 2 nmole/gram in ALH 83102 and 371 pmole/gram in Murchison is the dominant member of the series: it is much less abundant than succinic acid, the most abundant meteoritic dicarboxylic acid (2). The tricarboxylic acids consist of at least 25 members and extend to at least 10C (Fig. S1A; not all identified homologs are shown). As with the keto monoacids the “straight-chained” (referring to the non-carboxyl carbons) compounds apparently predominate. The one observed tetracarboxylic acid (1, 2, 3, 4-butanetetracarboxylic acid) (Fig. 1), to date, has been seen in only one extract. However, the analytical procedures used, generally for lower molecular weight compounds, must be developed or changed to search specifically for other possible tetracarboxylic acids.

Interstellar Formation Mechanisms. Although there may be multiple formation mechanisms for all soluble meteoritic organic compounds, the chemistry of interstellar nitriles, HCN/CN, ketene, and water may have played an important role in the formation of at least some compound classes (Fig. 24). A range of straight-chained nitriles (e.g., CCN, HCCN, CCCN, etc.), ketene, CN, and HCN are known interstellar molecules (14): laboratory studies show that addition of CN radical to unsaturated hydrocarbons to give nitriles is an exothermic process (15). On the formation of meteoritic keto monoacids, one possibility is the addition of ketene (a strong electrophile) to the terminal carbons of nitriles (radicals) to give the precursors of straight-chained members. In the case of pyruvic acid, experiments show that the reaction between ketene and HCN to give pyruvonitrile (CH_3COCN), a pyruvate precursor, proceeds readily at low temperatures (16). In addition, a radical, and another pyruvate precursor, $\text{CH}_2\text{C}(\text{O})\text{CN}$, is a theoretical intermediate in the

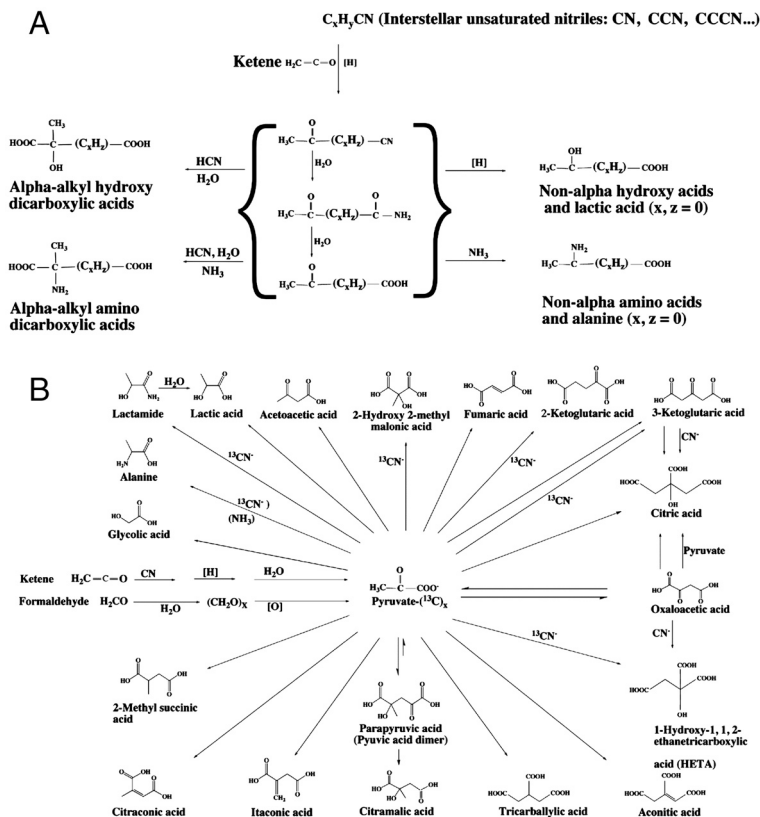


Fig. 2. A possible mode of formation of meteoritic compounds from known interstellar molecules and subsequent aqueous chemistry. (A) Addition of ketene (neutral or ionic) to interstellar nitriles and hydrogenation producing keto compounds. If addition is to terminal carbons, straight-chained keto acids are eventually produced. If addition is to internal carbons, branched keto acids (e.g., 3-methyl-4-oxopentanoic acid, Fig. 1) are the result. At any stage compounds may be saturated or unsaturated. (B) Observed laboratory pyruvate reaction products: a possible synthetic route to some known meteoritic compounds and prebiotic citric acid cycle precursors. All products were identified by GC-MS as their tBDMs and/or TMS derivatives. Depending on the particular experiment, all listed reaction products were observed labeled with ^{13}C from either reactant pyruvate ($x = 0, 1, 2, \text{ or } 3$) or $K^{13}CN$. Where pyruvate is the sole reactant, reaction products are fully labeled with ^{13}C when fully labeled ($U-^{13}C$) pyruvate is used, e.g., oxaloacetic acid, acetoacetic acid, 2-methyl succinic acid, etc. 2-Methyl succinic acid contains one ^{13}C (i.e., its mass increases by one amu) when $^{13}C_1$ -pyruvate is the reactant; it contains two ^{13}C when $^{13}C_2$ -pyruvate is the reactant; and four ^{13}C when starting with $^{13}C_{2,3}$ -pyruvate. This result is consistent with a substitution of the oxygen on carbon-2 of one molecule of pyruvate by a second molecule of pyruvate (at carbon-3) followed by decarboxylation and hydrolysis of the second pyruvate. 2-Ketoglutaric acid (^{13}C -labeled) is observed in pyruvate- $K^{13}CN$ reaction mixtures. To date, 2-ketoglutaric acid has not been seen in pyruvate-only solutions but is seen in pyruvate-oxaloacetate solutions implying that it can also be formed by pyruvate alone (i.e., after pyruvate produces oxaloacetate). 3-Ketoglutaric acid is seen in both pyruvate-only solution and pyruvate + KCN solution. Due to the GC coelution of cis and trans aconitate and the similarity of their mass spectra, the identity of the observed isomer(s) is not yet determined. Also, the presence or absence of malic acid (another citric acid cycle compound) is not yet confirmed. [In addition to the keto acid formation scheme in (A), meteoritic pyruvate formation might have also taken place by the oxidation of formaldehyde-water reaction products as shown].

reaction of CN radical with ketene (17). Similar reactions with higher nitriles and ketene (or other acetylating agents) may give the higher homologs (Fig. 2A). Addition of ketene in some form (ionic, radical, or neutral) to internal unsaturated carbons of the nitriles could give branched keto acids. After formation, the keto acids or their nitrile and/or amide forms (Fig. 2A) should then yield a variety of other meteoritic compounds including “non- α ” acids (*SI Text*). Ketene addition to the nucleophilic nitrogen of amino acids may be responsible for the presence of acetylated amino acids in meteorites (8). In terms of nitrile reactions with molecules other than ketene, theoretical calculations show that it is energetically favorable for molecules such as NH_3 and H_2O (among others) to also add to the terminal carbon of CCN (18): after further processing this could result in an amino and hydroxy acid, respectively. This scenario may also apply to higher nitrile homologs, again resulting in non- α acids.

A ubiquitous and important source of energy, interstellar radiation, should be considered in tricarboxylic acid formation. The exposure of interstellar grains and comets to various forms of radiation inspired research into how such phenomena would affect the production of organic compounds (19). A series of experiments employing the irradiation of various starting compounds, including HCN, were shown to produce some compounds

also found in meteorites (19, 20). The list includes some of the compounds we report here such as tricarballic acid and citric acid (20). Tricarballic acid (and other tricarboxylic acids) is also produced by aqueous reactions of pyruvate (outlined below). A portion of the meteoritic tricarboxylic acids may have formed from successive additions of HCN to unsaturated mononitriles. An analogy is the postulated formation of meteoritic succinic acid with HCN and acrylonitrile (21). For example, sequential beta additions of HCN to interstellar (22) cyanoallene, CH_2CCHCN , followed by aqueous hydrolysis could have produced tricarballic acid (Fig. 1).

The Formation of Prebiotic Compounds by Pyruvate Chemistry: Laboratory Reactions. To determine if subsequent reactions of keto acids could be partly responsible for the presence of hydroxy tricarboxylic acids (e.g., citric acid) and other known meteoritic compounds we began a series of reactions with keto acids under conditions related to those of carbonaceous meteorite parent bodies (*SI Text*). In the experiments a reactant, most often sodium pyruvate, was placed in a solution of carbonate buffer or sodium bicarbonate with or without KCN and/or peroxide and allowed to react at 2 °C or 22 °C. In some cases peroxide was added at later stages of the reactions. Many of the same products are seen at

either temperature. To verify products of the reactions and possibly elucidate reaction mechanisms ^{13}C -labeled reactants were also used. Labeled reactants were K^{13}CN , $^{13}\text{C}_1$ -pyruvate (i.e., the carboxyl carbon is carbon-13), $^{13}\text{C}_{2,3}$ -pyruvate, $^{13}\text{C}_3$ -pyruvate or fully labeled ^{13}C -pyruvate ($\text{U-}^{13}\text{C}$). In contrast to many other organic acids, it has been shown that even at -20°C the sodium salt of pyruvic acid is reactive with formation of the dimer (11) (Fig. 1) and other compounds, probably oligomers (11, 12). At 22°C dimer and oligomer formation can be seen in several minutes (12).

In our reactions with pyruvate a variety of compounds were produced (Fig. 2B, Fig. S3A and B, and Table S2) and many are known to have important biochemical functions: all were found to be ^{13}C labeled to various degrees when starting with labeled reactants. Measurements of molar abundances and yields are in progress: Table S2 gives a crude measure (detector responses) of possible abundance relationships. All of the identified compounds, with the possible exceptions of oxaloacetic acid, aconitic acid, and 3-ketoglutaric acid, are also now found in carbonaceous meteorites. While we do consider it likely that several of the shown meteoritic compounds were formed from pyruvate chemistry, in general there may be multiple routes to individual meteoritic compounds (2).

The range of identified pyruvate products (Fig. 2B, Fig. S3A and B, and Table S2) include hydroxy acids such as the 2C glycolic acid; the 4C keto acids acetoacetic acid and oxaloacetic acid; citramalic acid (5C); the unsaturated 5C isomers itaconic acid and citraconic acid. Mesaconic acid, an isomer of itaconic and citraconic acid is, for now, a tentative identification—by comparison of its National Institute of Standards and Technology (NIST) mass spectrum (tBDMS-derivative) with our spectra. In addition, mesaconic acid's mass spectrum is distinguished from those of the latter two acids by a significant fragment representing loss of a carboxyl carbon—in the form of CO. Also found are the hydroxy tricarboxylic acids, citric acid, and 1-hydroxy-1,1,2-ethanetricarboxylic acid (HETA). As in meteorites, citric acid is of relatively low abundance. Fumaric acid and small amounts of succinic acid are also produced; trace amounts of maleic acid (the cis isomer of fumaric acid) may be present in some samples. The expected pyruvate dimer, and the corresponding compound from Murchison, is shown in Fig. 3. Other observations: higher acids (>6C) are tentatively identified; urea is derived only from CN^- —its mass increases by one amu when K^{13}CN is a reactant; we also see citric acid production in aged 1,3-Acetone dicarboxylic acid. In addition to alanine, other amino acids (e.g., aspartic acid) are possibly present in low amounts—they may be formed by the reaction of NH_3 (from CN^-) with the corresponding keto acid.

Fig. 4 illustrates the production of oxaloacetate from ^{13}C -labeled pyruvate. This result is important from a prebiotic point of view because of the critical role of oxaloacetate in biology (e.g., the citric acid cycle) and its very labile nature. To date, there have been no demonstrations of its abiotic synthesis from smaller precursors or an explanation of how such a fragile molecule could persist long enough for incorporation into early biological structures. It can be seen (Fig. 4) that the mass of oxaloacetate increases by one amu when either $^{13}\text{C}_1$ - or $^{13}\text{C}_3$ -labeled pyruvate are the reactants and by three amu when using $^{13}\text{C}_{2,3}$ pyruvate. Efforts to determine the synthesis mechanism are ongoing: one possible mechanism involves the formation of a di-keto dicarboxylic intermediate that decarboxylates to oxaloacetate.

In the case of citrate formation from pyruvate, there could be at least three possible mechanisms at work (Fig. 2B): two follow from the production of oxaloacetate from pyruvate (Fig. 2B). The first involves the possible reaction of pyruvate with the produced oxaloacetate. In early work, a starting mixture of these two compounds (in Na_2CO_3) was said to give 35% yield of citric acid (23). The proposed mechanism involved the nucleophilic addition

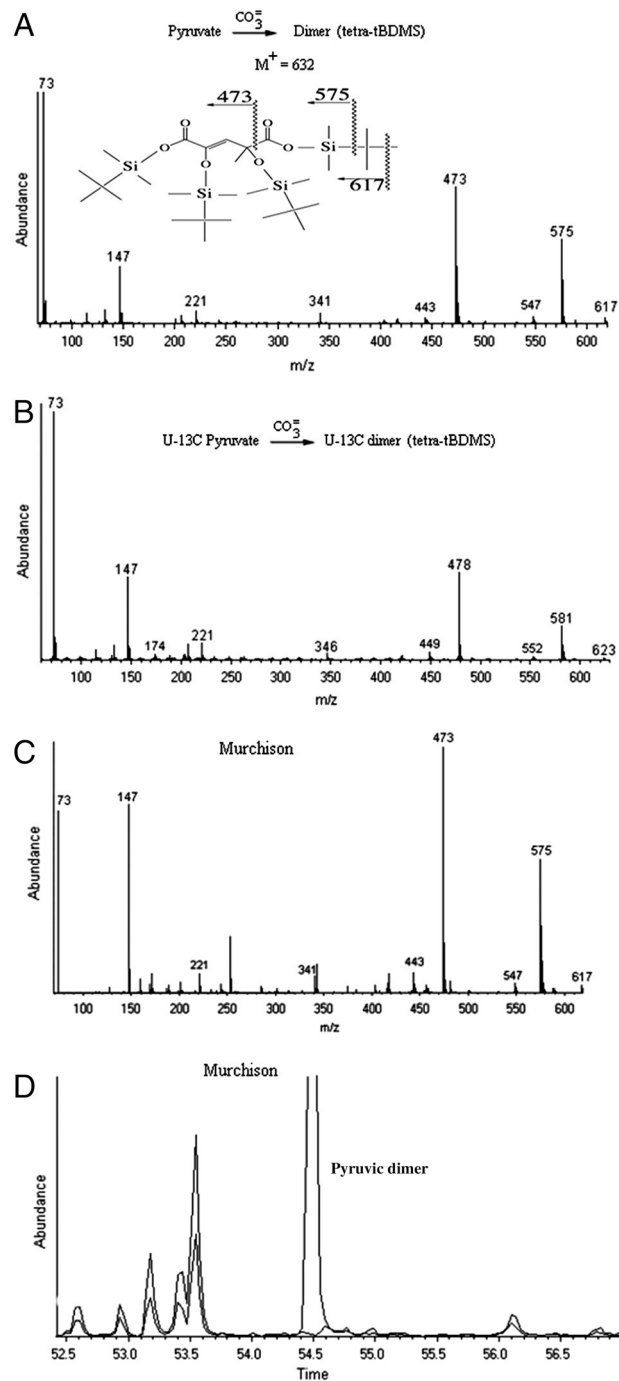


Fig. 3. (A) Mass spectrum of parapyruvate (pyruvate dimer) from a solution of pyruvate (0.062 M) and KCN (0.07 M) (7 d at 22°C in carbonate). The dimer is shown as its tBDMS [$-\text{Si}(\text{CH}_3)_2-\text{C}(\text{CH}_3)_3$] derivative. (B) Parapyruvate from solutions of $\text{U-}^{13}\text{C}$ -pyruvate. (C) Mass spectrum of the corresponding compound from the Murchison meteorite. Extra fragments are from coeluting compounds. (D) In Murchison, a chromatogram from single ion searches of 575 amu (dimer) and 574 amu. Some of the unlabeled compounds may be amides of parapyruvate and isomers.

of pyruvate to oxaloacetate (to give a 7C compound) with a subsequent loss of carbon-1 (decarboxylation) of the pyruvate moiety. Results of our experiments that react ^{13}C -pyruvate with (unlabeled) oxaloacetate are consistent with such a mechanism (Fig. S4) and perhaps crudely similar to the biological sequence of pyruvate—acetyl CoA + oxaloacetate—citrate.

Evidence of a second mechanism (also in pyruvate-only solutions) is actually the most definitive under the present

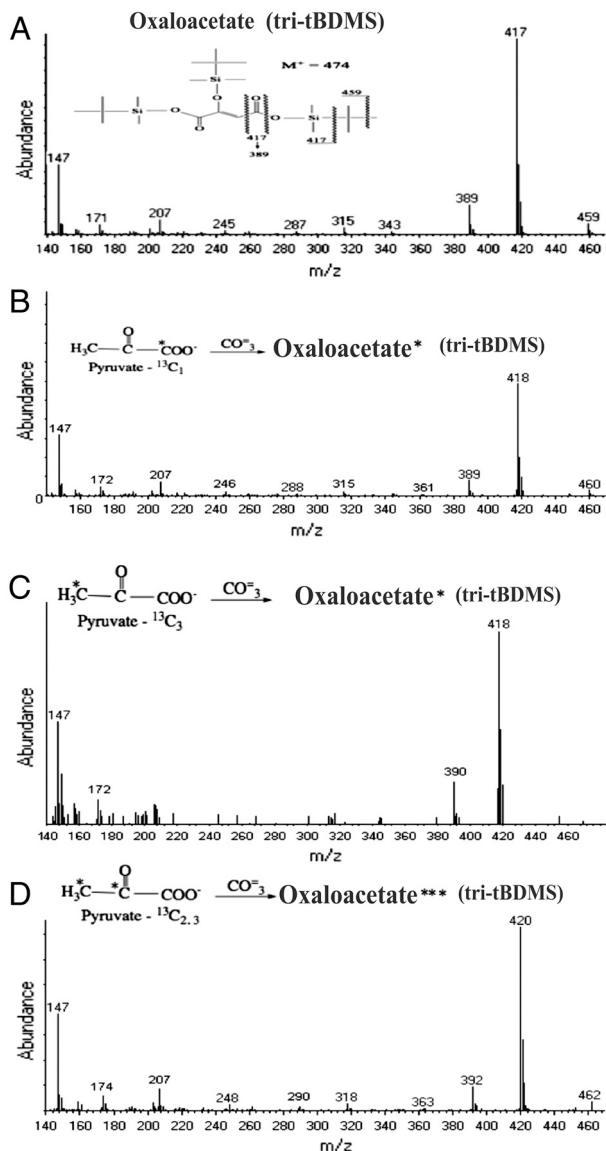


Fig. 4. Oxaloacetate standard after tBDMS derivatization and oxaloacetate produced from ^{13}C -labeled (*) pyruvate. **A.** Mass spectrum of standard, tri-tBDMS-derivative. **(B–D).** Spectra showing the mass increase of oxaloacetate produced from the indicated labeled pyruvate.

experimental conditions—it is seen directly. In contrast to the above loss of carbon-1 by the pyruvate moiety, here the mass of the resulting citrate does increase by one amu when $^{13}\text{C}_1$ -pyruvate is used, i.e., the starting carboxyl group is likely preserved. When $^{13}\text{C}_{2,3}$ -pyruvate is the reactant the mass of citrate increases by five amu.

In a third possible mechanism in initially pyruvate-only solutions, a portion of citrate could also be formed exclusively from the produced oxaloacetate: this reaction is known and likely involves nucleophilic attack of one molecule of oxaloacetate on the keto carbon of another followed by loss of OC-COOH (24, 25) (we also find citrate in oxaloacetate-only solutions). In the reaction of oxaloacetate with KCN we found an expected product, the smaller homolog of citric acid, HETA (Figs. 1, 2B): the MW of HETA increases by one amu when K^{13}CN is used.

Conclusions

We reported the presence of unique classes of organic compounds—some are labile and of potential prebiotic significance—in carbonaceous meteorites. Early solar system reactions of precursors

such as CN/HCN , ketene, and nitriles could have led to members of each class. We suspect that through subsequent reactions, especially of pyruvate, many observed meteoritic compounds were formed. Although we find pyruvate dimer and other possible pyruvate reaction products, only further isotope measurements and quantification of homologs within compound classes will tell if there is a “spike” in the abundances of suspected pyruvate products.

However, in a hint of possible meteoritic pyruvate chemistry, it is interesting to note that in the Tagish Lake meteorite, the three unsaturated isomers, citraconic acid, mesaconic acid, and itaconic acid are present but not the corresponding straight-chained analogs (26). This observation is in contrast to other known branched unsaturated meteoritic compounds and hints at a unique source. Also, in our previous meteorite extracts, citramalic acid (a possible product of pyruvate—Fig. 2B) is sometimes more abundant than its straight-chained isomer (2-hydroxy glutaric acid). It should be noted that the vast majority of these extractions were done under relatively mild conditions (i.e., without the use of acids, high temperatures, etc.). Such conditions result in less destruction of hydroxy compounds such as citramalic acid.

Competing formation hypotheses would also have to account for relatively large, multifunctional, and polar molecules including citric acid, HETA, and pyruvate dimer at apparently low temperatures. In addition, the presence and survival on the meteorite parent body of such labile keto compounds as acetoacetate, α -ketoglutarate, and possibly oxaloacetate, could have required a late stage synthesis and preservation and/or a very localized and continuous synthesis such as from pyruvate. Long-term/low temperature reactions of pyruvate—yielding the above compounds—possibly accounts for its low abundance relative to larger homologs in meteorites (Fig. S1A). Of course, these and other relatively labile compounds such as amides (8) could be preserved in meteorites (from any time of formation) simply because of very low temperatures and/or only very intermittent water activity.

Pyruvate dimer is more stable than pyruvate and its delivery by meteorites could have allowed a longer-term source of pyruvate in prebiotic chemistry given that the dimer can dissociate (enzymatically and nonenzymatically) into pyruvate with the equilibrium seemingly favoring the dimer at moderate pH (27). Although conditions (low temperature, etc.) on meteorite parent bodies were obviously favorable for the production and storage of the present compounds, pyruvate (and therefore its reaction products) could theoretically have been produced on the ancient Earth. For example, high temperature laboratory simulations of prebiotic hydrothermal vents produced small amounts of pyruvic acid using a FeS catalyst (28). Pyruvate and α -ketoglutarate (among other compounds) were also produced by photolysis in the presence of zinc sulfide (29).

If subjected to high temperatures and pressures such as those of Earth’s hydrothermal vents, pyruvic acid can also produce a suite of compounds including amphiphiles. Some of the properties of such mixtures resemble those of some Murchison meteorite components and may be of prebiotic significance (30). Under similar conditions, citric acid (as the initial reactant - in water) will decompose to many smaller compounds (31, 32): many of which are also identified in this work including citramalic acid, mesaconic acid, itaconic acid, citraconic acid, and pyruvic acid. More work must be done in order to determine how applicable such high temperature/pressure mechanisms are to those of the present low temperature pyruvate syntheses of the same compounds. For example, at this stage, data on some of the above acids in our pyruvate reactions (Fig. 2B) seem more consistent with formation from the pyruvic dimer as opposed to citrate decomposition. There seems to be an inverse abundance relationship between the dimer and some compounds including the above (except for pyruvic acid). Citric acid is always produced at much lower abundances (sometimes at trace levels) than the majority of identified compounds (e.g., Table S2). In addition, the loss of

^{13}C -containing fragments in mass spectra also seem to correlate the dimer with citramalic acid, mesaconic acid, and possibly other compounds [in the above high temperature pyruvic acid reactions (30), pyruvic acid dimers, trimers, etc., were also suggested as the starting point for further syntheses]. In contrast to mesaconic and citraconic acid, itaconic acid (significant in citric acid decomposition) is apparently not consistently present in our low temperature pyruvate reactions, depending on experimental conditions. A possible formation mechanism for 2-methylsuccinic acid from pyruvate, at least consistent with ^{13}C labeling, was given in the legend of Fig. 2B (some of these possible relationships were not pointed out in Fig. 2B). However, further work will tell if compounds of the same carbon number as citric acid, such as acnitic acid and tricarballic acid, derive from the citric acid produced in pyruvate solutions. It is also possible that a portion of some of the identified compounds in the above high temperature citric acid decomposition experiments (31) also derived from the produced pyruvate.

Whatever the ultimate synthetic mechanisms, the convincing demonstration that pyruvic acid can also arise if meteoritic (or other) citrate undergoes hydrothermal vent conditions on the early Earth (31), adds another potential source for this important compound. The present results show that if pyruvate is present and allowed to react at low to mild temperatures and moderate pH, a variety of products (many still unknown) with possible value to the origin/evolution of biochemical cycles will be produced.

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Materials and Methods

Most standard compounds were purchased commercially; we synthesized 3-methyl-4-oxopentanoic acid and 2-methyl-4-oxopentanoic acid by modification of published methods (33). Initial meteorite extractions, compound fractionation, GC-MS conditions and sample preparation procedures are similar to those used previously (8). The compounds are fractionated primarily by acidity: the most acidic fraction, the one containing tricarboxylic acids (including citric acid), was treated with barium chloride to precipitate sulfate and dried after removal of cations with cation exchange resin. To open possible lactone rings some samples are brought briefly to alkaline pH with ammonium hydroxide and thoroughly dried. Control procedures (ion exchange, etc.) with pyruvic acid and acetoacetic acid standards demonstrated $\leq 1\%$ recoveries. Therefore, to preserve as much of the more labile compounds as possible, an extraction of ALH 83102 (inset of Fig. S1A) was done using a sequential ether-ethanol-pyridine extraction of a dried water extract. tBDMs derivatives (34) were prepared as previously described (8); TMS derivatives were prepared with similar procedures and solvents. Compounds in some extracts were derivatized to ISP esters by published methods (35). In preparation for isotope measurements, compounds were further purified (e.g., Fig. S2) from the fractionated meteorite extract containing keto acids (Fig. S1A) using ion chromatography methods (36). The techniques of isotope measurements are similar to those in previous methods (37, 38).

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