



## Geochemical roots of autotrophic carbon fixation: Hydrothermal experiments in the system citric acid, H<sub>2</sub>O-(±FeS)-(±NiS)

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**Abstract**—Recent theories have proposed that life arose from primitive hydrothermal environments employing chemical reactions analogous to the reductive citrate cycle (RCC) as the primary pathway for carbon fixation. This chemistry is presumed to have developed as a natural consequence of the intrinsic geochemistry of the young, prebiotic, Earth. There has been no experimental evidence, however, demonstrating that there exists a natural pathway into such a cycle. Toward this end, the results of hydrothermal experiments involving citric acid are used as a method of deducing such a pathway. Homocatalytic reactions observed in the citric acid-H<sub>2</sub>O experiments encompass many of the reactions found in modern metabolic systems, i.e., hydration-dehydration, retro-Aldol, decarboxylation, hydrogenation, and isomerization reactions. Three principal decomposition pathways operate to degrade citric acid under thermal and aquathermal conditions. It is concluded that the acid catalyzed  $\beta\gamma$  decarboxylation pathway, leading ultimately to propene and CO<sub>2</sub>, may provide the most promise for reaction network reversal under natural hydrothermal conditions. Increased pressure is shown to accelerate the principal decarboxylation reactions under strictly hydrothermal conditions. The effect of forcing the pH via the addition of NaOH reveals that the  $\beta\gamma$  decarboxylation pathway operates even up to intermediate pH levels. The potential for network reversal (the conversion of propene and CO<sub>2</sub> up to a tricarboxylic acid) is demonstrated via the Koch (hydrocarboxylation) reaction promoted heterocatalytically with NiS in the presence of a source of CO. Specifically, an olefin (1-nonene) is converted to a monocarboxylic acid; methacrylic acid is converted to the dicarboxylic acid, methylsuccinic acid; and the dicarboxylic acid, itaconic acid, is converted into the tricarboxylic acid, hydroaconitic acid. A number of interesting sulfur-containing products are also formed that may provide for additional reaction. The intrinsic catalytic qualities of FeS and NiS are also explored in the absence of CO. It was shown that the addition of NiS has a minimal effect in the product distribution, whereas the addition of FeS leads to the formation of hydrogenated and sulfur-containing products (thioethers). These results point to a simple hydrothermal redox pathway for citric acid synthesis that may have provided a geochemical ignition point for the reductive citrate cycle. Copyright © 2001 Elsevier Science Ltd

### 1. INTRODUCTION

The primitive prebiotic Earth presumably had environments where organic synthesis was an intrinsic component of local geochemistry. Primitive biochemistry, and ultimately life, may have developed from such environments. The question of life's origins, therefore, is as much geochemical as biologic. At a minimum, the physical conditions of Earth's earliest environments must place a constraint or constraints on any biologic theory that proposes an initiation point for life. Furthermore, the selection of a specific environment as being more likely or ideal than others as life's emergence point must also be ranked in terms of viability in terms of the potential for geochemical promotion and development of useful, albeit primordial, biochemistry.

Over the past two decades, interest has developed in the possibility that deep submarine hydrothermal vent systems were ideal environments for the emergence of earliest life (e.g., Corliss et al., 1981; Holm, 1992). The perceived benefits afforded to this environment include protection from intense asteroid bombardment and intense UV, as well as providing a source of thermal and chemical energy in addition to potentially catalytic minerals. It has been shown via theoretical calculations (e.g., Shock et al., 1996)

that the synthesis of a broad array of organic molecules through the reduction of CO<sub>2</sub> is thermodynamically favorable in present-day hydrothermal vent fluids, provided that kinetic barriers to methane formation exist (Shock, 1992).

Wächtershäuser (1988a, 1990, 1992) has formulated a detailed theory describing how biochemistry may have begun, using as a primary energy source the reduction of CO<sub>2</sub> with the oxidation of pyrrhotite (FeS) to pyrite (FeS<sub>2</sub>) (Wächtershäuser, 1988b). This theory is commonly referred to as the Iron-Sulfur World Theory. Central to Wächtershäuser's thesis is the role that the iron sulfide must play as both a catalyst and a source of energy for the earliest life attempting to thrive using the volcanic exhalations of mantle-derived volatiles, e.g., CO<sub>2</sub>, CO, COS, H<sub>2</sub>S, N<sub>2</sub>, and NH<sub>3</sub>. The special role of transition metal sulfides in the origin of life has also been proposed by Russell and Hall (1997); specifically, they propose that FeS bubbles may have operated as the first membrane, solving the compartmentalization problem (i.e., proto-membrane) as well as providing a useful primordial catalyst.

Primordial carbon fixation has been proposed by Wächtershäuser (1988a, 1990, 1992), Russell and Hall (1997), and Morowitz et al. (2000) to be accomplished via the reductive citrate cycle (RCC), a carbon-fixation pathway used to this day by a few organisms, e.g., *Thermoproteus*, an anaerobic archaeobacteria (Buckel, 1999). Although there are a number of routes

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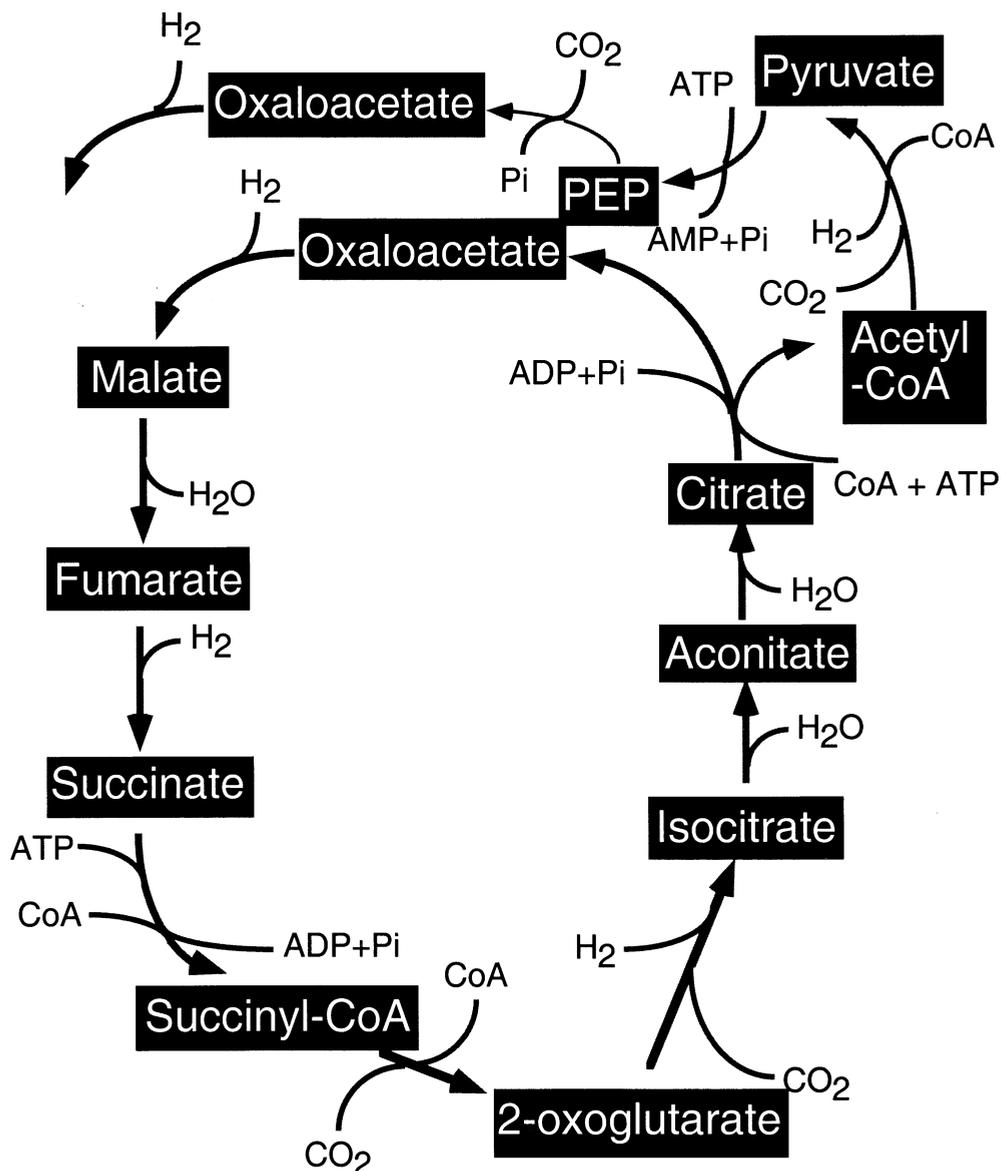


Fig. 1. A cartoon of the reductive citrate cycle (RCC). The cycle is as follows: Beginning with acetyl CoA, carbon insertion leads to the formation of pyruvate. Phosphorylation of pyruvate (at the cost of one mol of ATP (adenosine triphosphate)) leads to the formation of phosphoenol pyruvate (PEP). Electrophilic addition of  $\text{CO}_2$  leads to the formation of oxaloacetate. Reduction of oxaloacetate forms malate. Dehydration of malate forms fumarate. That is then reduced to form succinate. Succinate is activated to succinyl CoA at the cost of one mol of ATP. Carbon insertion leads to the formation of 2-oxoglutarate. A second carbon insertion leads to the formation of isocitrate. Isocitrate is isomerized through aconitate to citrate. The splitting of citrate to form one mol of acetyl CoA and oxaloacetate completes the cycle. This final step reveals the autocatalytic nature of the network in that the cycle doubles the number of  $\text{CO}_2$  acceptors with each revolution.

available for anaerobic carbon fixation, the RCC has the perceived benefit of being strongly network catalytic, i.e., the number of  $\text{CO}_2$  acceptors doubles with each turn of the cycle (see Fig. 1). It is proposed that after the RCC started, it operated with such kinetic advantage as to out-compete all other reactions.

The critical geochemical question regarding how to ignite an archaic RCC remains unanswered. Not knowing how or if biochemistry did originate with the RCC as a basis, it is not possible, a priori, to point within the RCC and identify a

particularly optimum initiation or ignition point. Therefore, in this paper an exploration into potential roots for the natural synthesis of citric acid in the context of prebiotic chemistry is considered. There exists considerable difficulty in geochemically synthesizing citric acid via a route similar to that of the extant RCC. Notwithstanding these difficulties, there has been some progress in identifying viable early pathways toward the RCC. For example, Heinen and Lauwers (1996) demonstrated the reduction of  $\text{CO}_2$  to form methane thiol, a reaction apparently coupled with the oxidation of FeS to form pyrite. Huber

and Wächtershäuser (1997) reported the synthesis of acetic acid in a high yield from methane thiol and CO, utilizing the catalytic qualities of coprecipitated FeS and NiS.

The next step, synthesis of pyruvic acid from thioacetyl groups, may also be geochemically viable. Nakajima et al. (1975) demonstrated a closely related reaction by forming phenylalanine from a thioester of phenyl acetic acid and octane thiol in a solution containing  $\text{Fe}_2\text{S}_2(\text{RS})_4$  (R = organic molecule) complex anions, albeit at low yield. (Note that this reaction requires the intermediate synthesis of phenylpyruvic acid and is, therefore, early proof of the synthesis of an  $\alpha$ -ketoacid.) More recently, Cody et al. (2000) reported the synthesis of pyruvate from methane thiol as a byproduct of high temperature and pressure carbonylation reactions utilizing FeS as a catalyst.

Notwithstanding these successes, the next step into the RCC is difficult, i.e., the carboxylation of pyruvate to form oxaloacetate followed by reduction of oxaloacetate to malate (Fig. 1). To the best of our knowledge, this reaction has not been demonstrated abiologically. In extant biochemistry, the first of these reactions is achieved by employing either the phosphoenol pyruvate (PEP) carboxykinase or PEP carboxylase enzymes (Boyer, 1973), requiring the conversion of adenosine triphosphate (ATP) to adenosine monophosphate (AMP) (Fig. 1). After this reaction, there remain several more difficult steps, including second and third  $\text{CO}_2$  additions to form 2-oxoglutarate and oxalosuccinic acid, and, ultimately, the isomerization of isocitrate to citrate (Fig. 1).

Given the complexities described above, it appears unreasonable that the prebiotic development of the RCC would have followed precisely the steps outlined in Figure 1. More likely is the scenario whereby in the primitive geochemical world, any set of reaction pathways (natural to their environment) that leads to the synthesis of compounds (e.g., citric acid) within the RCC might have provided an ignition point. Therein lies the basis of an experimental program, i.e., establishing the chemical reactions intrinsic to aqueous conditions that may have provided the foundations for the origin of life.

Any experimental approach directed toward identifying a likely ignition point for a primordial autocatalytic carbon fixation cycle must meet two conditions. First, it is necessary (but not sufficient) to demonstrate that critical reactions and/or specific reaction pathways are feasible nonenzymatically. Second, one must demonstrate that such chemical reactions could occur under realistic (natural) conditions, e.g., concentrations, temperatures, and pressures, among others. In work described below we set out to first establish the former condition by identifying a viable chemical route that could, in principle, jumpstart the RCC in the absence of enzymes. We only coarsely address the second condition by utilizing experimental conditions that grossly mimic those of hydrothermal systems, i.e., high temperature and pressure aqueous conditions with the presence of minerals to catalyze critical reactions. It is hoped that the insights gained from these experiments will lead toward more complicated and realistic simulations of the chemistry that is possible within recirculating hydrothermal systems.

Simplistically, the experimental protocol would start with an aqueous fluid bearing  $\text{CO}_2$ ,  $\text{H}_2$ , and CO and would contain catalytic mineral phases and from these synthesize citric acid. Given an equation such as  $6\text{CO}_2 + 9\text{H}_2 = (\text{CH}_2)_2\text{COH}(\text{COOH})_3 +$

$5\text{H}_2\text{O}$ , it is recognized that there certainly will be environments where the free energy of the system could be lowered by converting  $\text{CO}_2$  and  $\text{H}_2$  into citric acid. The specific pathway for such a synthesis, however, is not defined. Whatever pathway does exist certainly requires a number of reactions and intermediates. Cast in strictly thermodynamic terms, the synthesis of citric acid would likely compete with multitudes of other similarly energetically favorable reactions.

Any experimental approach to citric acid synthesis is bound to fail without careful consideration of pathway and mechanism. To establish some guidance and constraints into the synthesis of citric acid, we first investigate the intrinsic degradative reactions of citric acid in water at high temperature and pressure. In particular, we seek to identify relatively stable intermediates that may provide viable sources for citric acid under appropriate conditions. These data are then used to design a viable forward-directed geosynthetic route for the formation of citric acid and/or potential entry into the RCC.

## 2. EXPERIMENTAL

Solutions were made ranging from highly concentrated (5.6 M) to relatively dilute (58 mM) with high-purity anhydrous citric acid (99.99% pure, Fischer Scientific) and distilled deionized water (17 m $\Omega$ ). In all cases, Optima-grade solvents (99.9% pure, Fischer Scientific) were used and not distilled further.

Low-pressure experiments (P = vapor pressure of the solution) were run in 4-mm i.d. glass tubes. Solutions were loaded into each glass tube reactor, frozen in liquid  $\text{N}_2$ , evacuated, and flame sealed. Each tube was then placed in a copper tube with  $\text{H}_2\text{O}$  added for pressure compensation and capped with Swagelok fittings. The copper-jacketed glass reactors were heated and held at reaction temperature in a convective oven. Following reaction, the reactors were quenched in ice water and opened immediately. The thermal rise time was estimated to be on the order of 5 min, the quench time was estimated to be < 1 min.

High-pressure experiments were run in 2.4-mm diameter gold-tube reactors. Before use, the gold-tube reactors were refluxed in hydrochloric and nitric acid and annealed at 900°C. before sealing. The tubes were blanketed with dry  $\text{N}_2$ , immersed in liquid  $\text{N}_2$ , and arc welded. All runs were weighed before and after a given pressure and temperature run to test for leakage.

Yoder (1950) has previously described the gas-media apparatus used to achieve the high pressures and temperatures in these experiments. In brief, argon gas pressures up to 200 MPa are generated with a gas compressor, whereas pressures > 200 MPa require a second-stage intensifier. Pressure was measured resistively to within  $\pm 10$  KPa with a calibrated manganin wire coil. A given reaction temperature was achieved with a long platinum-wound furnace mounted on a thin-walled mullite tube with continuous monitoring for the duration of each experiment. The temperature variation was precise to within  $\pm 0.1^\circ\text{C}$  and accurate to within  $\pm 0.3^\circ\text{C}$ .

The following procedure was applied for all runs. First, the desired pressure was attained, then the temperature was raised to the desired target. The rate of temperature increase was 20°C/min up to within 10°C of the target temperature, followed by 5°C/min to the designated temperature. At the termination of the run, the furnace current was turned off and the temperature dropped to room temperature in < 3 min as a result of the water-cooled jacket outside of the pressure vessel. Following the run, the sample tubes were stored in a refrigerator (T = -87°C) until analysis.

Immediately before opening, the gold-tube reactors were immersed in liquid  $\text{N}_2$  to freeze the volatiles, specifically  $\text{CO}_2$ . Each reactor was opened at both ends and transferred to a 4-dram vial. At this point, one of two different analytical schemes was employed. For analysis with gas chromatography-mass spectrometry, approximately 1 mg of pentadecane was weighed into each sample vial as a concentration standard. All organic acids were converted to their volatile propyl esters with a solution of 14% (vol.)  $\text{BF}_3$ -propanol complex in propanol at 90°C with standard esterification procedures (Blau and King, 1977).

The esterified reaction products were analyzed with a Hewlett-

Packard 6890 Series gas chromatograph interfaced with a 5972 Series quadrupole mass spectrometer. Product concentrations were obtained from the mass spectrometer total ion current with calibration curves from which the response factors of the compounds had been determined with pure standards. In the case of the few compounds unavailable commercially, response factors were assumed to be equal to that of the standard. Chromatography was performed with a 14% cyano-propyl/86% dimethyl silicone capillary column, the stationary phase chosen for its excellent chromatographic separation of olefinic isomers. Identification of unknown compounds relied on the interpretation of fragmentation behavior with both electron-impact and chemical-ionization mass spectrometry; the latter method employed either methane or isobutane as the ionization gas.

Analyses of underivatized products in aqueous solutions were performed with high-performance liquid chromatography that employed a Hewlett-Packard 1050 HPLC, with a Aminex HPX-87 H-ion exclusion column, a 4 mM H<sub>2</sub>SO<sub>4</sub> buffer solution, and UV detection at 210 nm. Reaction products were run at ~5 to 10 mmol concentrations and yields were quantified with calibration curves derived from aqueous solutions of each compound at a series of known concentrations (typically over the range of 0.1–100 mM). Reproducibility between runs, as determined with triplicates, was excellent for the relatively high-volume glass-tube samples. The sample-to-sample variation was higher for the relatively small-volume, high-pressure gold-tube runs.

The minerals used in this study, iron and nickel sulfide, were synthesized from puratronic-grade Fe metal (99.998%), Ni metal (99.995%), and S (99.9995%) with standard, solid-state procedures (Kullerud, 1971). At the termination of the synthesis, a small portion of the charge was mounted in epoxy and polished with diamond abrasive paste. The polished section was examined optically in reflected light to ensure that the target phase was the only phase present. The chemical composition of the synthetic sulfides was determined by electron microprobe analysis with a JEOL Superprobe (JXA-8800), and the crystal structure of the target phase was confirmed with X-ray diffraction. Analysis of the residual sulfide revealed a slight excess of sulfur in each phase, i.e., Fe = 48.5% in the case of FeS, and Ni = 49.0% in the case of NiS.

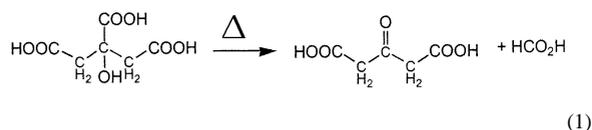
The synthesized mineral fractions were crushed in an agate mortar. Number-averaged particle diameter was determined with optical microscopy, digital image acquisition, and particle-analysis software. The average particle radius was 13.8 μm for the FeS and 15.0 μm for NiS. The synthesized sulfides were stored in an evacuated desiccator until use.

### 3. BACKGROUND CHEMISTRY

There have been numerous studies of organic reactions related to those operating in the citric acid-H<sub>2</sub>O system at high temperatures, including particularly relevant early papers by Linstead and Mann (1931), Johnson and Heinz (1949), Arnold et al. (1950), and Brown (1950). Specific research into the decomposition of citric acid in water under high-temperature conditions (T = 230–400°C, pressures from 27.5–34 MPa, and concentrations ranging from 0.01–0.1 mol/L) has also been explored (Carlsson et al., 1994). Given these and other studies, it is clear that there exist three significant pathways for citric acid decomposition.

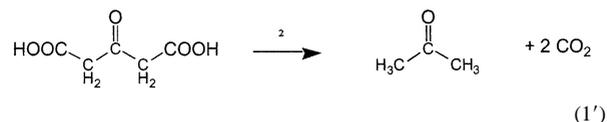
#### 3.1. Citric Degradation Pathway 1

First, there is the thermal reaction pathway, common to a hydroxy acids (e.g., March, 1991) that leads to the formation of 3-oxo-pentanedioic acid via Reaction 1 (note that Δ refers to promotion by heat):



3-oxo-pentanedioic acid, a β keto acid, is highly susceptible to decarboxylation (March, 1991). Two successive decarboxylations will ultimately lead to the formation of acetone via Reaction 1':

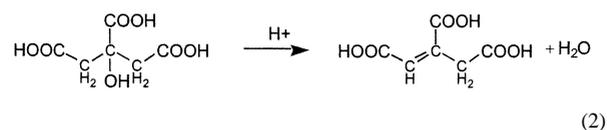
Under high-temperature aqueous conditions (e.g., 250°C ~4 MPa), aliphatic ketones such as acetone have been shown to be relatively stable (e.g., Siskin et al., 1990), although susceptible to reversible



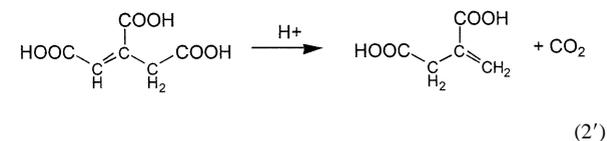
Aldol condensation reactions. Recent work by Seawald (2001), however, has shown that acetone can decompose (oxidize) to acetic acid and CO<sub>2</sub> (± CH<sub>4</sub>) under aquathermolytic (T = 300°C, P ~35 MPa) conditions when in the presence of the mineral buffer assemblage, hematite-magnetite-pyrite.

#### 3.2. Citric Acid Degradation Pathway 2

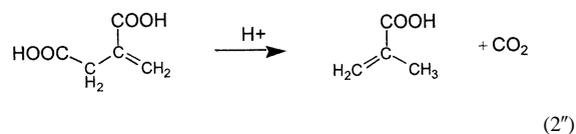
A second pathway, an acid catalyzed series of reactions, has been explored in recent work described by Carlsson et al. (1994). The pathway starts with the elimination of the alpha hydroxyl, yielding the tricarboxylic olefin, aconitic acid, via Reaction 2:



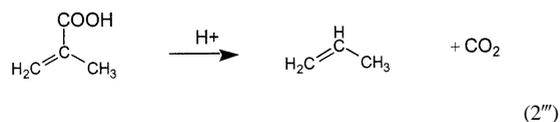
Aconitic acid (both *cis* and *trans*) is not stable and will decarboxylate (slowly) even at room temperature. (It is recommended by the supplier that aconitic acid be kept frozen until needed.) As shown at elevated temperatures, aconitic acid decarboxylates so rapidly that its steady-state concentration is extremely low (although detectable). The decarboxylation of aconitic acid follows a classic acid-catalyzed mechanism for βγ unsaturated acids (e.g., Arnold et al., 1950; Brown, 1950) diagrammed in Reaction 2':



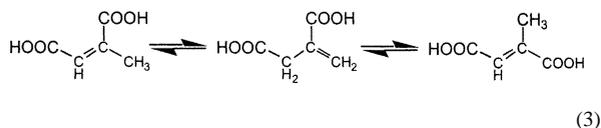
whereby aconitic acid decarboxylates to itaconic acid. Itaconic acid is also βγ unsaturated and may further decarboxylate to methacrylic acid via Reaction 2'':



Methacrylic acid is αβ unsaturated and, consequently, is less prone to decarboxylation, an important point highlighted by Carlsson et al. (1994) when considering the industrial conversion of citric acid to a value-added product such as methacrylic acid. Under extreme conditions or extensive periods of time, methacrylic acid may decarboxylate to propene as shown in Reaction 2''':



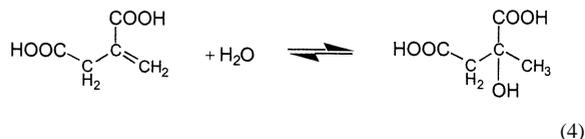
It should be noted that itaconic acid (Reaction 2') is considerably more stable than aconitic acid under high-temperature aqueous conditions. Consequently, the decomposition of citric acid via this pathway will lead to a significant steady-state concentration of itaconic acid. Itaconic acid, under acidic conditions, is susceptible to double-bond migration, leading to the formation of two other isomers, the *cis* (citraconic) acid and *trans* (mesaconic) acids via Reaction 3:



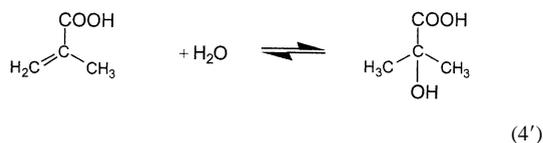
Note that the isomerizations depicted in reaction 3 are written as equilibria. That this is true was shown many decades ago in the classic paper by Linstead and Mann (1931) and subsequently re-explored by Sakai (1976). Specifically, Sakai confirmed that under aquathermolytic conditions (170°C), citraconic acid would isomerize to form itaconic acid and mesaconic acid.

This experiment was reproduced with the same experimental parameters reported by Sakai (1976) to reiterate this point. Following Sakai (1976), we present the products as isomer percentages evolving as a function of time (Fig. 2). It is seen that citraconic acid rapidly isomerizes to form itaconic acid and mesaconic acid. The results shown in Figure 2 are reasonably close to those that reported by Sakai (1976); however, there is a slight difference in the apparent kinetics. Sakai's data appeared to show that all three isomers were coupled via equally fast equilibria. Qualitatively, however, it is suggested from the data shown in Figure 2 that while the equilibration between citraconic and itaconic acid is fast, the corresponding equilibrium with mesaconic acid is considerably slower. Carlson et al. (1994) came to a similar conclusion in their study.

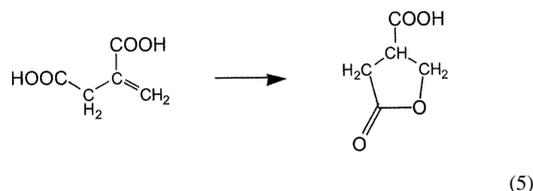
Reaction 2, 2', 2'', and 3 all involve protonated (carbocation) intermediates, where the proton donor might be either the acid or water. Under high-temperature aqueous conditions, it would be expected that water addition across the double bond should also occur. Given that these are ionic reactions, protonation is expected to occur on the least-substituted carbon; hence, the hydroxyl will add to the most-substituted carbon, i.e., a Markovnikov substitution (e.g., March, 1991). Hydration of aconitic acid would yield citric acid (the reversal of Reaction 2). Hydration of any of the three unsaturated diacids (Reaction 3; Fig. 2) yields citramalic acid via Reaction 4:



Similarly, addition of water across the double bond, in the case of methacrylic acid, would yield hydroxy isobutyric acid, e.g., Reaction 4':



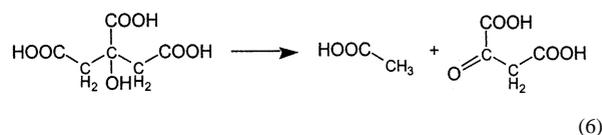
Another important reaction along pathway 2 is the intramolecular condensation of itaconic acid to form the lactone, paraconic acid, via Reaction 5:



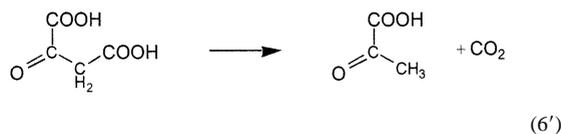
Although the yield of paraconic acid is typically low (except at very high pressures, e.g., 500 Mpa; see Table A3), the synthesis paraconic acid is significant in that it demonstrates that intramolecular condensation of a carboxylic acid with an olefin to form an ester is possible. Intermolecular olefin-acid condensations could provide an important n-molecular polymerization pathway in an otherwise unimolecular degradative reaction scheme.

### 3.3. Citric Acid Degradative Pathway 3

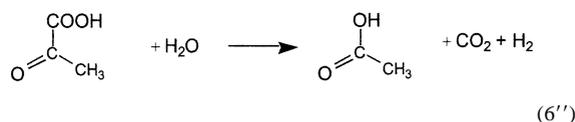
Under either acidic or basic aquathermolytic conditions, citric acid may decompose through a reaction whereby the 2, 3 carbon-carbon bond in citric acid is heterolytically cleaved and the hydroxyl proton transferred to the resultant carbanion, yielding acetic acid and oxaloacetic acid, essentially a reverse Aldol reaction (March, 1991), as summarized in Reaction 6:



This reaction is potentially interesting as it mimics a critical reaction in the RCC (Fig. 1), i.e., extant organisms that utilize the RCC require cleavage of citric acid to form oxaloacetic acid plus acetyl Co-A, a reaction promoted by the enzyme complex, citrate lyase, at a cost of converting ATP to ADP. In the abiotic reaction, oxaloacetic acid (also a  $\beta$  keto-acid) will rapidly decarboxylate to yield pyruvate plus  $\text{CO}_2$  via Reaction 6':



Hydrolytic attack of the keto-acid bond in pyruvate will yield acetic acid,  $\text{CO}_2$ , and  $\text{H}_2$  via the oxidative decarboxylation shown in Reaction 6'':



It is evident from Reaction 6'' that pyruvate has potential as a hydride donor. A number of extant microorganisms such as *Clostridia pasteurianum* utilize the oxidative decarboxylation of pyruvate, i.e., via Reaction 6'' as a source of reducing power (see for example, Buckel, 1999).

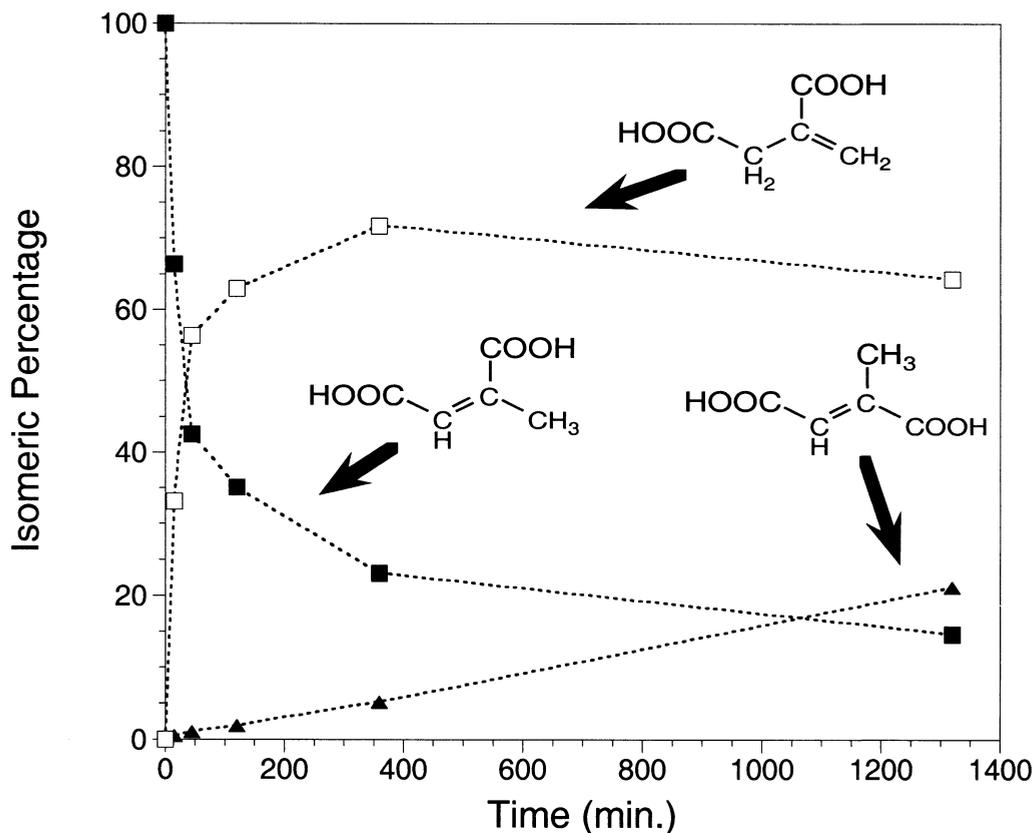


Fig. 2. The isomerization of citraconic acid at 170°C to form itaconic and mesaconic acids, thereby demonstrating the equilibration among the unsaturated dicarboxylic acids.

#### 4. CITRIC ACID REACTIONS UNDER HYDROTHERMAL CONDITIONS AT AMBIENT AND ELEVATED PRESSURES

The previous work of Linstead and Mann (1931), Sakai (1976), and Carlsson et al. (1994) have quite thoroughly explored most of the critical aspects of the aqueous chemistry of citric acid at high temperatures that are relevant to the present work. The study of Carlsson et al. (1994) explored citric acid degradation under a range of conditions, including variable concentration (0.1 and 0.5 mol/L solutions), variable temperature ( $T = 230, 280, 300,$  and  $320^{\circ}\text{C}$ ), and pressure ranging from  $\sim 28$  to  $34$  MPa. In their study, considerably more effort was spent on studying the aquathermolytic chemistry of itaconic acid. In the case of the itaconic acid-water experiments, the concentration range was broadened from 0.01 mol/L up to 0.5 mol/L, the temperature range was increased to 350 to  $400^{\circ}\text{C}$ , and the effects of addition of NaOH, NaCl, KOH, and  $\text{H}_2\text{SO}_4$  were all explored at pressures ranging from 28 to 34 MPa. Experiments by Sakai (1976) focused entirely on the aquathermolytic chemistry of the dicarboxylic acids, were run at lower temperatures ( $170^{\circ}\text{C}$ ), and at ambient pressure (i.e., vapor pressure in sealed glass tubes).

To fill in the gap in the data derived from the two more recent studies, we have set out to explore both the activity of water, pressure, and the addition of NaOH on citric acid decomposition reaction. For the purposes of comparison, experiments were run for 2 h and held at  $200^{\circ}\text{C}$ . Reactant solutions spanned from highly concentrated (5.6 mol/L) to comparably dilute (58 mM). The rationale for exploring such a range of concentrations (hence water activity) was two-fold. First, there was reasonable expectation that the extent of reaction under these conditions might vary as a function of the activity of water, e.g., hydration reactions (Reaction 4 and 4') would be expected to be favored at higher  $\text{H}_2\text{O}$  activities. Second, there is a practical need to derive a systematic picture of the role of water activity on reaction

progress under high-pressure conditions. Specifically, there are practical limits on how dilute one can go when exploring aqueous organic reactions at high pressures due to the small volume of the reactors. For dilute solutions (10 mM and lower in concentration), it can become difficult to recover and analyze quantitatively the distribution of products, given the small amount of initial citric acid present in the gold tubes. In contrast, high concentrations of reactant may promote alternate reaction pathways, e.g., bimolecular reactions vs. unimolecular reactions of reactant with water.

Before discussing the results, it is important to reiterate that molecular transformations of citric acid under moderate hydrothermal conditions are both thermodynamically and kinetically controlled. Consequently, the distribution of compounds, observed at the quench stage of a given experimental run, constitute a snapshot of an evolving system. Unfortunately, the use of welded gold-tube reactors does not provide for the continuous monitoring of reaction products, such as would benefit the determination of reaction kinetic-rate parameters and rate laws. It is critical, therefore, that in all further discussions, the reader recognize that the distribution of observed products is a dynamic function of the reaction time of the experiment.

In the first set of experiments, solutions A through E (5.6 mol/L, 1.1 mol/L, 0.58 mol/L, 0.280 mol/L, and 58 mM) were heated to  $200^{\circ}\text{C}$  in vacuum-sealed glass tubes. The reaction pressure was not measured, but it is assumed not to be  $> 2.0$  MPa (approximately the vapor pressure of pure  $\text{H}_2\text{O}$  at  $200^{\circ}\text{C}$ ) (Haar et al., 1984). Three experiments were run at each concentration, and the results averaged. These are presented in Figure 3 (see also Table A1). It is noted that the amount of citric acid remaining relative to the total product distribution after 2 hr of reaction at  $200^{\circ}\text{C}$  ranges from  $45 \pm 4\%$  down to  $28 \pm 6\%$ , moving from 5.6 mol/L to 58 mM. Recall that citric acid decomposition along pathway 2 is initiated by the dehydration (Reaction 2) to aconitic acid. Dehydration reactions are promoted by the protonation of a

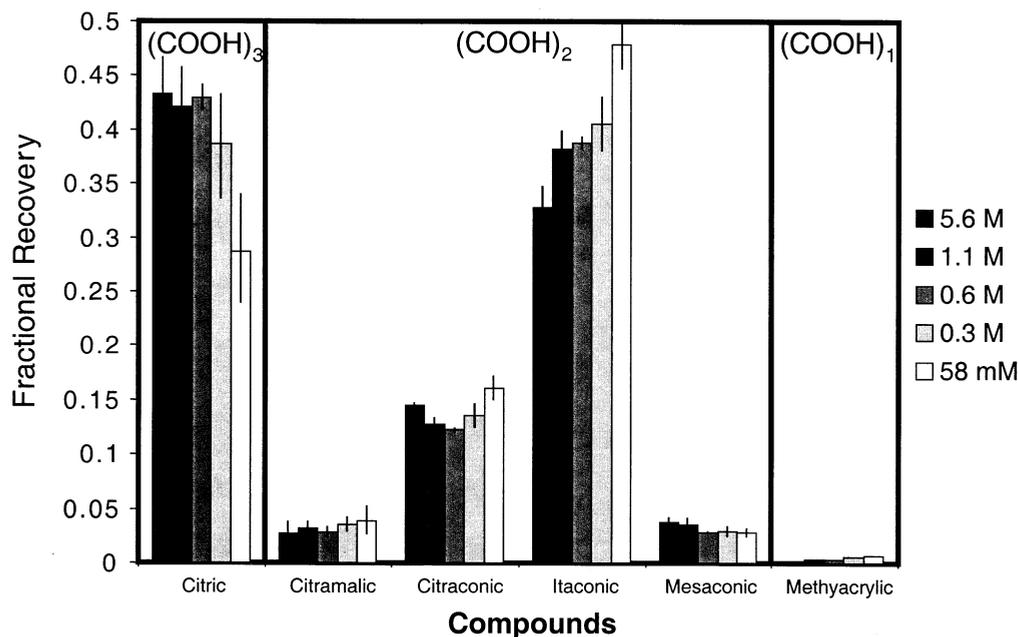


Fig. 3. Reaction of citric in water at 200°C for two hours in sealed-glass tubes. The pressure is controlled by the vapor pressure of water, estimated to be less than 2 MPa. Five solutions ranging from extremely concentrated to relatively dilute were studied. The reactant and products are grouped by the number of carboxyl groups on each molecule. The decomposition of citric acid through to methacrylic acid involves two decarboxylations. Error bars ( $2\sigma$ ) are included for each compound.

hydroxyl group (e.g., Carey and Sundberg, 1990). The apparent enhancement of citric acid degradation with increased activity of water, therefore, suggests that water may be the primary proton donor in this system.

Within 2 h, the aquathermolytic degradation of citric acid, (COOH)<sub>3</sub> (Fig. 3), yields, predominantly, dicarboxylic acids, (COOH)<sub>2</sub> (Fig. 3), including abundant alcohol (citramalic acid via Reaction 4), the three unsaturated dicarboxylic acid isomers (itaconic, citraconic, and mesaconic acid), and a trace of the unsaturated monocarboxylic compound (methacrylic acid (COOH)<sub>1</sub>) (Fig. 3). Pyruvic acid (Reaction 6') and acetic acid (Reaction 2) were detected in all runs in trace abundance.

Citramalic acid forms from the addition of water to the protonated double bond of the unsaturated dicarboxylic acids (itaconic, citraconic, and mesaconic). The data in Figure 3 weakly indicate a correlation between the activity of H<sub>2</sub>O and formation of this hydrated product; therefore, the degree of protonation correlates with the activity of H<sub>2</sub>O. A more convincing correlation is revealed with formation of both itaconic acid and methacrylic acid. Note that both these compounds are formed via the acid-catalyzed  $\beta\gamma$  decarboxylation reaction (Reaction 2 and 2'). The reasonable implication is that the increases in the activity of H<sub>2</sub>O corresponds with an increased promotion of this important decarboxylation reaction.

It is noted that there exists the possibility of a near-thermodynamic, albeit metastable, equilibria among the dicarboxylic acids: citramalic, itaconic, citraconic, and mesaconic acid. Certainly, given the reaction scheme in 3.2, the magnitude of population of the various constituents within the dicarboxylic acid assemblage, (COOH)<sub>2</sub> (Fig. 3), indicates that the decarboxylation reactions that feed (Reaction 2) and drain (Reaction 2') the dicarboxylic isomer "level" of citric acid decomposition are slower than the various isomerization reactions that occur within this molecular level. Such a conclusion has been reached previously in the work of Carlsson et al. (1994).

Finally, it is noted that even at the highest concentration solutions, there was no evidence in the product distribution of bimolecular reactions or reaction pathways that deviated in mechanism from the lower concentration solutions. Were reaction pathways available for the formation of high-molecular products from bimolecular reactions involving citric acid or its decomposition products, such products would clearly be favored at higher concentrations.

Pressure might be expected to be as significant as the activity of H<sub>2</sub>O in favoring specific reaction pathways (e.g., Asono, 1991). To explore the effects of elevated pressure on these reactions, the same solutions, A–E, were used. For these experiments, however, each solution was sealed in a welded gold tube and pressurized externally with the aforementioned gas pressure apparatus. For the purposes of comparison, a pressure of 100 MPa was chosen as reasonable, although pressures from 50 up to 500 MPa were also explored (Table A3).

The high-pressure data (Fig. 4; Table A2) exhibit considerably greater sample-to-sample variance compared with the ambient pressure data discussed above (Fig. 3). The increased variance results from the comparably smaller quantities of citric acid present in the relatively small sample volume gold-tube reactors, which leads to increased error associated with the precision of sample loading, recovery, and analytical detection. Notwithstanding these difficulties, the quality of the results are sufficient to make quantitative comparisons with the ambient pressure runs.

The following changes in reactivity with pressure are observed. Following 2 h of reaction, the percentage of citric acid to the total assemblage of products drops significantly relative to data obtained at ambient pressure (Fig. 3). At higher pressures, the data range from a high of  $37 \pm 4\%$  down to a low of  $7 \pm 7\%$ , moving from 5.6 mol/L to 58 mM initial citric acid concentrations. In general, the recovery of citric acid is statistically lower at the higher pressures. Consistent with these data, the degree of hydration, evident via the formation of the tertiary alcohol, citramalic acid (Fig. 4), is increased at higher pressures at all solution concentrations. Although the variance of the citramalic acid contribution to the products is high, the statistics support the conclusion that the yield of citramalic acid increases with the activity of water.

As was the case in the ambient pressure runs, after 2 h of reaction at 200°C, dicarboxylic acids dominate the product distribution. Although itaconic acid remains predominant, mesaconic acid has increased relative to citraconic acid compared to the ambient pressure runs. The contribution of methacrylic acid to the total product distribution increases with the activity of water at both pressures. At the higher pressures, however, the yield of methacrylic acid shifts from being a trace constituent to a significant species in the distribution at high H<sub>2</sub>O concentrations. Also similar to the lower pressure experiments (Fig. 3), there is no evidence that high solution concentrations lead to the

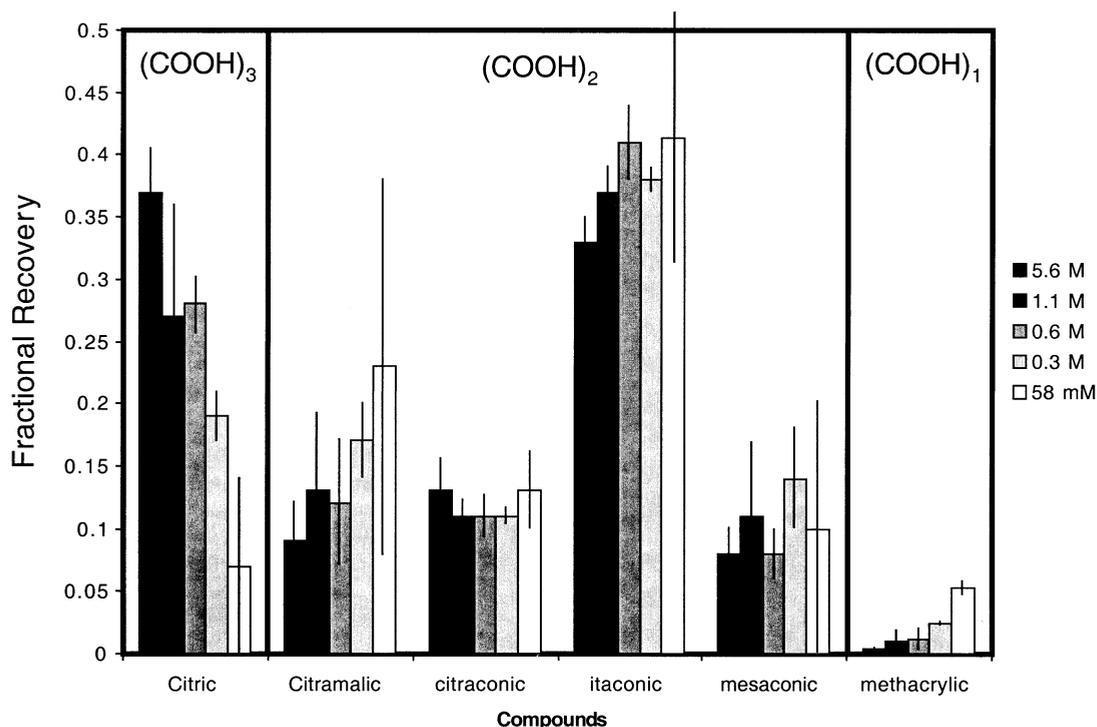


Fig. 4. Reaction of citric in water at 200°C for 2 h in sealed-gold tubes. The pressure is controlled by a gas-pressure apparatus and held to 100 MPa. Five solutions ranging from extremely concentrated to relatively dilute were studied. The reactant and products are grouped by the number of carboxyl groups on each molecule. The decomposition of citric acid to methacrylic acid involves two decarboxylations. Error bars ( $2\sigma$ ) are included for each compound.

development of any reaction pathways that differ from those at lower solution concentration.

As a consequence of the decarboxylation reactions, the amount of  $\text{CO}_2$  in the fluid phase necessarily increases. In the case of the most concentrated solutions, one might consider whether the system remains single phase. Whereas in pure  $\text{CO}_2$ - $\text{H}_2\text{O}$  fluids, the miscibility behavior is well known (e.g., Greenwood and Barnes, 1966), the phase behavior of concentrated solutions of organic acids, such as citric acid in water with  $\text{CO}_2$ , is not known. To ensure that the system remained monophasic, experiments were performed employing a hydrothermal diamond anvil cell. Continuous monitoring of the evolving system, both visually and with Raman spectroscopy, revealed no evidence of phase separation even after complete decarboxylation of citric acid in a 6 mol/L solution. The detailed results of these studies will be presented in a subsequent paper.

While the overall effect of increased pressure on the citric hydrothermal reactions is subtle, it is clear that increased pressure enhanced decarboxylation reactions (particularly at higher activities of water) as evidenced by the increase in both dicarboxylic and monocarboxylic contributions to the product distribution. It is notable that at least for these acid catalyzed reactions, increased pressure such as might be experienced on the floor of a deep ocean, appears to provide no sanctuary to protect against decarboxylation.

The observation that pressure should favor decarboxylation might appear counterintuitive from a thermodynamic point of view, i.e., given the increased molecularity of the reaction. From a kinetic point of view, however, enhanced decarboxylation rates are reasonable, given the ionic nature of the reaction. The  $\beta\gamma$  decarboxylation reaction is initiated through protonation, either intramolecular or from the solvent medium. The formation of a carbocation will generally be favored with pressure due to the electrorestrictive effect of the cation on the solvent; that is, solvation of a cation or anion by the solvent results in a reduction in volume of the system (Asano, 1991). Up to a point, therefore, increased pressure will favor  $\beta\gamma$  decarboxylation by enhancing the rate of protonation. The same argument explains the pressure

enhancement for the formation of citramalic acid and the dehydration of citric acid. Both the hydration of olefins and the protonation of hydroxyls pass through a carbocation intermediate that experiences enhanced stabilization at higher pressures.

##### 5. EFFECT OF PH ON CITRIC ACID REACTIONS AT ELEVATED TEMPERATURES AND PRESSURES

Although no specific attempt was made to buffer the pH in the experiments described above, as will be detailed below, the presence of organic acids both as products and reactants ensures that the pH will not drift significantly. Citric acid and its dominant carboxylated thermal decomposition products are relatively weak acids, e.g., for citric acid ( $\text{pK}_1 = 3.14$ ,  $\text{pK}_2 = 4.77$ , and  $\text{pK}_3 = 6.39$ ) and for itaconic acid ( $\text{pK}_1 = 3.85$  and  $\text{pK}_2 = 5.45$ ). While to the best of our knowledge the  $\text{pK}$  of methacrylic acid is not known, the  $\text{pK}$  of acrylic acid is 4.25 (Kortum et al., 1961). The chemistry described above in Section 4.2, therefore, clearly falls into the acid-catalyzed domain.

Two important questions arise. What is the effective pH at reaction conditions and does the pH shift with the conversion of citric acid to itaconic acid and to (ultimately) methacrylic acid? Precise answers to these questions are not possible. What can be stated is that were such reactions to occur at (or near) standard-state conditions, the pH would not drift significantly because the respective  $\text{pK}_a$ s of the principal products are similar. For example, the calculated equilibrium pH for a 100 mM solution of citric acid in water at standard state (making no correction for ionic strength and neglecting contributions from the second dissociation constant) is 2.1; the same calculation for itaconic acid yields a pH of 2.4.

Of course, the pH of pure citric acid solutions at room temperature and pressure are easily measured and are on the order of 1.6 (e.g., a 100 mM solution of citric acid has a measured pH of 1.57, as opposed to 2.10 calculated above, the difference reflecting the simplifying assumptions employed in the calculation). It is not, however, a simple task to state precisely what the pH is at 200°C at either ambient or high

pressure, because the various  $pK_a$ s are subject to change with temperature and pressure. It is well known, however, that the  $pK_a$ s of strong and relatively weak acids (e.g., citric, acetic, and carbonic acids) tend to decrease with increased temperature.

To the best of our knowledge, the  $pK_1$  of citric acid at high temperatures (e.g.,  $T \geq 200^\circ\text{C}$ ) has not been measured. With the values of  $pK_1$  and  $\Delta H_1^\ddagger$  for citric acid near ambient temperatures (as reported by Yadav et al., 1989), and measured values for the ion product of water ( $K_w$ ) over the desired temperature range (Marshall and Franck, 1981), the value of  $pK_1$  of citric acid at  $200^\circ\text{C}$  can be estimated from the isocolumbic method (e.g., Park et al., 1998). Doing this, we calculate that the first dissociation constant of citric acid will decrease by a factor of  $\sim 4$ , i.e.,  $pK_1(298\text{ K}) = 3.14$ , whereas  $pK_1(473\text{ K}) = 3.75$ . The magnitude of this decrease is similar to that of other organic acids. For example, acetic acid's dissociation constant decreases by a factor of 6 over the same temperature range (Fisher and Barnes, 1972) and carbonic acid's first dissociation constant decreases by a factor of 7 (Park et al., 1998). Over the same temperature interval, water becomes a much stronger acid, e.g., the dissociation constant of water increases by  $\sim 4500$  times (Marshall and Frank, 1981).

As it stands, there currently exist insufficient thermodynamic data for a robust calculation of the combined pressure, temperature, and concentration effects on the various  $pK_a$ s of the reactants and products considered in this study. Consequently, it is not possible to define an equilibrium pH for the system. It can be qualitatively concluded, however, that both the effects of temperature and pressure on the various  $pK_a$ s of the products of these reactions are in the same direction and of similar magnitude; therefore, it appears unlikely that the pH would drift enormously by virtue of the extent of reaction.

What does happen when the pH is imposed, for example, by the addition of a strong base like NaOH? Sakai (1976) observed the following interesting behavior in his studies of the aquathermolytic chemistry involving the dibasic isomers (see Reaction 3). With the progressive addition of NaOH, the equilibrium distribution of the dicarboxylic acid isomers initially shifted systematically, as might be expected. At 0.5 mol/L equivalent of NaOH (1 mol of NaOH to 1 mol of dicarboxylic acid), there was a discontinuity in trend of the NaOH-promoted isomer redistribution. When the quantity of NaOH reached the molar equivalent of COOH groups, there was a sharp discontinuity in the product distribution. That these discontinuities occurred at 0.5 and 1 mol/L equivalent points of NaOH addition, was considered by Sakai to be a direct consequence of moving through the protonated and monoanionic domain into the monoanionic and dianionic domain, and finally into the fully dissociated domain.

Similarly interesting behavior was observed by Carlsson et al. (1994) at higher temperatures ( $T = 350\text{--}400^\circ\text{C}$ ) and pressure ( $P = 34\text{ MPa}$ ) in the system itaconic acid in water. Specifically, they were able to show that the addition of a small amount of NaOH (e.g., 0.20 mol NaOH/mol itaconic) actually increased the rate of  $\beta\gamma$  decarboxylation of itaconic acid. Larger concentrations of NaOH (e.g., 5.0 mol NaOH/mol itaconic), however, decreased the rate of decarboxylation significantly. The interpretation offered to explain this behavior is that the basicity of the double bond in itaconic acid increases due to the loss of the carboxyl proton, thus it becomes more susceptible to protonation intramolecularly. The decrease in decarboxylation rate with higher concentrations (hence, activity) of NaOH was interpreted by Carlsson et al. (1994) to be governed by the magnitude of the second dissociation constant, i.e., at a pH much beyond  $pK_2$ , the concentration of the less-acidic  $\beta$  carboxyl proton obviously decreases; hence, the probability of intramolecular protonation of the double bond decreases.

Both of these studies reveal interesting behavior related to the degree of ionization of the polycarboxylated acids. To add a bit more information to the chemistry specifically involving citric acid, we explored the role of the addition of NaOH to the aquathermolytic degradation of citric acid at high pressures. Solutions were prepared such that citric acid concentration was fixed at 110 mM, while the concentration of NaOH was increased incrementally. The solution concentrations were 0,  $\sim 1$ ,  $\sim 2$ ,  $\sim 3$ , and  $\sim 4$  mol NaOH/mols citric acid. The room temperature pH for each solution was 1.6, 3.4, 4.2, 5.3, and 13.0, respectively. The jump in pH moving to the last solution results from exceeding the limit in the buffer capacity of citric acid, i.e., beyond a NaOH/citric molar ratio of 3, the amount of NaOH exceeds the number of COOH groups.

The results of experiments with residence times held at 2 h, temper-

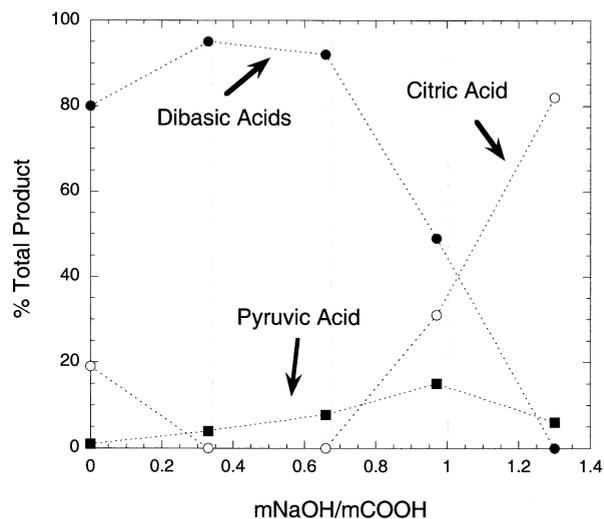


Fig. 5. The distribution of products from decomposition reactions ( $200^\circ\text{C}$ , 200 MPa, 2 h) of solutions of citric acid in water (110 mM) with addition of NaOH. The room temperature pH is 1.6 (no NaOH), 3.4 ( $\sim 1$  NaOH), 3.4 ( $\sim 1$  NaOH/citric), 4.2 ( $\sim 2$  NaOH/citric), 5.3 ( $\sim 3$  NaOH/citric), and 13.0 ( $\sim 4$  NaOH/citric). The products derived from the acid catalyzed pathway are grouped together as dibasic (dicarboxylic) acids; the only observed product of the retro-Aldol pathway is pyruvic acid (Pathway in 3.3). Citric acid recovery drops to zero with the addition of a small amount of NaOH.

atures fixed at  $200^\circ\text{C}$ , and pressures of 200 MPa are presented in Figure 5. Several important points arise from a cursory examination of these data. First, it is clear that the acid catalyzed pathway operates effectively even in the presence of relatively high levels of base. For example, even at  $pH_{25^\circ\text{C}} = 5.3$ , considerable amounts of the dicarboxylic acids were synthesized (Fig. 5). Trace quantities of itaconic and mesaconic acids are present even in the  $pH_{25^\circ\text{C}} = 13$  solution (see Table A4). Second, even when the acid-catalyzed pathway appears completely suppressed by reduction of hydronium ion activity, other pathways (e.g., 3.3) will still operate to consume citric acid as evidenced by the formation of pyruvic acid.

These results indicate that in the absence of a source for citric acid, there will likely always be a sink for any citric acid introduced to hydrothermal conditions. Finally, these data indicate that the addition of some base actually accelerates citric acid decomposition. Specifically, with the addition of a small amount of NaOH, citric acid recovery dropped to zero; however, when the molar amount of NaOH added equaled or exceeded the number of acidic functional groups, the recovery of citric increased dramatically. These data are in general agreement with the conclusions of Carlsson et al. (1994) and Sakai (1976) derived from NaOH/itaconic acid reactions. In the present case, it is inferred that a small amount of base enhances the decarboxylation of aconitic acid, driving the system to consume citric faster.

From the discussion above, it is clear that at low- or high-water activities, low or high pressures, or low or high pH, citric acid is doomed to decompose when subjected to elevated temperatures. The efficiency of the aquathermolytic degradative pathways indicates that the concentration of citric acid in any given environment must ultimately be low in the absence of significant sources.

## 6. MOVING FROM DECOMPOSITION TO SYNTHESIS VIA HETEROCATALYSIS

Currently there are prolific biotic sources of citric acid. The fermentation of molasses and other sugars by microorganisms such as *Aspergillus niger* and *Aspergillus terreus*, for example, is reported to yield  $\sim 10^8$  Kg/yr of citric acid (Leeper et al., 1991). Whether there ever existed such a prolific abiotic syn-

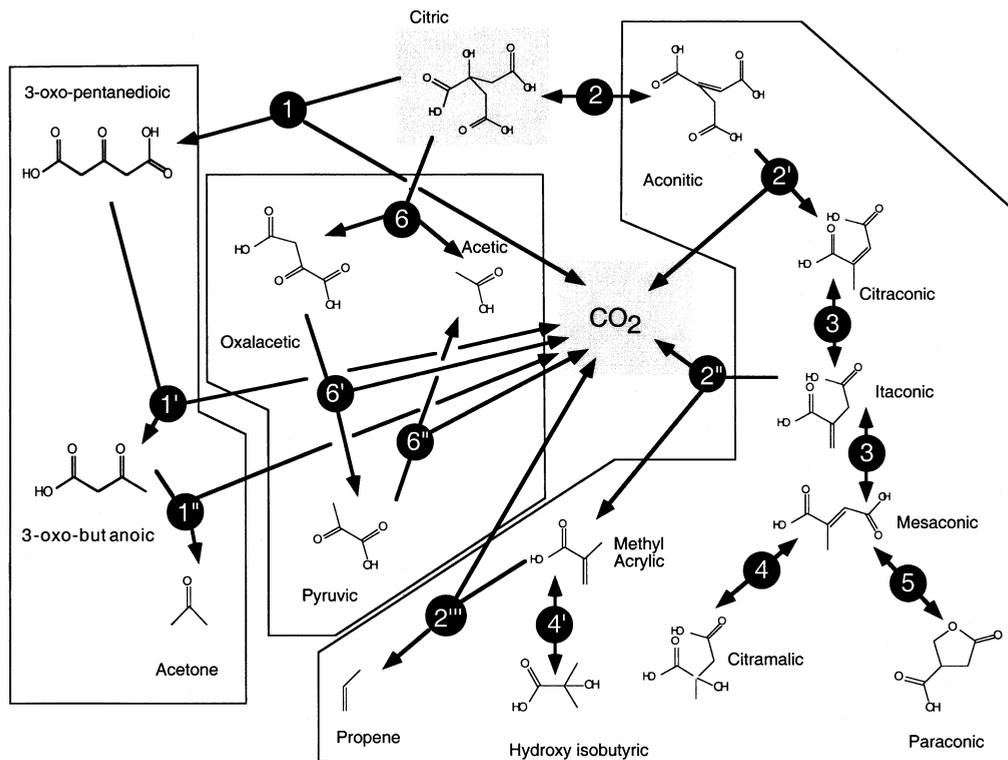


Fig. 6. A compilation of the important decomposition pathways for citric acid. Each reaction is numbered to correspond with discussions in the text. A solid line encloses each distinct pathway, described in detail within the text. The end products of each pathway are acetone + CO<sub>2</sub>, acetic acid + CO<sub>2</sub>, and propene + CO<sub>2</sub>.

thetic route for citric acid on the primitive Earth appears unlikely. From a purely theoretical point of view, however, it has been reasonably argued that organosynthesis up to 10<sup>8</sup>–10<sup>9</sup> Kg C/yr could have occurred at deep sea hydrothermal systems located near and at spreading centers (Shock, 1992).

From the discussions above, it is recognized that the three principal degradation pathways of citric acid lead to either acetone and CO<sub>2</sub> (pathway 1, section 3.1), acetic acid and CO<sub>2</sub> (pathway 2, section 3.2), or propene and CO<sub>2</sub> (pathway 3, section 3.3), as summarized in Figure 6. If any of these pathways were reversible, then one could envision starting with acetone, propene, and/or acetic acid and generating citric acid under certain circumstances. The question arises as to which if any of these pathways has the highest probability of operating in a reverse sense under the natural conditions of hydrothermal systems that might have existed on the primitive, prebiotic Earth.

One of the most straightforward means of synthesizing citric acid is through the Strecker synthesis (e.g., March, 1991); this reaction can be used to readily form alpha hydroxy and amino acids through reaction of HCN with an appropriate carbonyl-containing compound. In the case of citric acid synthesis, the carbonyl-bearing compound would be 3-oxo-1,5 pentanedioic acid (the product of Reaction 1, Section 3.1). The Strecker method has been used to synthesize citric acid in high yield (e.g., Winkel et al., 1989). The facility of this reaction suggests that reversal of pathway 3.1 might provide a viable route for citric acid synthesis.

Success would require that under geochemically reasonable conditions, one could first form acetone and, second, convert acetone into 3-oxo-1,5 pentanedioic acid. This first task may not be difficult under hydrothermal conditions. Seawald (2001), for example, has shown that acetone forms readily from propene in aquathermolytic experiments buffered (and likely catalyzed) by the mineral assemblage, hematite-magnetite-pyrite. A source of propene under hydrothermal conditions is not difficult to envision. Hydrocarbons, such as methane, ethane, propane, and higher carbon number compounds, can form from CO<sub>2</sub> and H<sub>2</sub> in the presence of appropriate transition metal catalysts (e.g., Berndt et al., 1996) via the Fischer-Tropsch synthesis (Fischer, 1935). In addition to purely saturated hydrocarbons, mono-olefins, alcohols, and carboxylic acids may also be important constituents in the product suite (e.g., McCollom et al., 1999). Furthermore, it has been shown that under hydrothermal conditions, saturated hydrocarbons may establish a metastable equilibrium with unsaturated derivatives, e.g., ethane and ethylene (Seawald, 1994). It is also significant that ethylene and propene have been identified as constituents in fluid inclusions contained within rocks associated with ancient hydrothermal systems (de Ronde et al., 1997).

The difficulties arise when considering a pathway from acetone to 3-oxo-1,5 pentanedioic acid. It is conceivable that under some set of conditions, CO<sub>2</sub> could carboxylate acetone, e.g., by virtue of the alpha carbonyl, the methyl groups in acetone are weakly acidic. Under basic conditions, ionization can occur, leaving the resultant carbanion susceptible to elec-

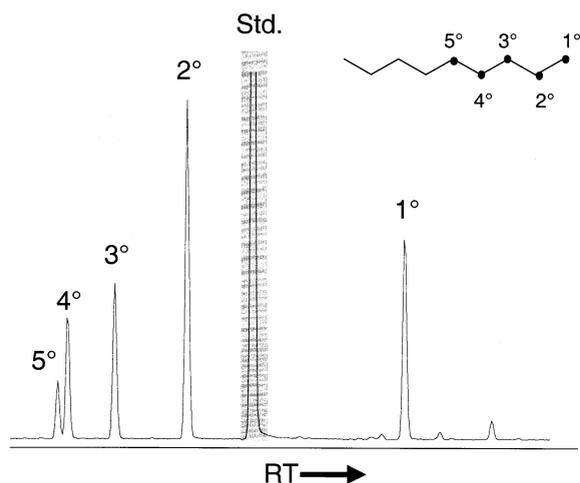


Fig. 7. A chromatogram showing the distribution of C10 acids (total concentration 6.1  $\mu\text{mol}$ ) formed from the Koch reaction involving 22.6  $\mu\text{mol}$  of 1-nonene, 62  $\mu\text{mol}$  of formic acid, and 113  $\mu\text{mol}$  of NiS as the catalyst. The intensity of each peak is given by the total ion current detected in the mass spectrometer. The reaction conditions were 250°C, 200 MPa, 6 h. The various isomers are labeled by their position along the original C9 chain of nonene, where the carbonyl insertion occurred, i.e., at the 1° through 5° carbons of nonene. The distribution of isomers indicates both that extensive double-bond migration occurs before carbonylation as well, which reveals that Markovnikov substitution prevails.

trophilic attack from  $\text{CO}_2$ . Note that this is in essence what occurs in the formation of oxaloacetic acid derived from pyruvic acid (e.g., Fig. 1). Provided that such chemistry could be promoted naturally, the formation of citric acid could arise from the Strecker reaction provided there existed a source of HCN.

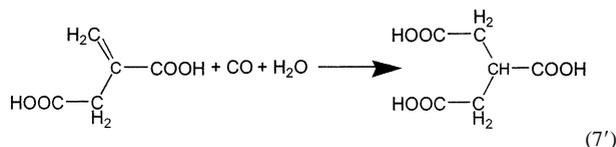
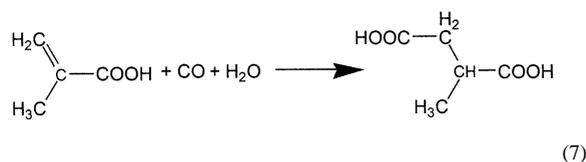
A pathway that might be more promising under hydrothermal conditions is the reversal of degradative pathway 3 (section 3.3 above), i.e., starting with propene and  $\text{CO}_2$ . The same conditions (e.g., heterocatalytic reduction of  $\text{CO}_2$  with  $\text{H}_2$ ) that lead to the formation of propene might also promote relevant and important hydrocarboxylation reactions. Specifically, the Koch or "oxo" reaction involves the bimolecular reaction of a surface-bound carbonyl group with a surface-bound alkyl group followed by hydrolysis, thus yielding a carboxylic acid. Any alkene can be carbonylated via the Koch reaction by reacting with CO in the presence of an appropriate catalyst and under favorable conditions (e.g., March, 1991).

As a demonstration of this chemistry, consider the reaction of 1-nonene ( $\text{CH}_3(\text{CH}_2)_6\text{CH}=\text{CH}_2$ ) with formic acid in the presence of NiS at a temperature of 250°C and a pressure of 200 MPa. After 6 h of reaction, the primary products are isomers of decanoic acid 1- through 5- (Fig. 7). The yield of  $\text{C}_{10}$  acids is 27.5% on the basis of the starting amount of 1-nonene. The distribution of isomers reveals a strong preference for carbonyl insertion at interior carbons; in fact, 76% of the acids are carboxylated at positions 2 to 5. This preference highlights a key mechanistic aspect of this chemistry. First, migration of nonene's double bond precedes the carbonyl insertion as evidenced by a distribution of isomers with the double bond predominantly in the interior carbons.

This chemistry is perfectly general, i.e., it should occur with

any olefin, providing that there are no steric restrictions and accepting that differences in kinetics will be likely. Propene, therefore, in the presence of CO-bearing fluids and catalytic minerals will react to form a mixture of n-butanoic acid and isobutyric acid. Whereas n-butanoic acid is not particularly useful, isobutyric acid may provide a route for the ultimate synthesis of citric acid.

The Koch reaction indicates the possibility of a natural pathway that would lead to progressively carboxylated products—ultimately, citric acid. For example, analogous to what is proposed for propene, carboxylation of methylacrylic acid could form methylsuccinic acid:



and hydrocarboxylation of itaconic acid to could form the tricarboxylic compound, hydroaconitic acid:

Addition of an OH- group to the tertiary carbon of hydroaconitic acid would yield citric acid.

Several issues arise regarding the promise of this pathway. First, it must be established experimentally that the carboxylated products are those desired; for example, as a consequence of reacting methacrylic acid in the presence of CO and an appropriate catalyst, we require the formation of methylsuccinic acid not dimethylmalonic acid. Second, there must not be any steric hindrance involving either alkylation of the catalyst surface or the carbonyl insertion.

To test the viability of such chemistry, a set of experiments were run with NiS, formic acid (as a source of  $\text{CO}_2$ ), CO,  $\text{H}_2$ , and  $\text{H}_2\text{O}$  and either methylacrylic or itaconic acid with the temperature set at 250°C, pressure at 200 MPa, and the residence time fixed at 6 h. A scheme depicting the reaction to form methylsuccinic acid from methylacrylic acid on a cartoon depiction of the NiS surface acid is shown in Figure 8. The results of a reaction starting with methacrylic acid and itaconic acid are presented in Figure 9a,b. In both experiments, a small amount of the desired carboxylated product was synthesized. For example, in the case of methylacrylic acid, a 0.9% yield of methylsuccinic acid was obtained, whereas in the case of itaconic acid, a 1.2% yield of hydroaconitic acid was obtained. No products related to carbonyl insertion at the tertiary (3°) position were observed, indicating a high degree of selectivity perhaps due to steric hindrance. It is reasonable to expect that with adjustment of environmental parameters and/or the mineral structure of the transition metal-bearing solid phase, it should be

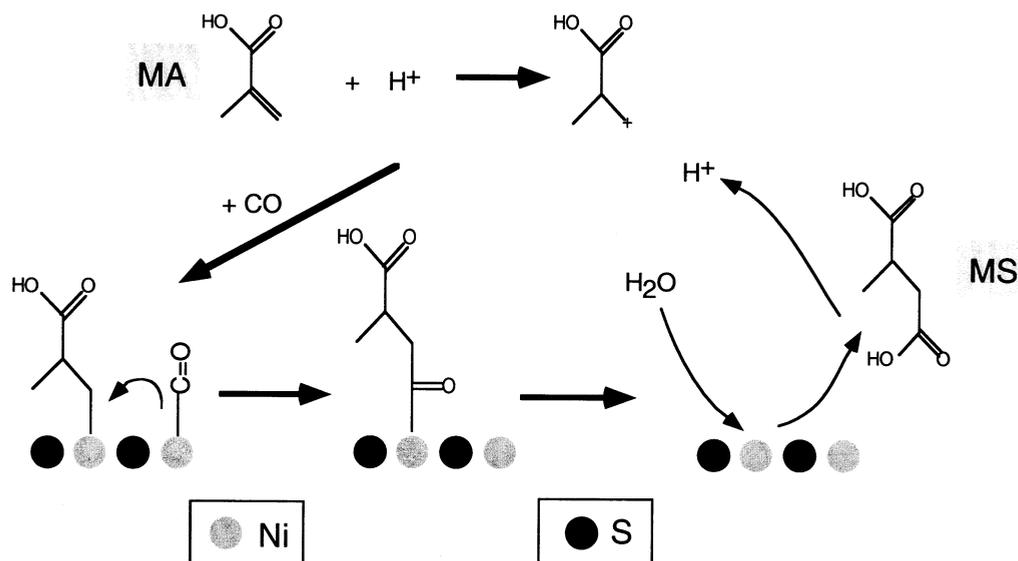


Fig. 8. A cartoon depicting the carbonylation of methacrylic acid (MA) on the surface of an NiS catalyst. The mechanism is broken down to sequential steps of protonation, surface carbonylation, carbonyl insertion, and finally hydrolysis to form the carbonylated product methylsuccinic acid (MS).

possible to increase the hydrocarboxylation yields substantially.

Also evident in Figure 9a,b are a number of other compounds, side products generated under the conditions chosen to promote the synthesis of the desired carboxylic acids. Identification of specific molecules in Figure 9a,b relied on interpretation of electron impact and soft (chemical) ionization mass spectrometric data (see Table A6). The majority of these products are sulfur containing, indicating that partial dissociation of NiS has occurred. Dissociation can occur either through the direct dissolution of the sulfide via complexation with the respective acids or via a disproportionation reaction involving the formation of nickel tetracarbonyl (Ni(CO)<sub>4</sub>) and S<sup>0</sup> (e.g., Able and Crosse, 1967). This latter pathway was shown to be significant in a set of similar reactions involving an alkane thiol, FeS, and formic acid (Cody et al., 2000) where the presence of S<sup>0</sup> was verified directly in the product suite. In the present case, only reduced sulfur compounds were identified.

For example, in the case of the methacrylic acid reaction (Fig. 9a), these additional compounds include 1-methylthioisobutyric acid, thiocitramalic acid, and isothiocitramalic acid (Table A6). The formation of methyl-isobutyric acid thioether reveals that under these experimental conditions, the catalytic reduction of CO to form methane thiol is possible. This chemistry is not unexpected (e.g., Heinen and Lauwers, 1996); the subsequent reaction of methane thiol with the surface-bound isobutyric acid group to form the thioether is also expected (e.g., Cody et al., 1999, 2000). Presumably, the methane thiol reacts with the surface-bound alkyl acid cation to produce the thioether, interrupting any potential carbonyl insertion reaction that would lead to a more carboxylated product. It is significant that the thioether forms only at the less-substituted position, which supports the proposed surface-catalyzed mechanism (e.g., Fig. 8).

The formation of the thio- and isothiocitramalic acids is

interesting. In terms of the oxidation state of carbon, thiol-substituted carbon is equivalent to an olefin. The presence of thiols, therefore, highlights the potential for a second carboxylation, possibly leading to a "one pot" synthesis of a tricarboxylic acid from a monocarboxylic acid. Note, however, that substitution of bisulfide, in the case of these two molecules, occurs on the more substituted positions as would be expected in a classic Markovnikov substitution and is consistent with the reaction with bisulfide or H<sub>2</sub>S occurring in solution not on a mineral surface.

In the case of itaconic acid experiments (Fig. 9 b), there are also other interesting products aside from hydroaconitic acid. The predominant product is methylsuccinic acid (recovered at a 14% yield based on the amount of initial itaconic acid) formed from the catalytic hydrogenation of itaconic acid. In addition to this compound, two other methylthio ether derivatives are present. The formation of the methyl-methylsuccinic acid thioether is analogous to that of the methyl-isobutyric acid thioether (Fig. 9 a) and is significant for the same reason, i.e., the methylthio- substitution is exclusively on the least-substituted carbon supporting a sterically hindered surface reaction. The other significant product is the thioether of methane thiol with methylacrylic acid. The formation mechanism for this compound is likely an oxidative decarboxylation of methyl-methylsuccinic acid thioether, in essence, the reverse of the Koch reaction. The potential significance of this reaction will be explored in greater detail the next section.

## 7. REACTIONS PROMOTED BY THE ADDITION OF FeS AND NiS TO THE CITRIC-H<sub>2</sub>O SYSTEM

In the experiments described above, NiS was shown to promote the Koch reaction, thus providing a potential pathway up from propene to a tricarboxylic acid. In the final set of experiments, the effects of the addition of FeS and NiS to the

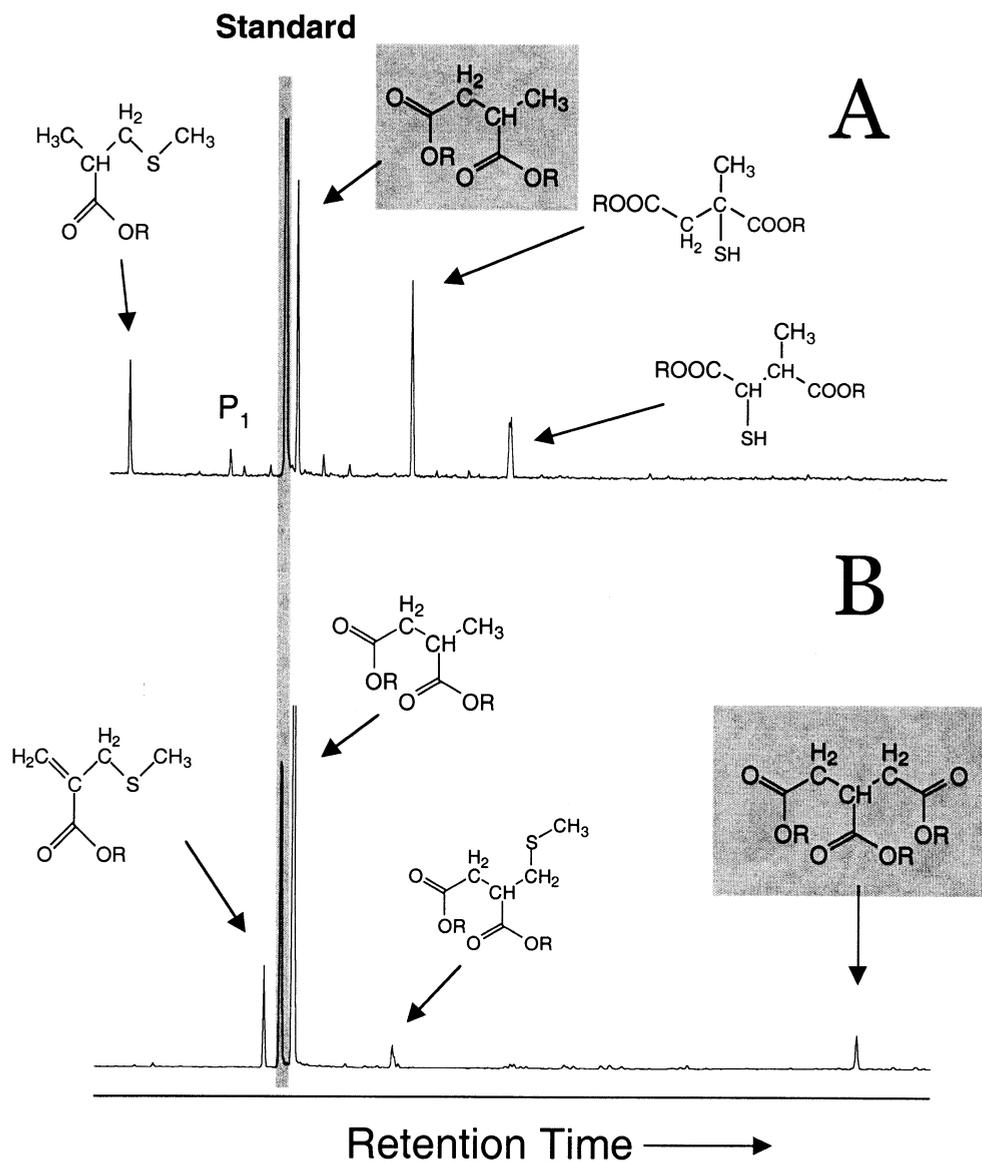


Fig. 9. Total ion current chromatograms of the propylated ( $R =$  propyl group) products of the Koch reaction for (A) 52  $\mu\text{mol}$  of methacrylic acid and (B) 39.6  $\mu\text{mol}$  of itaconic acid run at 250°C, 200 MPa, 6h with 126  $\mu\text{mol}$  of formic acid as a source of CO and 170  $\mu\text{mol}$  of NiS as the solid-phase catalyst. (A) In addition to the target product, methylsuccinic acid (gray box, 0.47  $\mu\text{mol}$  or 0.9% conversion), additional sulfur-containing molecules are formed (see text and Appendix, Table A6, for details). (B) The target product, hydroaconitic acid (gray box, 0.44  $\mu\text{mol}$  or 1.1% conversion), is accompanied by other sulfur-containing organic compounds (see text and Appendix, Table A6, for details). The standard (STD) is pentadecane.

citric- $\text{H}_2\text{O}$  system in the absence of a source of CO (via formic acid) was explored in experiments run for 2 h at 200°C and at 50, 100, and 200 MPa. For each set of conditions, three gold-tube reactors containing concentrated solutions of citric acid and  $\text{H}_2\text{O}$ , with either FeS, NiS, and a control containing no sulfide, were run concurrently to minimize any run-to-run experimental uncertainties. Following reaction, the runs were extracted, derivitized, and analyzed with the same batch of  $\text{BF}_3$ -Propanol complex and GC/MS instrumental parameters (the product distribution is listed in Table A5).

Representative chromatograms of the control and the FeS-loaded runs (200°C and 200 MPa) are shown in Figure 10, revealing substantial differences between the two reactions. For

example in the FeS-containing experiment, hydroxyisobutyric acid (HI in Fig. 10) is clearly evident by the presence of a peak at the shortest elution time (Fig. 10). The formation of hydroxyisobutyric acid occurs through the hydration of methylacrylic acid (Reaction 4'). The addition of FeS also promotes hydrogenation reactions yielding methylsuccinic acid (MS in Fig. 10 and the product in Reaction 7) and hydroaconitic acid (HA in Fig. 10 and the product in Reaction 7'). These products are formed through the hydrogenation of the unsaturated dibasic acids (predominantly itaconic acid) and aconitic acid, respectively. The source of reducing power is related to the partial dissolution of FeS via reaction with citric acid.

Reducing power can arise in two ways. First,  $\text{H}_2$  can be

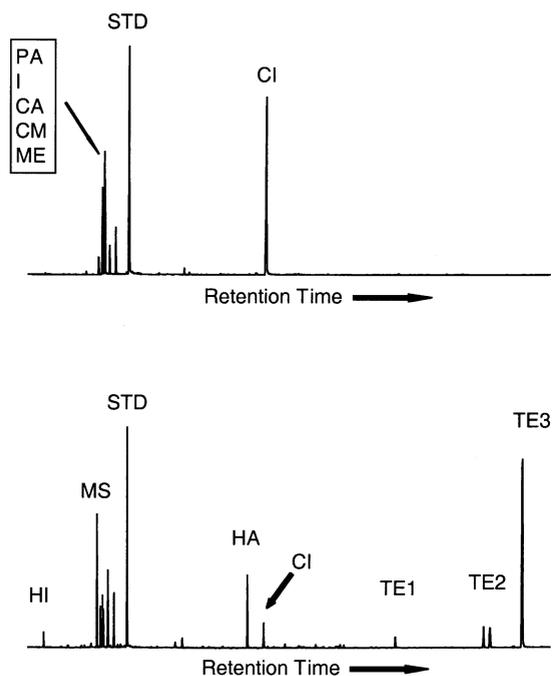


Fig. 10. Total ion chromatograms of propyl esters of run products from concentrated solutions of (A) citric acid in H<sub>2</sub>O (5.6 M) and (B) citric acid in H<sub>2</sub>O (5.6 M) plus FeS. Both reactions are run at 200°C and 200 MPa with residence times fixed at 2 h. All products are designated with abbreviated names: HI = hydroxyisobutyric acid, PA = paraconic acid, I = itaconic acid, CA = citraconic acid, CM = citramalic acid, Me = mesaconic acid, HA = hydroaconitic acid, CI = citric acid, TE1 = thioether of methylsuccinic acid, and TE3 = thioether of isobutyric acid and hydroaconitic acid (see text and Appendix, Tables A5 and A6, for details). The standard (STD) is pentadecane.

generated via subsequent reaction of the liberated H<sub>2</sub>S with the undissolved FeS, yielding pyrite (e.g., Schoonen and Barnes, 1991). This reaction would liberate H<sub>2</sub>, yielding a reaction similar to that proposed and demonstrated by Wächtershäuser (1988b) (see also Heinen and Lauwers, 1996) as the important source of reducing power in archaic metabolic chemistry. Note that no pyrite was detected in the solids following reaction, although analysis of the residual FeS indicated that the mol.% of Fe dropped to 47.5%. Interestingly, Schoonen and Barnes (1991) noted in their study that the transformation of Fe<sub>1-x</sub>S to pyrite was inhibited in the presence of sodium citrate. Another reasonable source of hydrogen would be from the oxidation of chelated ferrous iron to form Fe(OH)<sup>2-</sup> with the concomitant production of hydrogen from water (e.g., Kubal and Panacek, 1995).

By whatever means the partial dissolution of FeS provides a source of hydrogen, the remaining sulfide mineral surface likely plays an important catalytic role in the hydrogenation reaction by facilitating hydrogen dissociation. The relatively high yields of hydroaconitic acid, for example, may require a surface-catalyzed reaction. At temperatures  $\geq 200^\circ\text{C}$ , aconitic acid is unstable; therefore, accumulation of significant quantities of aconitic acid in solution would not be expected. Dehydration of citric acid promoted on the surface of FeS, followed by rapid hydrogenation of aconitic acid before decarboxylation, might explain the relatively high yields of hydroaconitic acid.

The addition of FeS also yields a new and interesting suite of compounds, designated here as TE1, TE2, and TE3 (see Fig. 10) and identified as thioethers on the basis of structural mass spectrometry (see Table A6). Compound TE1 is identified as a thioether of dipropyl methyl succinate and propyl isobutyrate (IB). Both TE2 compounds are identified as thioether isomers of dipropyl methylsuccinate. Compound TE3 is identified as a thioether of tripropyl hydroaconitate and propyl isobutyrate. The formation of thioethers from the reactions of olefinic acids and H<sub>2</sub>S liberated from the decomposition of FeS is not unexpected (e.g., March, 1991, and references therein).

Sulfidization of olefins can occur through electrophilic, nucleophilic, and radical mechanisms (March, 1991). Nucleophilic addition of bisulfide generally occurs under basic conditions. See Gershbein and Hurd (1947) and Vairavamurthy and Mopper (1987) for examples relevant to the current system. Under the present conditions, the pH is assumed to be low. Electrophilic substitution is classically Markovnikov and the 3°-S-3° isomers of TE1 through TE3 are expected; thus, one peak per thioether would be expected in the chromatogram. Sulfidization through a radical mechanism (possibly promoted by the mineral surface operating as an initiator) classically leads to anti-Markovnikov substitutions (March, 1991). In the case of TE1, therefore, two isomers, e.g., MS(1°)-S-IB(1°) and MS(2°)-S-IB(1°), are possible, although only one is observed. Similarly, two isomers of TE2 are expected, e.g., MS(1°)-S-MS(1°) and MS(1°)-S-MS(2°), and are observed (Fig. 11). The abundance of the 1°-S-2° isomer should be twice that of the 1°-S-1°; however, the two isomers of TE2 have a relative abundance of ~55 and 45 mol.%. Compound TE3, under radical sulfidization conditions, will exhibit only a single isomer HA(2°)-S-IB(1°). The presence of the two TE2 isomers suggests, although it does not prove, a radical mechanism.

We have already seen that steric hindrance can lead to less probable substitutions (e.g., Figs. 8 and 9). If TE3 is sulfur-linked through the 2° carbon of hydroaconitic acid, then it is possible that there exists an FeS-catalyzed pathway that promotes conversion of citric to isothiocitric acid. This is analogous chemistry to the isomerization of citric to isocitric acid, an important step in the oxidative citrate cycle. Although this reaction is improbable in solution, it is catalyzed by the Fe<sub>3</sub>S<sub>4</sub> cluster in the aconitase enzyme (Beinert and Kennedy, 1989; Werst et al., 1990). The demonstration of a primitive analog of this reaction under conditions relevant to the ancient geochemical world would establish a compelling connection to extant biochemistry. Work is in progress to sort out the details of sulfur linking in this system and will be reported on separately.

Whereas the addition of FeS has a profound effect on the product distribution, the addition of NiS has only a slightly perturbative effect on the product distribution compared with the control. This difference is seen in Figure 11, where the distribution of products in each run is presented with pressure. It is seen that the addition of NiS appears to enhance, slightly, the decomposition of citric acid to the various dicarboxylic compounds relative to the control. In all cases, the effect of changing pressure appears to have a very small effect on the overall product distribution. Most significant is the complete lack of any sulfur-containing species detected in the NiS-containing runs. As we have already seen in Figure 9, NiS, in the presence of carbon monoxide (derived from formic acid)

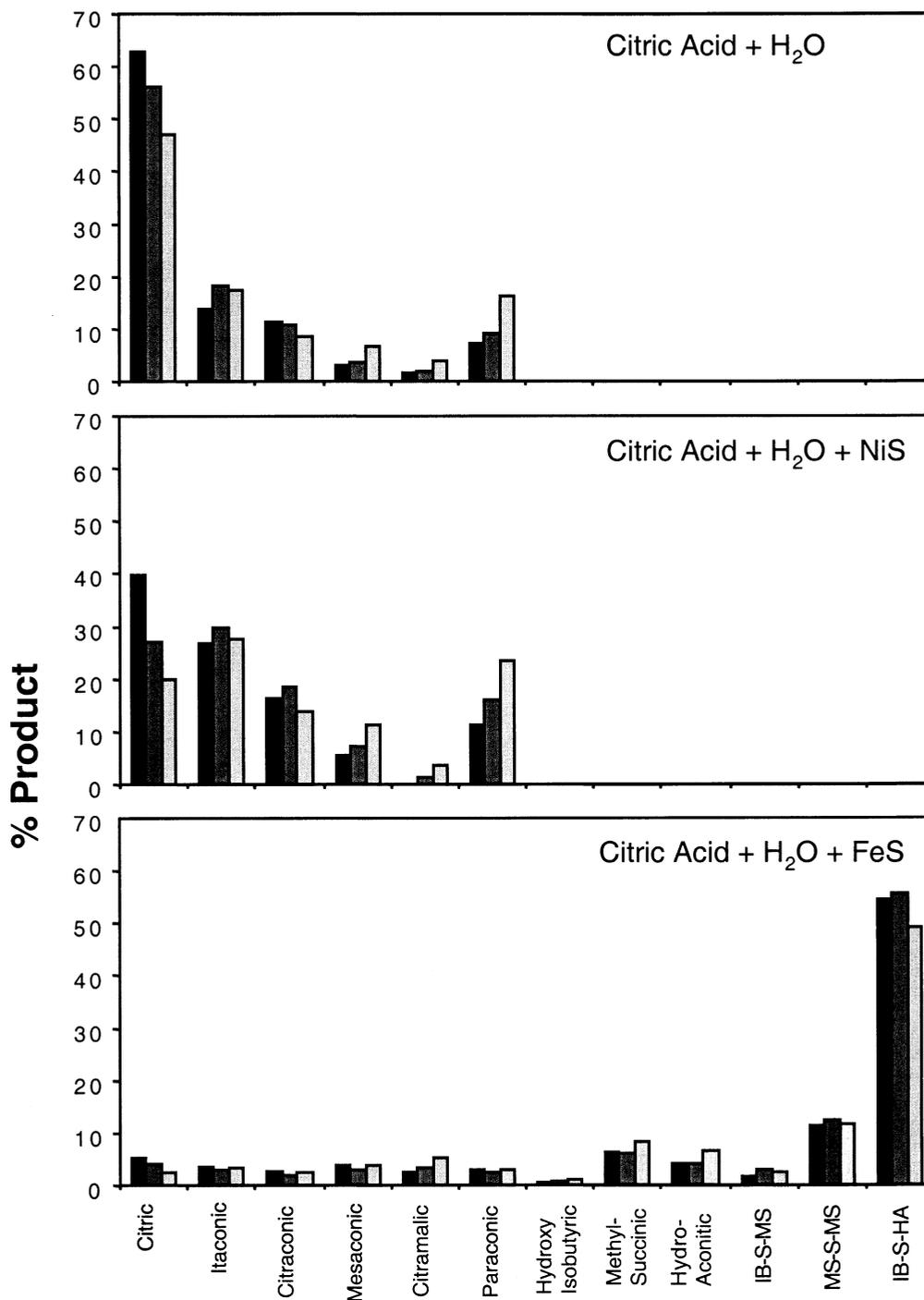


Fig. 11. Product distribution (Table A5, Appendix) for reactions of citric acid in 200°C water at pressures of 50 (black), 100 (dark gray), and 200 MPa (light gray), 2 h, with and without the addition of minerals (FeS and NiS). Citric acid + H<sub>2</sub>O + FeS is at the bottom. Product recovery is normalized to 100%, reflecting the amount of citric acid initially present each reaction.

and organic acids, yields sulfur-containing organic species. Certainly, unsaturated compounds such as itaconic acid and its isomers (see Reaction 3) will react readily with H<sub>2</sub>S to form thiols. The implication is that in the absence of metal carbonylation, minimal H<sub>2</sub>S is generated.

The production of sulfur-containing species is significant for

two reasons. First, it demonstrates a potentially useful set of reaction pathways that diverge from the general trend of decomposition manifested in Figure 6. Second, the introduction of sulfur into the citric acid-H<sub>2</sub>O reaction network may validate an important postulate of Wächtershäuser's theory (Wächtershäuser, 1988a, 1992), specifically that in an H<sub>2</sub>S-rich fluid,

classic RCC chemistry may be replaced with analogous thio-reactions. The proposed benefit in this shift is that it may activate kinetically sluggish, but thermodynamically viable, reactions, as well as lower thermodynamic barriers in traditionally endergonic reactions (Wächtershäuser, 1992).

## 8. IMPLICATIONS

The purpose of this paper is to test the theoretical proposal for a geochemical origin of anabolic biochemistry (e.g., Wächtershäuser, 1992; Russell and Hall, 1997) by experimentally verifying that there exist viable geochemical pathways that could lead into anabolic metabolism. The results described above may offer a viable solution to the so-called "induction period" problem discussed by Wächtershäuser (1992). Specifically, the problem of how to initiate or "ignite" an archaic RCC for carbon fixation. In the absence of a kinetically favored pathway, the concern has been raised that the development of an archaic RCC may have taken an enormous amount of time and perhaps occurred by pure chance (Wächtershäuser, 1992). After initiation, however, such a cycle presumably operated with such kinetic advantage as to out-compete other reactions: in essence, a Darwinian "survival of the fittest" chemical dynamic evolution.

The chemistry described above clearly points to a viable route for entry into the RCC via the synthesis of citric acid from a simple molecule like propene. The availability of such chemistry, in an FeS- and NiS-catalyzed hydrothermal world, indicates that ignition of the RCC pathway might not have been difficult or even fortuitous. After citric acid was formed, other biochemically significant pathways could have been initiated. For example, the retro-Aldol reaction (Reaction 6) is viable over a wide range of pH (Fig. 5); thus, citric acid provides a source for both oxaloacetic acid and pyruvic acid (Reactions 6 and 6'). En route to the formation of citric, citramalic acid may provide an additional source of pyruvic acid via a reaction similar to Reaction 6 (Carlsson et al., 1994). These alpha keto acids in the presence of  $\text{NH}_3$  and a source of reducing power (e.g., the oxidative decarboxylation of pyruvate shown in Reaction 6'', the oxidative decarboxylation of methyl-methylsuccinic acid thioether, or the oxidation of  $\text{Fe}^{2+}$  by  $\text{H}_2\text{O}$ ) provide a straightforward route for the synthesis of the amino acids, alanine and aspartic acid, from pyruvic and oxaloacetic acid, respectively (e.g., Morowitz, 1992; Maughn and Miller, 1998; Brandes et al., 1999). The potential development of a wider range of more evolved biochemical pathways has been outlined in detail by Wächtershäuser (1988a, 1992).

The results of this study provide a necessary, but not yet sufficient, solution for life's geochemical origins. It remains to prove that such chemistry could viably occur under conditions that more realistically mimic those of the primitive Earth. To do this, however, considerably more must be known regarding these critical initial conditions. Acknowledging this deficiency, the chemistry described above does indicate that the most archaic carbon-fixation chemistry may have employed a relatively simple and facile hydrothermal redox pathway leading from propene to citrate, and only later evolved the reductive acetyl CoA-to-citrate pathway, possibly from the top down, i.e., from a subsequent bifurcation of the citric acid decomposition reactions.

As Wächtershäuser (1992) noted in his treatise on the evolutionary biochemistry in an Iron-Sulfur World, the development of the archaic reductive citrate cycle and subsequent prelife biochemical function must follow some sort of biochemical phylogeny where primitive molecular pathways evolve and change. Thus, even the most primitive extant reductive citrate cycle chemistry may be far removed from the archaic carbon-fixation chemistry. In the very beginning, it appears reasonable that this chemistry must have been intrinsic to the geochemical conditions of the ancient Earth and must have been both kinetically and thermodynamically favorable for primitive biochemistry and, ultimately, for life to emerge. The hydrothermal chemistry intrinsic to the citric acid- $\text{H}_2\text{O}$  system appears well suited as a starting point for primitive metabolism.

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#### APPENDIX 1: Identification of Thioether products in the system citric-H<sub>2</sub>O with the addition of FeS

The compounds TE1, TE2, and TE3 observed in experiments with citric and H<sub>2</sub>O in the presence of FeS elute considerably later than the tripropyl derivative of citric acid (Figure 9). Structural mass spectrometry with both electron impact and chemical ionization modes serves as the basis for their identification. Both TE2 and TE3 molecules are isomers because they share a common molecular ion of  $m/z = 462$  (Table A3). The separate designation of TE2 vs. TE3 is based on their electron impact fragmentation behavior. The two TE2 compounds exhibit an intense  $m/z = 216$  ion; the TE3 compound(s) exhibits an intense  $m/z = 301$  ion (Table A3). The  $m/z = 216$  ion is reasonably inferred to be dipropyl methylsuccinate; the  $m/z = 301$  ion is identified as the tripropyl hydroaconitate cation. In all the cases, the  $m/z = 462$  ion exhibits both strong  $M + 1$  and  $M + 2$  ions, revealing the presence of sulfur within the molecule. The molecular ion of TE1 is  $m/z = 376$ , and its strongest ion is  $m/z = 215$ , reasonably inferred to be a dipropyl methylsuccinate carbocation. As is the case with TE2 and TE3, the TE1 molecular ion also exhibits significant  $M + 1$  and  $M + 2$  ions. In all four cases, the lower mass fragments (e.g., the  $m/z = 113$  ion) are similar to those exhibited by propyl esters of citric, citramalic, and the unsaturated dibasic acids. Given these data, the following assignments for compounds TE1, TE2, and TE3 are proposed. Compound TE1 is identified as a thioether of dipropyl methyl succinate and propyl isobutyrate (IB). Both of the TE2 compounds are identified as thioether isomers of dipropyl methylsuccinate. Compound TE3 is identified as a thioether of tripropyl hydroaconitate and propyl isobutyrate.

Table A1. Citric-H<sub>2</sub>O reactions, 200°C, 2h, sealed glass ampules, P ~ 2 MPa (vapor pressure of solution).

Solution	Conc. (M)	CI(I) $\mu$ mol	CI(F) $\mu$ mol	ITA $\mu$ mol	CA $\mu$ mol	ME $\mu$ mol	CM $\mu$ mol	MA $\mu$ mol	Recovery
A1	5.6	5.6	0.999 (43.6)	0.7903 (34.5)	0.341 (14.9)	0.098 (4.3)	0.068 (3.0)	0.0032 (140)	2.2927 (41)
A2	5.6	5.6	1.431 (49.4)	0.890 (30.7)	0.413 (14.3)	0.095 (3.3)	0.066 (2.3)	0.0028 (100)	2.8978 (52)
A3	5.6	5.6	1.287 (45.3)	0.942 (33.2)	0.412 (14.5)	0.111 (3.9)	0.083 (2.9)	0.0037 (130)	2.8390 (51)
B1	1.1	4.4	1.081 (38.0)	1.143 (40.2)	0.381 (13.4)	0.120 (4.2)	0.108 (3.8)	0.0125 (440)	2.8449 (65)
B2	1.1	4.4	1.454 (44.8)	1.202 (37.1)	0.394 (12.2)	0.098 (3.0)	0.086 (2.7)	0.0090 (280)	3.2424 (74)
B3	1.1	4.4	1.493 (43.5)	1.281 (37.4)	0.429 (12.5)	0.111 (3.2)	0.106 (3.1)	0.0100 (290)	3.4300 (78)
C1	0.57	4.3	1.084 (43.7)	0.963 (38.8)	0.303 (12.2)	0.069 (2.8)	0.056 (2.2)	0.0083 (330)	2.4833 (58)
C2	0.57	4.3	1.397 (43.5)	1.230 (38.3)	0.387 (12.0)	0.090 (2.8)	0.10 (3.1)	0.0110 (340)	3.2130 (75)
C3	0.57	4.3	1.386 (41.5)	1.319 (39.5)	0.416 (12.5)	0.101 (3.0)	0.105 (3.2)	0.0124 (370)	3.3395 (78)
D1	0.28	14.0	4.051 (34.9)	4.955 (42.7)	1.658 (14.3)	0.400 (3.5)	0.465 (4.0)	0.0672 (580)	11.596 (83)
D2	0.28	14.0	5.98 (44.2)	5.1123 (37.8)	1.670 (12.3)	0.328 (2.4)	0.374 (2.8)	0.0703 (520)	13.534 (97)
D3	0.28	14.0	5.025 (37.2)	5.572 (41.2)	1.904 (14.1)	0.436 (3.2)	0.511 (3.8)	0.0703 (520)	13.518 (97)
E1	0.06	5.8	1.334 (26.6)	2.485 (49.5)	0.837 (16.7)	0.154 (3.1)	0.174 (3.5)	0.0412 (820)	5.0250 (87)
E2	0.06	5.8	1.690 (33.9)	2.2728 (45.6)	0.734 (14.7)	0.119 (2.4)	0.140 (2.8)	0.0291 (580)	4.9841 (86)
E3	0.06	5.8	1.326 (25.4)	2.5390 (48.7)	0.869 (16.7)	0.160 (3.1)	0.277 (5.3)	0.0428 (820)	5.2128 (90)

\*CI(I) = citric acid (initial), CI(F) = citric acid (final), ITA = itaconic acid, CA = citraconic acid, ME = mesaconic acid, CM = citramalic acid, MA = methacrylic acid. All products analyzed with HPLC and UV detection,  $\lambda = 210$  nm.

\*\*Absolute concentration as measured in  $\mu$ mol, normalized concentration, percent, in parenthesis. Methacrylic acid normalized concentration presented in ppm.

Table A2. Citric-H<sub>2</sub>O reactions, 200°C, 2h, welded Au tubes, P = 100 MPa.

Solution	Conc. (M)	CI(I)* $\mu$ mol**	CI(F) $\mu$ mol	ITA $\mu$ mol	CA $\mu$ mol	ME $\mu$ mol	CM $\mu$ mol	MA*** $\mu$ mol	Recovery $\mu$ mol
A1	5.6	58.40 (100)	17.24 (39.2)	14.56 (33.1)	6.68 (15.2)	2.67 (6.1)	2.77 (6.3)	0.12 (270)	44.0 (75.3)
A2	5.6	58.40 (100)	14.00 (33.3)	14.90 (35.5)	5.50 (13.1)	4.00 (9.5)	0.80 (1.9)	0.20 (480)	42.0 (71.9)
A3	5.6	56.30 (100)	13.40 (42.8)	10.60 (33.9)	3.30 (10.5)	2.70 (8.6)	1.20 (3.8)	0.10 (320)	31.3 (55.6)
B1	1.1	34.65 (100)	11.03 (37.2)	11.68 (39.4)	3.63 (12.2)	1.43 (4.8)	1.71 (5.8)	0.18 (610)	29.7 (85.6)
B2	1.1	35.06 (100)	3.33 (19.5)	6.40 (37.4)	1.77 (10.3)	2.76 (16.1)	2.79 (16.3)	0.06 (350)	17.1 (48.8)
B3	1.1	35.29 (100)	5.88 (25.7)	8.94 (39.1)	2.46 (10.7)	2.71 (11.8)	2.46 (10.7)	0.47 (2050)	22.9 (64.9)
C1	0.57	17.55 (100)	4.40 (29.5)	6.63 (44.4)	1.98 (13.3)	0.85 (5.7)	0.93 (6.2)	0.15 (1000)	14.9 (85.1)
C2	0.57	18.25 (100)	3.53 (28.6)	4.97 (40.3)	1.21 (9.8)	0.90 (7.3)	1.68 (13.6)	0.05 (410)	12.3 (67.4)
C3	0.57	17.99 (100)	3.12 (25.3)	4.65 (37.7)	1.24 (10.0)	1.20 (9.7)	1.85 (15.0)	0.27 (2190)	12.3 (68.4)
D1	0.28	10.02 (100)	0.02 (0.3)	3.37 (43.5)	1.98 (25.6)	0.79 (10.2)	1.21 (15.6)	0.37 (4780)	7.74 (77.3)
D2	0.28	8.99 (100)	0.47 (16.9)	1.03 (36.9)	0.29 (10.4)	0.45 (16.1)	0.53 (19.0)	0.02 (720)	2.79 (31.0)
D3	0.28	9.28 (100)	1.20 (19.6)	2.39 (39.1)	0.68 (11.1)	0.69 (11.3)	0.93 (15.2)	0.22 (3600)	6.11 (65.8)
E1	0.06	1.96 (100)	0.05 (3.6)	0.66 (47.5)	0.23 (16.6)	0.12 (8.6)	0.25 (18.0)	0.08 (5760)	1.39 (70.9)
E2	0.06	2.06 (100)	0.02 (2.7)	0.23 (30.7)	0.08 (10.7)	0.08 (10.7)	0.31 (41.3)	0.03 (4000)	0.75 (36.4)
E3	0.06	2.00 (100)	0.19 (15.3)	0.56 (45.2)	0.14 (11.3)	0.12 (9.7)	0.15 (12.1)	0.08 (6450)	1.24 (62.0)

\* CI(I) = citric acid (initial), CI(F) = citric acid (final), ITA = itaconic acid, CA = citraconic acid, ME = mesaconic acid, CM = citramalic acid, MA = methacrylic acid. All products analyzed using HPLC using UV detection,  $\lambda = 210$  nm.

\*\* Absolute concentration in  $\mu$ mol, normalized concentration (percent) in parenthesis.

\*\*\* Normalized concentration of methacrylic acid is presented as ppm.

Table A3. Citric-H<sub>2</sub>O reactions, 200°C 2 h duration, variable pressure and concentration analysis via GC/MS.

Pressure (MPa)	Concentration (M)	Citric (i) $\mu\text{mol}$	Citric* (f) $\mu\text{mol}$	ITA** $\mu\text{mol}$	CA $\mu\text{mol}$	ME $\mu\text{mol}$	CM $\mu\text{mol}$	PA $\mu\text{mol}$	Recovery <sup>†</sup> $\mu\text{mol}$
50	56	51.0	9.74 (34)	6.75 (24)	7.34 (26)	1.61 (6)	0.24 (1)	3.01 (10)	28.69 (56)
100	56	49.7	8.99 (37)	6.13 (25)	5.16 (21)	1.38 (6)	0.21 (1)	2.73 (12)	24.60 (49)
500	56	49.7	7.83 (35)	3.95 (18)	1.50 (7)	1.75 (8)	0.83 (4)	6.68 (30)	22.54 (45)
50	11	49.2	9.33 (31)	8.43 (31)	4.14 (15)	1.81 (7)	0.77 (3)	2.71 (10)	27.19 (55)
100	11	49.5	21.05 (37)	15.92 (28)	7.09 (12)	3.96 (7)	2.01 (3)	7.58 (13)	57.61 (116)
500	11	50.6	4.97 (22)	4.03 (18)	1.06 (5)	2.17 (10)	2.13 (10)	7.78 (35)	22.14 (44)
50	2.7	25.4	4.47 (29)	5.39 (35)	1.87 (12)	1.07 (7)	0.61 (4)	1.79 (12)	3.23 (13)
100	2.7	24.7	6.34 (33)	6.18 (32)	2.16 (11)	1.45 (7)	0.71 (4)	2.65 (14)	19.50 (79)
500	2.7	24.8	13.26 (15)	2.76 (21)	0.61 (5)	1.49 (11)	2.05 (15)	4.31 (33)	13.26 (53)
50	1.1	19.5	8.29 (27)	3.35 (40)	1.03 (12)	0.60 (7)	0.22 (3)	0.84 (10)	8.29 (44)
100	1.1	—	—	—	—	—	—	—	—
500	1.1	19.7	9.74 (21)	1.6 (16)	0.36 (4)	0.82 (8)	1.76 (8)	3.18 (33)	9.74 (49)
50	0.27	4.8	1.30 (21)	2.4 (39)	0.76 (13)	0.43 (7)	0.49 (8)	0.70 (12)	6.08 (127)
100	0.27	5.1	0.59 (17)	1.30 (38)	0.40 (12)	0.32 (9)	0.35 (10)	0.50 (14)	3.46 (69)
500	0.27	5.1	0.24 (12)	0.36 (18)	0.08 (4)	0.20 (10)	0.37 (19)	0.73 (37)	1.98 (39)

\* In parenthesis is the percentage recovery normalized to total analyzed.

\*\* ITA = itaconic acid, CA = citraconic acid, ME = mesaconic acid, CM = citramalic acid, PA = paraconic acid. All products were converted to propyl esters and analyzed with gas chromatography/mass spectrometry.

<sup>†</sup> Absolute concentration determination was performed with an external standard, pentadecane. The wide variation in recoveries reflects the difficulty of reliably extracting quantitative yields. Normalized yields appear to be more reliable.

Table A4. Citric-NaOH-H<sub>2</sub>O reactions, 200°C, 2 h duration, 200 MPa, 0.11 M analysis via GC/MS.

Citric (I) $\mu\text{mol}$	NaOH $\mu\text{mol}$	pH <sub>25</sub> <sup>*</sup>	Citric (f) $\mu\text{mol}$	Pyr** $\mu\text{mol}$	MS $\mu\text{mol}$	ITA $\mu\text{mol}$	CA $\mu\text{mol}$	ME $\mu\text{mol}$	CM $\mu\text{mol}$	PA $\mu\text{mol}$	Recovery
16.93	20.71	3.4	ND	0.349 (4.5)	0.129 (1.7)	1.575 (20.3)	2.737 (35.2)	1.767 (22.8)	0.014 (0.2)	1.196 (15.4)	7.77 (46)
21.15	41.92	4.2	ND	0.498 (7.7)	0.971 (1.5)	1.280 (19.9)	1.770 (27.5)	2.130 (33.1)	0.014 (0.2)	0.641 (10.0)	6.43 (30)
21.88	64.00	5.3	4.02 (30.4)	1.98 (15.0)	0.55 (4.2)	1.75 (13.2)	1.10 (8.3)	3.48 (26.3)	0.10 (0.8)	0.25 (1.9)	13.23 (61)
18.02	70.30	13.0	8.43 (90.4)	0.64 (6.9)	0.197 (2.1)	0.0216 (0.2)	ND	0.033 (0.4)	ND	ND	9.32 (52)

\*\* Pyr = pyruvic acid, MS = methylsuccinic acid, ITA = itaconic acid, CA = citraconic acid, ME = mesaconic acid, CM = citramalic acid, MA = methacrylic acid. All products converted to propyl esters and analyzed with gas chromatography and mass spectrometry. Absolute concentrations are in  $\mu\text{mol}$ s determined via the use of an external standard (pentadecane), normalized concentration, as percent of total recovery, is included in parenthesis.

Table A5. Product distributions from experiments with citric acid-H<sub>2</sub>O(5.6M) ± FeS ± NiS temperature = 200°C, time = 2 h, Pressure = 50, 100, 200 MPa.

P(MPa)	Cat. μmol	CI(i) μmol	CI* μmol	ITA μmol	CA μmol	ME μmol	CM μmol	PA μmol	HI μmol	MS μmol	HA μmol	TE1 μmol	TE2 μmol	TE3 μmol	Recovery μmol
50	—	26.04	13.40 (62.8)	2.95 (13.8)	2.46 (11.5)	0.68 (3.2)	0.34 (1.6)	1.54 (7.2)	—	—	—	—	—	—	21.35 (82)
100	—	26.82	13.50 (56.1)	4.40 (18.3)	2.60 (10.8)	0.89 (3.7)	0.48 (2.0)	2.19 (9.1)	—	—	—	—	—	—	24.06 (90)
200	—	26.30	8.52 (47.1)	3.16 (17.4)	1.55 (8.6)	1.21 (6.7)	0.71 (3.9)	3.04 (16.3)	—	—	—	—	—	—	18.09 (69)
50	NiS 39.8	26.7	4.54 (39.7)	3.07 (26.9)	1.87 (16.4)	0.64 (5.6)	0.00 (0.0)	1.31 (11.5)	—	—	—	—	—	—	11.43 (43)
100	NiS 35.2	26.9	2.15 (27.1)	2.37 (29.8)	1.48 (18.6)	0.57 (7.1)	0.11 (1.4)	1.26 (15.9)	—	—	—	—	—	—	7.94 (30)
200	NiS 31.6	26.7	2.08 (19.9)	2.91 (27.8)	1.44 (13.8)	1.20 (11.5)	0.38 (3.6)	2.46 (23.5)	—	—	—	—	—	—	10.47 (39)
50	FeS 33.0	26.3	1.26 (5.4)	0.87 (3.7)	0.63 (2.7)	0.92 (3.9)	0.61 (2.6)	0.68 (2.9)	0.16 (0.7)	1.49 (6.4)	1.00 (4.3)	0.19 (1.6)	1.34 (11.4)	6.41 (54.6)	15.57 (89) <sup>†</sup>
100	FeS 35.2	26.9	1.08 (4.0)	0.83 (3.1)	0.54 (2.0)	0.81 (3.0)	0.91 (3.4)	0.64 (2.4)	0.19 (0.7)	1.61 (6.0)	1.13 (4.2)	0.40 (3.0)	1.67 (12.5)	7.42 (55.5)	17.24 (99) <sup>†</sup>
200	FeS 34.7	26.9	0.73 (2.6)	0.91 (3.3)	0.70 (2.5)	1.10 (3.9)	1.48 (5.3)	0.83 (3.0)	0.32 (1.2)	2.34 (8.3)	1.88 (6.7)	0.35 (2.5)	1.61 (11.5)	6.91 (49.3)	19.18 (104) <sup>†</sup>

\* CI(I) = citric acid (initial), CI(F) = citric acid (final), ITA = itaconic acid, CA = citraconic acid, ME = mesaconic acid, CM = citramalic acid, PA = paraconic acid, HI = hydroxyisobutyric acid, MS = methylsuccinic acid, HA = hydroacontic acid, TE1 = thioether of isobutyric and methylsuccinic acids, TE2 = thioethers of two methylsuccinic acids, TE3 = thioether of isobutyric and hydroacontic acids. All products were converted to propyl esters and analyzed with GC/MS. Absolute concentration are determined via use of an external standard, pentadecane, and are in μmols, the normalized concentrations (percent) are in parenthesis.

<sup>†</sup> NOTE: Each thioether (T1, T2, and T3) corresponds to two molecules of citric acid and contribute 2X to total recovery. The response factors of the thioethers under electron impact are not known and were assumed to be equal to 1.0.

Table A6. Electron Impact Mass Spectral Fragmentation Pattern of Sulfur-Containing Organic Products.

Label	Compound	MW*	Fragmentation Pattern m/z (% relative intensity)
None	Methylthio-acrylic acid propyl ester	174	174(40,M); 131(15,M-CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> ); 115(25,M-CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> O); 86(100),83(50)
None	1-methylthio-isobutyric acid propyl ester	176	176(40,M); 134(15,M-CH <sub>3</sub> CHCH <sub>2</sub> ); 117(25,M-CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> O); 88(60); 61(100,CH <sub>3</sub> S-CH <sub>2</sub> )
None	Thiocitramalic acid dipropyl ester	248	200-199(50,M-CH <sub>3</sub> SH-H); 172-170(20,M-SH-CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> ); 129(100);111(30),88(30),83(50)
None	Isothio-citramalic acid dipropyl ester	248	199(80,M-M-CH <sub>3</sub> SH-H); 172-170(35,M-SH-CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> ); 129(75); 116(70),74(100,CH <sub>3</sub> CHCHSH <sup>+</sup> )
None	CH <sub>3</sub> -S-MS dipropyl ester	362	188(30,M-CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> O-CH <sub>3</sub> ); 146(188-CH <sub>3</sub> CHCH <sub>2</sub> ); 129(30,188-CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> O); 86(100);61(20,CH <sub>3</sub> S-CH <sub>2</sub> <sup>+</sup> )
HA**	Hydro-Aconitic Acid Tri-Propyl ester	302	261(5,M-CH <sub>2</sub> CH=CH <sub>2</sub> ); 243(25,M-CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> O); 215(3,M-CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> O <sub>2</sub> C); 201(40,M-Propyl-O <sub>2</sub> CCH <sub>2</sub> ); 159(100); 141(90); 113(25)
TE1	MS-S-IB Tripropyl Ester	376	376(15,M); 317(20,M-CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> O); 288(15,M-CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> O <sub>2</sub> C); 275(10,M-Propyl-O <sub>2</sub> CCH <sub>2</sub> ); 215(100,Propyl-O <sub>2</sub> CH <sub>2</sub> C(CH <sub>3</sub> )CO <sub>2</sub> -Propyl+); 201(30); 187(25); 169(20); 155(25); 145(30); 131(30); 113(45)
TE2	MS-S-MS Tetrapropyl Ester	462	462(5,M); 403(19,M-CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> O); 375(5,M-CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> O <sub>2</sub> C); 315(30); 216(98,Propyl-O <sub>2</sub> CCH <sub>2</sub> CH(CH <sub>3</sub> )CO <sub>2</sub> -Propyl); 187(40); 156(80); 145(60); 129(30); 113(100)
TE2	MS-S-MS Tetrapropyl Ester	462	462(5,M); 403(10,M-CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> O); 375(5,M-CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> O <sub>2</sub> C); 315(30); 216(100,Propyl-O <sub>2</sub> CCH <sub>2</sub> CH(CH <sub>3</sub> )CO <sub>2</sub> -Propyl); 187(40); 156(80); 145(60); 129(30); 114(95)
TE3	HA-S-IB Tetrapropyl Ester	462	462(5,M); 403(3,M-CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> O); 361(5,M-Propyl-O <sub>2</sub> CCH <sub>2</sub> ); 301(100,Propyl-O <sub>2</sub> CCH <sub>2</sub> C(CO <sub>2</sub> -Propyl)CH <sub>2</sub> CO <sub>2</sub> -Propyl+); 215(55,Propyl-O <sub>2</sub> CH <sub>2</sub> C(CH <sub>3</sub> )CO <sub>2</sub> -Propyl+); 201(45); 173(50); 155(30); 145(35); 131(45); 113(70)