Rate-determining processes in acid-catalyzed decarboxylation reactions

by

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A thesis submitted in conformity with the requirements for the degree of Doctorate of Philosophy
Department of Chemistry
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Abstract

The acid-catalyzed decarboxylation reactions of indole- and pyrrole-carboxylic acids require the addition of one equivalent of water to the carboxyl group and a proton to the heterocyclic ring carbon at the position $\alpha$ to the carboxyl. Where $\alpha$-protonation is thermodynamically favoured over $\beta$-protonation, the magnitude of the observed $^{12}\text{C}/^{13}\text{C}$ kinetic isotope effect (CKIE) is greater than where the $\beta$-position is protonated. This can be understood in terms of a mechanism involving a protonated hydrated precursor to carbon-carbon bond cleavage, where the difference in energy of intermediates and transition states control the proportioning of the intermediates. The intrinsic CKIE on the carbon-carbon bond-breaking step that produces protonated carbonic acid (PCA) is independent of the site of protonation. The interpretation of the observed CKIEs can be generalized based on intermediates from isomeric carboxylic acids whose energetics vary predictably with their sites of protonation. The relative free energy barriers to reversion and formation of PCA control the magnitude of the observed CKIEs and correlate with reactivity. The reported data implicate the formation of PCA as the initial product of carbon-carbon bond cleavage. Application of the principle of microscopic reversibility implies that electrophilic aromatic substitution based on PCA should be an accessible route to carboxylation of aromatic substrates. Over the course of
the project, new methods were developed for the simultaneous pressure detection and mass spectral analysis of carbon dioxide released as a final product. Specifically, headspace gas analysis and compound-specific isotope analysis of carbon dioxide have been coupled as a result. The evaluation for new decarboxylation mechanisms in general has led to a clearer understanding of how the intervention of hydrated intermediates leads to formation of PCA and its subsequent rapid conversion to carbon dioxide.
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Statement of Authorship and Publication Status

Chapter 1

Title: Pressure-monitored headspace analysis combined with compound-specific isotope analysis to measure isotope fractionation in gas-producing reactions

Authors: Scott O. C. Mundle, Adelle A. Vandersteen, Georges Lacrampe-Couloume, Ronald Kluger, Barbara Sherwood Lollar

Note: Experimental procedures were performed by Scott O. C. Mundle and Adelle A. Vandersteen. Data interpretation was carried out by Scott O. C. Mundle, Adelle A. Vandersteen, Georges Lacrampe-Couloume, Ronald Kluger and Barbara Sherwood Lollar. The manuscript was written by Scott O. C. Mundle and Barbara Sherwood Lollar, with input from all co-authors.

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Authors: Adelle A. Vandersteen, Scott O. C. Mundle, and Ronald Kluger

Note: Kinetic experiments were carried out by Adelle A. Vandersteen, with additional data provided by Scott O. C. Mundle. Interpretation of results and manuscript writing was performed by Adelle A. Vandersteen and Ronald Kluger.

Title: Carbon Kinetic Isotope Effects Reveal Variations in Reactivity of Intermediates in the Formation of Protonated Carbonic Acid

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### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>$^{12}\text{C}/^{13}\text{C}$</td>
<td>Carbon-12 to Carbon-13 isotopic ratio</td>
</tr>
<tr>
<td>CKIE</td>
<td>Carbon kinetic isotope effect</td>
</tr>
<tr>
<td>CSIA</td>
<td>Compound-specific isotope analysis</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>DMBA</td>
<td>2,4-dimethoxybenzoic acid</td>
</tr>
<tr>
<td>GC-IRMS</td>
<td>Gas chromatography-coupled isotope ratio mass spectrometer</td>
</tr>
<tr>
<td>$H_0$</td>
<td>Hammett acidity function</td>
</tr>
<tr>
<td>I-2-C</td>
<td>Indole-2-carboxylic acid</td>
</tr>
<tr>
<td>I-3-C</td>
<td>Indole-3-carboxylic acid</td>
</tr>
<tr>
<td>MTh</td>
<td>2-(2-mandelyl)thiamin</td>
</tr>
<tr>
<td>P-2-C</td>
<td>Pyrrole-2-carboxylic acid</td>
</tr>
<tr>
<td>P-3-C</td>
<td>Pyrrole-3-carboxylic acid</td>
</tr>
<tr>
<td>PCA</td>
<td>Protonated carbonic acid</td>
</tr>
<tr>
<td>SKIE</td>
<td>Solvent kinetic isotope effect</td>
</tr>
<tr>
<td>UV-vis</td>
<td>Ultraviolet-visible</td>
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Chapter 1
Catalysis and decarboxylation

1.1 Decarboxylation and the formation of carbon dioxide

Although the term “decarboxylation” means ‘the loss of a carboxyl group’, it has become interchangeable with a reaction that occurs by cleavage of a carbon-carbon bond of a carboxylate to produce carbon dioxide (CO$_2$). Decarboxylation is an important reaction in biological, organic and environmental settings and decarboxylase enzymes catalyze metabolic processes. This electrophilic substitution usually results in the replacement of the carboxyl group by a proton from a Brønsted acid donor. In some cases, protonation can precede the carbon-carbon bond-breaking step where a site of unsaturation is present at the α-β position relative to the carboxyl carbon atom (Scheme 1.1).

\[
\begin{align*}
\text{a} & \quad R^\ominus \xrightarrow[\text{CO}_2]{\text{R}} \quad R^\ominus + \xrightarrow[H^\ominus]{\text{CO}} \quad RH + \text{CO}_2 \\
\text{b} & \quad \begin{array}{c}
\alpha \beta \\
\text{H}^\ominus \xrightarrow[\text{CO}_2]{\text{H}} \quad \alpha \beta \xrightarrow[\text{CO}_2]{\text{H}} \quad \alpha \beta + \text{CO}_2
\end{array}
\end{align*}
\]

\textbf{Scheme 1.1.} Decarboxylation reactions proceeding by a) loss of CO$_2$ followed by protonation of the residual carbanion (R$^-$) or b) protonation of an α-β site of unsaturation followed by loss of CO$_2$.

Upon cleavage of the carbon-carbon bond, the immediate products are a carbanion and carbon dioxide. Generation of this nucleophile-electrophile pair may lead to recombination, reversing the bond-breaking step. Computational calculations$^{1,2}$ along with carbon kinetic isotope effects (CKIEs)$^{3,4}$ and carboxyl exchange$^{5,6}$ have supported the possibility of internal return of carbon
dioxide in decarboxylation reactions. Enzymes and synthetic catalysts might overcome this
reversibility by attenuating the reactivity of either the carbanion or carbon dioxide in order to
promote throughput of the reaction.

Factors leading to the stabilization of the incipient carbanion (R\(^-\), Scheme 1.1.a) through inductive
effects or electron delocalization lead to acceleration of the reaction. An example of carbanion-
based stabilization is amine-catalyzed decarboxylation of \(\beta\)-ketoacids\(^7\) (Scheme 1.2).

\[
\begin{align*}
\text{O} & \quad \text{O} \quad \text{NRH}_2 \\
\text{R} & \quad \text{NH} & \quad \text{O} \quad \text{O} \quad \text{R}^+ \\
\text{NH} & \quad + & \quad \text{CO}_2
\end{align*}
\]

**Scheme 1.2.** Amine-catalyzed decarboxylation of \(\beta\)-ketoacid acetoacetate

Reversible formation of the imine intermediate, as confirmed by *in situ* reduction with sodium
cyanoborohydride, helps to delocalize carbanion character upon carbon-carbon bond cleavage.

For the pathway shown in Scheme 1.1.b, the ground state energy of the unsaturated reactant is
destabilized upon protonation, leading to a lower barrier for the carbon-carbon bond-breaking step.
In the decarboxylation of cinnamic acids, acids facilitate protonation at the \(\alpha\) position prior to loss
of carbon dioxide (Scheme 1.3). The formation of the double bond upon cleavage of the carbon-
carbon bond occurs with obliteration of the positive charge.

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{H} & \quad \text{H} & \quad \text{O}^+ \\
\text{H} & \quad + & \quad \text{CO}_2
\end{align*}
\]

**Scheme 1.3.** Decarboxylation of cinnamic acid produces styrene and carbon dioxide

Pre-associated Brönsted acid catalysis can provide another means of reducing residual carbanion
character by supplying the necessary proton immediately upon carbon-carbon bond-breaking. In
the case of the decarboxylation of mandelylthiamin (MTh), pyridinium ions catalyze the reaction by $\pi$-stacking with the substrate prior to the rate-limiting carbon-carbon bond cleavage step\textsuperscript{4,8,9} (Scheme 1.4). The nascent carbanion becomes rapidly protonated and the carbon dioxide generated from the reaction no longer can recombine with the carbanion. The result is enhanced separation and forward commitment of the reaction.

![Scheme 1.4. Pyridinium-catalyzed decarboxylation of mandelylthiamin via pre-association](image)

In most discussions of decarboxylation, the effects on the departure of the carbon dioxide molecule are considered in terms of entropy with the notion that the heterolytic cleavage is favoured by the increased entropy upon separation of carbon dioxide. Due to its highly electrophilic nature and its poor solubility in water, the generation of carbon dioxide as a direct product can be problematic\textsuperscript{10}. Thus, it is possible that some decarboxylation reactions may be accelerated by making carbon dioxide less electrophilic and by increasing its solubility. Knowles proposed that the hydrate of carbon dioxide is a more feasible reaction species than carbon dioxide for biotin-dependent enzymes.\textsuperscript{10}

### 1.2 Alternatives to the direct formation of carbon dioxide

Hydrates of carbon dioxide include the various protonation states of carbonic acid. These are much more soluble in water than carbon dioxide (Scheme 1.5). The concentration of dissolved carbon dioxide in equilibrium with the atmosphere at physiological pH and temperature is 10 $\mu$M, whereas that of bicarbonate is 200 $\mu$M.\textsuperscript{10}
Although carbonate derivatives undergo hydration and exchange through nucleophilic addition and expulsion of water,\textsuperscript{11} they are less electrophilic than carbon dioxide. Thus, making them viable intermediates in decarboxylation reactions to promote forward throughput. In the reverse carboxylation reaction, entropic barriers can be overcome by utilizing these more soluble carbonate derivatives. When decarboxylation is observed in more acidic conditions, the energetically unfavourable direct formation of carbon dioxide is circumvented by the generation of protonated carbonic acid (PCA) as the immediate product of carbon-carbon bond cleavage.

**Scheme 1.5.** Different protonation states of the hydrate of carbon dioxide

![Scheme 1.5. Different protonation states of the hydrate of carbon dioxide](image)

1.2.1 Acid-catalyzed decarboxylation

A common view of decarboxylation is that carbon dioxide results directly from carbon-carbon bond-breaking regardless of the reaction conditions; however, depending on the protonation state of the carboxyl group, this is unlikely. Many examples can be found of unexplained observations of increasing rates of decarboxylation as a function of increasing solution acidity.\textsuperscript{12-22} Often these reactions are measured under conditions requiring acidity functions to correct effects due to the increased concentration of hydronium and counter ions.\textsuperscript{23}

An early observation of the acid-catalyzed decarboxylation of mesitoic acid was presented by Klages and Lickroth\textsuperscript{16}; however, any meaningful mechanistic insight was not reported until fifty years later. Schubert proposed that a water molecule is involved in the decarboxylation process and that it acts as a proton shuttle concerted with carbon-carbon bond-breaking.\textsuperscript{24} Although this mechanism leads to the observed mesitylene product, it also produces the prohibitively high-
energy protonated carbon dioxide molecule upon carbon-carbon bond cleavage and is therefore impossible (see Section 1.2.3).

Dunn and Lee\textsuperscript{12} presented the acid-catalyzed decarboxylation of pyrrole-2-carboxylic acid that follows the same pattern of increasing rate upon increasing acidity. In addition, they measured the carbon kinetic isotope effect (CKIE, $^{12}$C/$^{13}$C) whose magnitude increases in acidic solutions. This observation is consistent with a change in rate-determining step from proton-transfer to carbon-carbon bond-breaking as acidity is increased. The mechanisms presented by those authors are shown Scheme 1.6.

\begin{center}
\begin{tikzpicture}
  \node[draw, align=center] at (0,0) (a){\textbf{a}}; \node[draw, align=center] at (1,0) (b){\textbf{b}};
  \node at (0,-0.5) {$\text{H}^+$}; \node at (1,-0.5) {$\text{H}^+$};
  \node[draw, align=center] at (0,0.5) {\text{Pyrrole-2-carboxylic acid}}; \node[draw, align=center] at (1,0.5) {\text{Protonated pyrrole-2-carboxylic acid}}; \node[draw, align=center] at (0,-0.5) {\text{Pyrrole}}; \node[draw, align=center] at (1,-0.5) {\text{Protonated pyrrole}}; \node[draw, align=center] at (0,1.5) {\text{Decarboxylation of pyrrole-2-carboxylic acid producing (a) carbon dioxide or (b) protonated carbon dioxide}};
\end{tikzpicture}
\end{center}

\textbf{Scheme 1.6.} Decarboxylation of pyrrole-2-carboxylic acid producing (a) carbon dioxide or (b) protonated carbon dioxide

The decarboxylation taking place under less acidic conditions proceeds by way of the zwitterionic pre-decarboxylation intermediate (Scheme 1.6.a); however, the availability of this intermediate in solution decreases as acidity increases so that pathway (b) becomes favoured. The concentration of the neutral and singly protonated state of the substrate begin to dominate in solution. From these intermediates, it is not possible to lose carbon dioxide directly and protonated carbon dioxide is not an alternative (see Section 1.2.3).

Instead of the generally accepted dissociative mechanism, our research group has presented evidence for an associative decarboxylation where carbon-carbon bond-breaking takes place in acidic solutions. In this associative mechanism, hydration of the protonated carboxyl group
followed by a proton transfer step leads to a pre-decarboxylation intermediate that is capable of undergoing carbon-carbon bond-breaking to produce PCA, the hydrated and protonated derivative of carbon dioxide. Pyrrole-2-carboxylic acid was used by Scott Mundle as a reaction standard for carbon kinetic isotope effect measurements. From these studies, an acid-catalyzed hydrolytic decarboxylation mechanism has been outlined\textsuperscript{25,26} and supported by computational analysis\textsuperscript{27,28} (Scheme 1.7).

\[ \text{Scheme 1.7. Acid-catalyzed hydrolytic decarboxylation of pyrrole-2-carboxylic acid} \]

1.2.2 Evidence for associative mechanisms and hydrated intermediates

In order for the carbon-carbon bond-breaking step to take place, it is necessary that the mechanism proceeds by way of an intermediate that is more reactive than the reactant. It is desirable to determine the energetics of this intermediate to test if the pathway is energetically feasible. For a substrate to undergo associative decarboxylation, addition of water into the carboxyl group must occur at a rate that is kinetically competent. The associative pathway for acid-catalyzed decarboxylation has elements in common with the well-documented mechanism of ester hydrolysis (Scheme 1.8). Studying these systems provides insights into the hydration of carboxylic acids.

\[ \text{Scheme 1.8. Ester hydrolysis proceeding through a tetrahedral intermediate} \]
Since these intermediates are difficult to observe in solution as a result of their low concentration, methods have been developed for estimating the free energies of formation of these tetrahedral intermediates based on the free energies of stable analogues. Cullimore and Guthrie have estimated the difference in energy between an intermediate resulting from addition of water to an ester and the corresponding mixed ortho acid-orthoester. Their results give a good estimate of the energy for the incorporation of a water molecule into the carboxyl group of a carboxylic acid.

The rates of formation and decomposition of the tetrahedral intermediates involved in the hydrolysis of dimethyl arylphosphonates provide a model for the breakdown of intermediates from ester hydrolysis and can also be used to infer the kinetic competence of the addition and loss of water in hydrolytic decarboxylation. Kluger and Chin found that at low pH the rate of formation of the tetrahedral intermediate is rapid relative to its decomposition. By varying the pH of the medium for this two-step reaction, the hydration step could be isolated and the rate constants calculated. The calculated rate constants are on the order of $k_{obs}$ for acidic decarboxylation of pyrrole-2-carboxylic acid, establishing the kinetic competence of the hydrolytic route.

The acid-catalyzed exchange of $^{18}$O from water into the carboxyl group of benzoic acid and trifluoroacetic acid has been demonstrated. This requires hydration and supports this as a kinetically competent process where acid-catalyzed decarboxylation is observed. Warren and Williams postulated a mechanism for the decarboxylation of phosphonoformic acid via a hydrated intermediate. The final bond-breaking step would also produce PCA as the initial product prior to its breakdown in solution to carbon dioxide. This is the first proposal of a decarboxylation that produces PCA. Surprisingly, those authors assumed that the route is unique to the phosphonoformic acid system while others produce protonated carbon dioxide.

### 1.2.3 Energetics of protonated carbon dioxide and protonated carbonic acid

Reports of acid-catalyzed decarboxylation had assumed the generation of protonated carbon dioxide as the immediate product of the carbon-carbon bond-breaking step. The authors of those studies were not aware that it is a prohibitively energetic species. Work from 1990 and later established that the proton affinity of carbon dioxide is -128 kcal/mol, close to that of methane.
Guthrie derived that protonated carbon dioxide would have a $pK_a$ ranging from -31 to -39, depending on the geometry, making any reaction pathway with it impossibly high in energy.

Protonated carbonic acid (PCA) is an energetically reasonable alternative product of a decarboxylation reaction, formed from the covalent hydrate of the protonated carboxyl group under acidic conditions. Pathways showing the generation of PCA versus protonated carbon dioxide for the model decarboxylation reaction of pyrrole-2-carboxylic acid are presented in Scheme 1.9.

The proton affinity of carbonic acid (-189 kcal/mol) suggests it is roughly 60 kcal/mol more stable than protonated carbon dioxide. Olah and White first reported the preparation and characterization of PCA by low temperature NMR analysis. Using the strong acid fluorosulfuric acid in the presence of antimony pentafluoride and sulfur dioxide (FSO$_3$H-SbF$_5$-SO$_2$) they observed the $C_3$ symmetric structure reflected by the formula C(OH)$_3^+$ upon addition of carbonic acid. Due to its stability, the authors proposed the importance of PCA as a potential biological intermediate in carboxylation and decarboxylation reactions.

While PCA is the immediate product in the hydrolytic acid-catalyzed decarboxylation of carboxylic acids, it would rapidly lose a proton to water to generate neutral carbonic acid. The
breakdown of carbonic acid upon proton migration leads to the observed carbon dioxide reaction product (Scheme 1.10).\textsuperscript{40}

Scheme 1.10. Decomposition of PCA produces carbon dioxide, water and a proton

1.3 Carbon kinetic isotope effects in decarboxylation

Identifying the rate-determining step in a decarboxylation reaction is critical for mechanistic understanding. One technique that is used for isolating this step is measurement of the kinetic isotope effect (KIE) on the reaction. A KIE occurs when isotopically substituted molecules react at different rates. Interpretations of these rate differences allow for mechanistic understanding. In the case of carbon-carbon bond-breaking, the rate for $^{12}\text{C}$ and $^{13}\text{C}$ are measured simultaneously to provide data needed to calculate the carbon kinetic isotope effect (CKIE). The isotope effect arises from differences in the zero point energy of the carbon-carbon single bond undergoing bond cleavage (Figure 1.1) and any differences in energies of transition states. Where effects on the scissile bond are studied, the ratio is a primary CKIE.
Figure 1.1. Reaction profile showing differences in the activation energy ($E_a$) for bond-breaking between $^{12}$C-$^{12}$C and $^{12}$C-$^{13}$C

Where carbon-carbon bond-breaking is involved in the rate-limiting transition state, a non-unity CKIE will be observed. Cleavage of the bond between $^{12}$C-$^{13}$C requires more energy compared to $^{12}$C-$^{12}$C, primarily due to the lower zero-point energy of the heavier isotope. This discrepancy in activation energy ($\Delta E_a$) is reflected in the observed rate of the reaction, with $k_{\text{obs}}^{13}$C $< k_{\text{obs}}^{12}$C.

CKIEs on decarboxylation reactions are among the most well-studied isotope effects, both in nonenzymatic and enzymatic systems. Decarboxylation reactions are attractive for two reasons: the heavy-atom involved in the bond-breaking step comes from a single carbon-carbon bond and the carbon dioxide produced from this bond-breaking step can be directly detected (see Section 1.4). Early studies presented the theory required to calculate the fractionation of carbon dioxide produced upon decarboxylation. When carbon-carbon bond-breaking is rate-determining, the carbon dioxide will be initially enriched in the lighter $^{12}$C isotope. Measurement of the ratio of $^{12}$C to $^{13}$C in the carbon dioxide at early stages of the reaction yields a CKIE. As the reaction reaches
completion, the $^{12}$C to $^{13}$C ratio in the carbon dioxide corresponds to that of the starting material. Bothner-By and Bigeleisen published a modification of the Rayleigh equation (1.1) that is used for calculating the CKIEs for decarboxylation reactions.42

$$k^{12}/k^{13} = \log (1 - f)/\log [1 - f(N_{x}/N_{x_0})]$$  \hspace{1cm} (1.1)

Parameters of the equation include the observed first-order reaction coefficients for the light ($k^{12}$) and heavy ($k^{13}$) isotopes of carbon, the abundance of each isotope ($N$) and the reaction progression ($f$). The magnitude of the resulting CKIE provides a means of characterizing the operative decarboxylation mechanism.

### 1.3.1 Magnitude and meaning of carbon kinetic isotope effects

The magnitudes for CKIEs are much smaller than the values for hydrogen/deuterium KIEs ($k_H/k_D$). This arises from the difference in reduced mass between the isotopic pairs. While deuterium has twice the mass of hydrogen, $^{13}$C is less than ten percent greater in mass than $^{12}$C. CKIEs for room temperature decarboxylation have been reported in the range of 1.03-1.06 when carbon-carbon bond-breaking is entirely rate-determining.41,43 Factors that attenuate the observed CKIE are of interest from both fundamental and experimental standpoints.

The effects on the magnitudes of KIEs are usually explained in terms of proton transfer reactions. In these cases, a proton undergoes transfer from a donor (A) to an acceptor (B) (Figure 1.2). If this process is rate-determining, a primary KIE is observed.

![Figure 1.2. Proton transfer from a donor (A) to an acceptor (B)](image-url)
Differences in the magnitude of $k_H/k_D$ reflect differences in the degree of bond-breaking in the transition state for this transfer reaction, leading to early or late transition states. An early transition state means that the proton is mostly bonded to the donor molecule while a late transition state has more bonding between proton and acceptor and resembles the product of this step. When proton transfer is symmetric between donor and acceptor, the KIE reaches a maximum value.\(^{44}\) Often, this same terminology is used to describe the transition states for cleavage of the carbon-carbon bond in decarboxylation reactions; however, bond-breaking here is purely dissociative as there is no acceptor molecule. Approximating the relationship between the magnitude of the CKIE and the transition state structure (bond-order) has led to results that are inconsistent for a variety of systems and this analysis does not often apply to multistep pathways.\(^{45-47}\) An alternative approach to investigate the magnitude of CKIEs comes from enzyme steady state theory.\(^{48-50}\) This permits an extension to mechanisms with multiple steps. Under these circumstances decarboxylation reactions would have a similar intrinsic maximum value for bond-breaking where observed variations in the magnitude arise from the reversible steps surrounding carbon-carbon bond cleavage. For enzymatic systems, heavy-atom isotope effects are typically measured by competitive techniques and this leads to the observed results being effects on $V_{\text{max}}/K_{m}$. The outcome is that these CKIEs do not always provide information on the rate-limiting step of the overall reaction. Rather, these CKIEs reflect steps leading up to and including the first irreversible step in the reaction. The preceding steps involve entropic factors such as substrate binding.\(^{41}\) In order to better understand how the magnitude of the CKIE is affected, separation of the substrate from the enzyme is necessary. To this end, small molecules of interest can be studied in solution to determine how the observed CKIE changes without the interference of entropic contributions from active site binding.

One example of such a study is the irreversible decarboxylation of substituted carboxybenzisoxazoles (Scheme 1.11).\(^{51}\)

![Scheme 1.11. Decarboxylation of 5-nitro-3-carboxybenzisoxazole](image)
The rate of the reaction is dramatically accelerated in the presence of non-polar solvents as well as with a catalytic antibody that mimics the hydrophobic active site of enzymes. It is believed this acceleration is the result of the charge-delocalized transition state being stabilized in organic solvents while the ground-state carboxylate is destabilized upon removal of hydrogen-bonding interactions with water. This results in a dramatic decrease in the activation energy required for the reaction. Lewis and coworkers compared the CKIE of the decarboxylation reactions in the presence of water, water/dioxane solvent and with a catalytic antibody. The isotope effects under all three conditions were similar (~1.046) and indicated that carbon-carbon bond cleavage is fully rate-determining. Despite the dramatic acceleration in the observed rates of the reaction in the presence of non-polar solvents, the unchanging CKIE indicates an irreversible, one-step reaction. In this case, there are no steps surrounding carbon-carbon bond-breaking that can attenuate the observed CKIE. Thus, its value is close to the values seen for intrinsic CKIEs.\cite{41,43}

Where changes in the magnitude of CKIEs are observed under changing reaction conditions, they can be explained in terms of the concept of commitment to catalysis. Reversibility in the carbon-carbon bond-breaking step can attenuate the reaction throughput and be reflected in the CKIE where this bond cleavage is rate-limiting. Such a system is apparent for the pyridinium-catalyzed decarboxylation of mandelylthiamin (Scheme 1.12).\cite{4,8,52} Upon cleavage of the carbon-carbon bond, the pre-associated pyridinium catalyst allows for rapid proton transfer and increased throughput of the reaction and leads to an increase in the observed CKIE from $\text{CKIE}_{\text{uncat}} = 1.058 \pm 0.0005$ to $\text{CKIE}_{\text{cat}} = 1.060 \pm 0.0005$.\cite{4} In the absence of pyridinium ions, the nascent carbanion and carbon dioxide are more likely to recombine.

Scheme 1.12. The decarboxylation of mandelylthiamin is accelerated by pre-association catalysis with pyridinium ions.
The significant change in CKIE between the catalyzed and uncatalyzed pathway is the result of changing the reversibility in the carbon-carbon bond-breaking step. Much like the internal return observed for proton transfer reactions, generation of the electrophilic carbon dioxide next to the nucleophilic carbanion can lead to considerable recombination. Cram and Haberfield\textsuperscript{3} presented evidence for the internal return of carbon dioxide that affects the stereochemistry of decarboxylation reactions. This idea of recombination is supported by calculations for the decarboxylation system of orotidine-5\textsuperscript{′}-monophosphate decarboxylase. In this system, there is essentially no barrier to the reverse reaction between carbon dioxide and the orotidine carbanion.\textsuperscript{2}

When the lifetime of the carbanion resulting from carbon-carbon bond cleavage is similar to that of diffusion, the observed CKIE for the reaction is impacted by the ratio of $k_{-1}/k_2$ (Scheme 1.12). Tittmann and co-workers\textsuperscript{54} have provided evidence that intermediates of thiazolium compounds and enzymatic thiamin derivatives have localized carbanion character. In order to overcome internal return, enzymes could utilize Brønsted acids to promote the reaction.

Further evidence for carbanion character comes from the theoretical work of Bernasconi. His Principle of Non-perfect Synchronization states that reaction progress and extent of delocalization of residual charge are independent.\textsuperscript{55} This can be used to understand how decarboxylation reaction pathways have unlinked carbanion character and carbon dioxide generation, allowing carbanions to form. Additional insights have come from Saunders and co-workers\textsuperscript{56} in their comparison of concerted E2 mechanisms with stepwise E1\textsubscript{CB} pathways. Those authors suggest that the E2 reactions are in fact very non-synchronous, thereby avoiding the energetic penalties associated with a truly concerted process.

\textbf{1.4 Methodology of measuring carbon kinetic isotope effects by headspace analysis}

Measuring isotope effects typically can require enrichment of the substrate molecule with the heavy atom isotope under study. In the case of carbon kinetic isotope effects (CKIEs), enrichment with the stable $^{13}\text{C}$ atom takes place (enrichment with $^{11}\text{C}$ and $^{14}\text{C}$ is also possible). Compared to natural abundance samples, enriched samples require less precise instrumentation; however, the desired materials require site-specific isotope incorporation and enriched samples are much more subject to errors from contamination as compared to natural abundance samples.\textsuperscript{41} Thus, where
possible, analytic methods that take advantage of the natural abundance of the heavy isotope are very desirable. In the case of isotope ratio mass spectrometry (IRMS), the heavy-to-light isotope ratio is simultaneously measured with no need for isotope enrichment. The inlet system is designed to alternate the flow into the ionization chamber between a sample and a standard.\textsuperscript{57} The coupling of IRMS instruments to gas chromatography (GC)\textsuperscript{58} and liquid chromatography (LC)\textsuperscript{59} techniques has further extended the detection abilities to a variety of reaction components with the carrier gas allowing a much more dilute sample to flow and be detected.

Early detection methods for the carbon dioxide generated from decarboxylation reactions were cumbersome. Owing to its high freezing point, carbon dioxide can be isolated via a vacuum line for isotopic analysis; however, this leads to low sampling rates and high substrate concentrations being required for detection. Fortunately, for decarboxylation reactions the gaseous carbon dioxide product is amenable to direct detection. By using gas-tight pressure-locked syringes, the headspace of sealed reaction bottles can be sampled and injected directly into the IRMS for analysis.\textsuperscript{60} Further technique development in this area has led to a new approach where both reaction rate and CKIE are measured simultaneously via continuous detection of carbon dioxide generated from decarboxylation reactions. This methodology can be extended to any gas-producing or gas-consuming reaction. In reactions where more than one gaseous product is formed or consumed, further modification to the method would be required.

1.4.1 Continuous measurement of reaction kinetics and isotopic ratio

The following publication outlines the methodology I helped develop for continuous headspace sampling of non-isobaric reactions for isotopic analysis. As a reliable and well-studied reaction, acid-catalyzed decarboxylation of pyrrole-2-carboxylic acid was selected for the study. An evaluation of the experimental error in the calculated isotope values (reported as $\varepsilon$) is also outlined. “Pressure-monitored headspace analysis combined with compound-specific isotope analysis to measure isotope fractionation in gas-producing reactions” is reproduced with permission from Scott O. C. Mundle \textit{et al.}, \textit{Rapid Communications in Mass Spectrometry} 2013, 27, 1778–1784. Copyright 2013 John Wiley & Sons, Ltd.
Isotope ratio mass spectrometry (IRMS) measures the natural abundance of stable isotopes in small molecules with great accuracy and sensitivity. Along with the theoretical framework of kinetic isotope effects (KIE) that account for the different reactive patterns of isotopes in chemical reactions and equilibria, significant advancement in the interpretation of biological, environmental, and geological processes has been achieved.\(^1\)\(^2\) Although other analytical approaches can measure reactive differences between isotopes of artificially enriched or radioactively labeled substrates, IRMS is the only technique capable of highly precise measurements at natural abundance, and on heavier nuclei (\(^{13}\)C/\(^{12}\)C, \(^{15}\)N/\(^{14}\)N, \(^{18}\)O/\(^{16}\)O).

In the IRMS measurement of the \(^{13}\)C/\(^{12}\)C isotope ratio of CO\(_2\), early work focused on the collection and measurement of CO\(_2\) produced directly from chemical\(^3\)\(^4\) and enzymatic\(^5\)\(^6\) decarboxylation reactions. The reactive patterns of isotopes can generally be modeled using the Rayleigh distillation \((R/R_0 = e^{-f/1000})\) equation.\(^2\) Based on this equation, isotope fractionation can be quantified using enrichment factors \((\varepsilon)\), fractionation factors \((\alpha\text{, where } \varepsilon = 1000(\alpha - 1))\), or carbon kinetic isotope effects (CKIE = \(\alpha^{-1}\)).\(^7\) For decarboxylation, these parameters are typically calculated by measuring the \(^{13}\)C/\(^{12}\)C isotope ratio at two time points from individual sacrificial samples that are quenched in acid to terminate the reaction after a specific time interval.\(^8\) The first point is obtained at 'low conversion' (ideally \(<10\%\) completion) and the second point at 'complete conversion' \((100\%\) completion).\(^9\) Reaction progress is determined by quantifying the produced CO\(_2\) with a manometer that is connected to the vacuum line used for purification, and the isotope ratios are measured using IRMS. A major disadvantage of this traditional approach is the large amount of substrate necessary for analysis, where samples containing 1 mg of produced CO\(_2\) are typically considered the lower reliable detection limit.\(^10\)

A significant improvement in IRMS technology has been achieved by combining gas chromatography and a combustion oven to the front-end of the mass spectrometer (Compound-Specific Isotope Analysis, CSIA) providing a means to isolate and convert organic materials directly into CO\(_2\) (via combustion).\(^11\) This dramatically reduces the
quantity of substrate necessary for analysis and allows measurement of single analytes from mixtures that are separable via gas chromatography. Slater et al.[12] developed an approach using CSIA to study the degradation of volatile chlorinated organic contaminants in groundwater using headspace analysis. In this approach, the gas phase above an aqueous solution in a ‘closed system’ is sampled using a gas-tight syringe and the \( ^{13}\text{C}/^{12}\text{C} \) isotope ratio is determined using CSIA. The volume of sample removed from the headspace is typically replaced with an inert gas to maintain a consistent pressure in the headspace throughout the experiment. Once equilibrium between the headspace and solution phase is established, enrichment factors (\( \varepsilon \) values) are determined by measuring the change in concentration and \( \delta^{13}\text{C} \) value of the reactant as the reaction progresses. Although the reaction is taking place in the solution phase, if partitioning between the aqueous phase and gas phase is fast compared with the chemical reaction taking place, the isotopic composition of the reactant in solution will be the same as the isotopic composition observed in the headspace throughout the reaction. In these cases, the \( \varepsilon \) value can be determined by following the reaction in either the solution or the headspace and is appropriate for any compound for which it has been demonstrated that there is no significant fractionation due to equilibrium partitioning between the headspace and solution phase.[12] The entire reaction can be followed using a single reaction vessel with multiple sampling events, dramatically decreasing the amount of material required to determine an \( \varepsilon \) value.

This technique is now in widespread use and considerable efforts have been applied to improve the precision of the isotopic measurements. Significantly, the largest source of experimental error typically results not from determination of the \( ^{13}\text{C}/^{12}\text{C} \) ratio, but from uncertainty in the reaction progress (\( f \)) which arises entirely from the x-axis of the Rayleigh equation (\( R/R_0 = f/100\% \)) used to calculate \( \varepsilon \) values.[8] The reaction progress is commonly determined for slow reactions by sampling the headspace sequentially for CSIA and for compositional analysis using gas chromatography, where the GC error is typically \( <5\% \) of the peak area.[12] Over the course of an experiment, as the headspace is sampled and an inert gas is injected to replace the volume of gas removed for CSIA and GC compositional analysis, the composition of the headspace is depleted in the target substrate. This depletion can be approximated by accounting for the dilution of the headspace caused by the inert gas:

\[
\text{Area}_{\text{CORR}} = \left( \text{Area}_{\text{HS}} + \text{Area}_{\text{DIL}} \right) / \text{Area}_{\text{GC}}
\]

where \( \text{Area}_{\text{HS}} \) is the total volume of headspace in the closed reaction vessel, \( \text{Area}_{\text{DIL}} \) is the volume of inert gas used to maintain consistent headspace pressure, \( \text{Area}_{\text{GC}} \) is the peak area obtained directly from GC, and \( \text{Area}_{\text{CORR}} \) is the peak area corrected for dilution of the headspace with an inert gas. This approach is only valid if the pressure remains constant throughout the experiment. Reactions that produce or consume volatile compounds or gas will lead to a net change in the pressure within the headspace. Therefore, as the pressure changes during the course of the experiment, the concentration of substrate within a given volume of gas sampled will not be constant. Significant error in determining the reaction progress can occur as the concentration of substrate within a given volume sampled from the headspace can be higher as a result of increased pressure inside of the closed system.

Decreasing the experimental error associated with the reaction progress (\( f \)) term of the Rayleigh calculation will improve confidence intervals for reported \( \varepsilon \) values; however, another area of concern arises from the statistical evaluation of \( \varepsilon \) values. The magnitude of statistical confidence intervals does not typically account for the experimental error in both the [\( \text{CO}_2 \)] and the \( ^{13}\text{C}/^{12}\text{C} \) inputs into the Rayleigh calculation. The statistical approximation of the confidence interval is typically based directly on the data point.[13] This can lead to the appearance of artificially small confidence intervals that become problematic when small differences in reported \( \varepsilon \) values are used to elucidate different processes or pathways.

We report: (1) a new experimental approach that combines the use of headspace and CSIA to accurately measure both the \( ^{13}\text{C}/^{12}\text{C} \) isotope ratios and the reaction progress for \( \text{CO}_2 \)-producing reactions and allows simultaneous determination of the \( \varepsilon \) value (or CKIE) and the rate constant for the reaction. This method can be applied to any process that produces or consumes a volatile organic compound or gas. (2) A new approach to evaluate the magnitude of statistical confidence intervals associated with \( \varepsilon \) values to better account for experimental variability in the Rayleigh calculation.

**EXPERIMENTAL**

**Chemicals**

Pyrrrole-2-carboxylic acid and perchloric acid (70%) were used as purchased. All acid solutions were diluted with distilled water.

**Kinetics of decarboxylation: UV-Vis spectrometry**

The rate of decarboxylation of pyrrole-2-carboxylic acid was measured for samples dissolved in a 26% (w/w) solution of perchloric acid. All measurements were carried out in solutions maintained at 25 °C. The reaction was monitored by the decrease in absorbance at 262 nm with a UV-Vis spectrometer with a temperature-controlled cell compartment (within ±0.1 °C). Data were collected with an interfaced computer and the observed first-order rate constants were calculated from non-linear regression fitting to the integrated first-order rate expression.

**Pressure-monitored headspace analysis**

CSIA and headspace analysis were used to monitor decarboxylation reactions that lead to a net change in pressure in closed systems. These reactions can generate \( \text{CO}_2 \) *in situ* with a significant degree of fractionation, providing an ideal model of a non-isobaric reaction that reflects product formation. An approach was developed to determine the reaction progress by monitoring the change in headspace pressure directly using a digital pressure gauge attached to a 60 mL serum bottle modified with a glass sidearm (Fig. 1). The analytical error associated with the pressure gauge was 0.25% of the reported pressure.
CO2 remaining, and enrichment factor equal to 1000 (\(\delta^{13}C\) values are converted into abundance ratios using \(\delta^{13}C = (N_{\text{sample}} - N_{\text{standard}})/N_{\text{standard}}\)). A plot of \(\log [1 + (N_{\text{sample}} - N_{\text{standard}})/N_{\text{standard}}(1 - f)]\) versus \(-\log (1 - f)\) generates a straight line with a slope of \(\varepsilon/1000 = (x - 1)\), where \(x = \text{CKIE}^{-1}\).

**RESULTS**

**Measuring reaction rates**

The observed rate constant for the decarboxylation of pyrrole-2-carboxylic acid in 26% (w/w) perchloric acid determined independently by UV-Vis spectroscopy (\(k_{\text{obs}} = 3.2 (\pm 0.1) \times 10^{-4}\) s\(^{-1}\)) corresponded well with the observed rate constants measured by the pressure method, which ranged between 3.1 \(\times 10^{-4}\) s\(^{-1}\) and 3.3 \(\times 10^{-4}\) s\(^{-1}\) (Fig. 2). The acidity of the solution combined with vigorous stirring ensured that the CO2 produced in the aqueous phase partitioned rapidly into the headspace. Although the acidity of the solutions used in this study favors partitioning of the CO2 to the headspace, this approach has been applied in less acidic solutions to show that the retention of CO2 in solution (from addition of water to form bicarbonate) is not significant up to pH 4 and hence this approach should be feasible up to that acidity.

**Compound-specific isotope analysis**

The \(\delta^{13}C\) value of the CO2 produced by decarboxylation increased from ~56.5% to ~26.2% over the course of the reaction. This is a classic enrichment trend producing a progressively more \(^{13}C\)-enriched \(\delta^{13}C\) value in the CO2 product as the reaction proceeds to completion (Fig. 3). Table 1 shows the \(\varepsilon\) values for individual experimental replicates with the corresponding \(r^2\)-values and 95% confidence intervals. The \(\varepsilon\) values were calculated from the derivation of Bothner-By and Bigeleisen of the Rayleigh equation for in decarboxylation reactions, where \(k_H\) and \(k_L\) are the observed first-order rate coefficients for reaction of the heavy and light isotopes, respectively. In this case, \(R\) and \(R_0\) are replaced with \(N_x\) and \(N_{x0}\) (\(\delta^{13}C\) values are converted into abundance ratios using \(\delta^{13}C = (N_{\text{sample}} - N_{\text{standard}})/N_{\text{standard}}\)).

\[
R/R_0 = \frac{f^{(x/1000)}}{f^{(x - 1)}} = f^{(x - 1)},
\]

\(R/R_0\) = \(f^{(x/1000)}\) = \(f^{(x - 1)}\), a system with low natural abundance of the heavy isotope (1 + R)/(1 + R0)\approx 1, where R and R0 are the \(^{13}C/^{12}C\) ratios of CO2 at time t and time zero respectively, f is the fraction of CO2 remaining, and \(\varepsilon\) (denoted in parts per thousand) is the enrichment factor equal to 1000(x - 1), where \(x = \text{CKIE}^{-1}\).

Bothner-By and Bigeleisen reported a derivation of this equation, (\(N_f/N_{x0}\)) = \(1 - (1 - f)(k_H/k_L)\), for determining the isotope fractionation for the accumulated CO2 produced in decarboxylation reactions.

**Figure 1.** Reaction vessel used to determine the enrichment factors for decarboxylation using pressure-monitored headspace analysis (recirculating water bath removed for purpose of the photograph).

**Figure 2.** Pressure (Torr) versus time (seconds) for the evolution of CO2 in the decarboxylation of pyrrole-2-carboxylic acid in 26% (w/w) perchloric acid at 25 °C (\(k_{\text{obs}} = 3.2 \times 10^{-4}\) s\(^{-1}\)) (open circles), \(k_{\text{obs}} = 3.3 \times 10^{-4}\) s\(^{-1}\) (open triangles), \(k_{\text{obs}} = 3.1 \times 10^{-4}\) s\(^{-1}\) (open squares). Different symbols denote individual experimental runs. The error bars are smaller than the plotted symbols.
The overall range of the individual replicate runs is between 32.9% to 80% reaction completion. The uncertainty in the values calculated for individual experiments and combined data at intervals from 20% to stages (>30%). The uncertainty in the ε values above 30% increases for individual bottles and, as has been seen, can be of the order of 1%. Combining all the data into a single regression analysis still produces very consistent ε values based on reaction progress intervals at least between 20% and 70%. There appears to be a slight deviation for the fit that includes data points at 80% reaction completion.

Table 1. Statistical analysis of calculated ε values for individual experiments and combined data at intervals from 20% to 80%, where n is the number of data points used in the calculation

<table>
<thead>
<tr>
<th>Reaction progress</th>
<th>Run 1</th>
<th></th>
<th>Run 2</th>
<th></th>
<th>Run 3</th>
<th></th>
<th>Combined data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ε (%)</td>
<td>± (95%CI)</td>
<td>n</td>
<td>ε (%)</td>
<td>± (95%CI)</td>
<td>n</td>
<td>ε (%)</td>
</tr>
<tr>
<td>80%</td>
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<td>0.6</td>
<td>15</td>
<td>-34.4</td>
<td>0.6</td>
<td>16</td>
<td>-33.9</td>
</tr>
<tr>
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<td>0.7</td>
<td>13</td>
<td>-33.4</td>
<td>0.4</td>
<td>13</td>
<td>-33.8</td>
</tr>
<tr>
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</tr>
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</tr>
<tr>
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<td>7</td>
<td>-34.1</td>
<td>0.7</td>
<td>7</td>
<td>-33.2</td>
</tr>
<tr>
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<td>n &lt; 5</td>
<td></td>
<td>n &lt; 5</td>
</tr>
</tbody>
</table>

r² > 0.99 for all individual fits and the combined fits used to calculate ε values.
FIGURE 5. Calculated ε values (%) versus reaction progress (%) from Table 1 for individual runs (open symbols) and all data combined (solid diamonds). Error bars represent 95% confidence interval. This type of statistical comparison of calculated ε values over reaction progress intervals demonstrates the effects of variation caused in later data points. This suggests that there is larger uncertainty in ε values than expressed based on the 95% confidence interval alone.

DISCUSSION

Improvement in the total uncertainty of an ε value can be achieved by reducing the experimental error in the reaction progress (f) term of the Rayleigh calculation for processes where the depletion of reactant, or the accumulation of product is monitored over the course of a reaction. Monitoring the depletion of a reactant is advantageous since equilibrium between the headspace and aqueous phase in a closed system can be achieved prior to initiating the reaction. For rapidly equilibrating materials, the distribution of stable isotopes in the headspace and aqueous phase along with the proportional change in concentration over time will give a good representation of the entire system.[22] However, if equilibration between the headspace and the aqueous phase is not sufficiently fast, the magnitude of the ε value will be suppressed to the extent that phase transfer becomes partially rate-limiting.[23]

Measuring ε values based on the accumulation of product is more complicated. Since the material being monitored is initially present entirely in the solution phase, it must be rapidly transferred into the headspace for analysis. Any delay in the transport of material to the headspace will ultimately result in mixing of the instantaneously formed product, with later stage products, leading to a suppression of the observed ε value. In addition, as the system equilibrates over the course of the reaction, based on the specific Henry’s Law constant of the product being formed, it will lead to an unequal distribution of material in the headspace and dissolved in the aqueous solution. In such a case, the reaction progress must be calculated from the total concentration in both the headspace and the aqueous phase, which will necessarily increase the experimental error associated with that measurement and ultimately the ε value on which it is based. In addition, substrate removal and headspace dilution from the inert gas used to maintain pressure in these closed systems, as discussed earlier, must be corrected in the reaction progress (f) term of the Rayleigh calculation. This increases the total uncertainty and must be considered in all cases, both isobaric and non-isobaric. In either case, the magnitude of the potential error associated with these calculations is greatest when large volume samples are retrieved from small headspaces. Since pressure-monitored headspace analysis determines the reaction progress (f) directly from the evolution of gas from the solution to the headspace, it completely eliminates the need to account for substrate removal and headspace dilution.

Cryogenic separation of CO2 on a vacuum line using sacrificial samples is still a standard approach to experimentally determine ε values for decarboxylation reactions.[9,24] Determining the reaction progress on a vacuum line requires extensive sample manipulations that can produce significant experimental error. CSIA has provided a significant improvement through decreased sample volume requirements and sample throughput. However, headspace analysis for CSIA cannot account for the pressure increase from CO2 produced over the course of the reaction. Measuring the reaction progress directly from the pressure change in the headspace over the course of the reaction eliminates uncertainty in the [CO2] measurement and the reaction progress parameter for the Rayleigh calculation. It also dramatically reduces the amount of substrate needed to produce reliable ε values. For example, in this work accurate ε values have been successfully achieved in individual experiments using 15 mg of pyrrole-2-carboxylic acid and reactions have been successfully monitored with as little as 6 mg of this substrate.[25] The traditional approach using sacrificial samples typically requires a minimum of 150 mg of substrate for a two-point determination for a single ε value calculation.[9] This approach also permits simultaneous determination of the rate constant obtained directly from the pressure change.

Pressure effects from co-produced volatile compounds and gases

Decarboxylation is an example of a reaction where the product being monitored is responsible for the change in pressure observed in the closed system. The effects of pressure must also be considered in other cases where a co-produced product generates a pressure change. For example, microbial microcosms involved in the isobaric degradation of a contaminant with methanogens can also produce a significant quantity of methane that can cause an increase in the pressure of the headspace.[26] This change in pressure will produce a disparity between the pressure in the experimental microcosm and the compositional standards used to determine the reaction progress using GC. In these cases, pressure-monitored headspace analysis can provide an approach to monitor the change in headspace pressure during the experiment, which can be used to provide an independent kinetic measure of the co-produced species and an approach to more accurately calculate the concentrations of all species being degraded or produced.

Calculating ε values that account for the magnification of error at later stages of the reaction

Bigeleisen and Allen[8] reported that the error in ε values arising from the error associated with determining the reaction progress is magnified at later stages of the reaction. They estimated that a 0.5% error in the reaction progress in the region above 80% reaction completion (or <20% starting
material remaining) will produce an error of 4–7% or larger in the \( \varepsilon \) value. This is consistent with the scatter observed at >80% reaction completion in Fig. 4(A). With our approach, reaction progress could not reliably be determined to a precision greater than \( \pm 5\% \) in the region above 80% conversion. Based on Fig. 5, data points beyond 75% reaction completion begin to have a greater impact on the calculation of the \( \varepsilon \) values; however, the \( \varepsilon \) values including all data points up to 80% reaction completion still fall within the calculated 95% confidence intervals for every interval except 30% and 60% where there is only a 0.1% disparity between the confidence intervals. As the reaction approaches completion, the increment of change in the concentration of substrate \( \text{con} \) is not necessarily re.

95% \( \text{con} \) up to 80% reaction completion still fall within the calculated conversion. Based on Fig. 5, data points beyond 75% reaction progress could not reliably be determined to a precision greater than \( \varepsilon \) values that arise from data points at later stages of the reaction are a direct indication that the error in the determined reaction progress is having a significant effect on the calculated \( \varepsilon \) value. This phenomenon is not necessarily reflected in the correlation coefficient \( (r^2) \) and can produce artificially accurate confidence intervals. This was avoided by recalculating the \( \varepsilon \) values on shorter reaction progress intervals across the entire reaction. This revealed the additional variation in the calculated \( \varepsilon \) values that arose from the larger uncertainty in data points at the end of the reaction progress (Fig. 5). This procedure is important when attempting to interpret degradation pathways based on different \( \varepsilon \) values\,[27,28] masking effects\,[22,29,30] and is especially important in cases with few data points or more scatter at later stages of the reaction\.[26,31]

If the range of \( \varepsilon \) values calculated on shorter reaction progress intervals is larger than the 95% confidence interval based on all the data points, the total error associated with this \( \varepsilon \) value is probably greater than is predicted by the statistical analysis. Second, there is interdependence in the data points within individual experimental replicates. Although increasing the number of data points used to calculate an \( \varepsilon \) value for an individual replicate will generally lead to a smaller 95% confidence interval, it does not provide any insight into the total variability of the experiment. The total uncertainty for an experiment can only be reasonably estimated from an \( \varepsilon \) value calculated by combining all the data from multiple replicates with overlapping confidence intervals into a single regression analysis.

CONCLUSIONS

The use of pressure-monitored headspace analysis and CSIA is an accurate, robust approach to determine isotope fractionation for reactions that produce or consume gas. Since this method does not require compensation for substrate removal and headspace dilution during sampling, it minimizes the propagation of experimental error over the course of the reaction. This error has the greatest impact in systems that require large samples from small headspace volumes and is amplified in cases where the pressure is changing in the headspace.

To address the assessment of total uncertainty associated with statistically calculated \( \varepsilon \) values, we have outlined a recommendation for processing the data via a rigorous assessment of error by examining the linearity of the fits over shorter reaction progress intervals. An accurate assessment of the uncertainty associated with \( \varepsilon \) values is necessary to ensure that reported small differences in these values are truly the result of the processes that control them and not equivalent values that appear distinct as a result of inadequate assessment of experimental error.

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REFERENCES


1.4.2 Headspace analysis using sacrificial reaction vials

For decarboxylation systems requiring elevated temperature and volatile acid solutions, a variation of the method is used (Chapter 2). Headspace analysis of the carbon dioxide produced from the reaction takes place using sacrificial reaction vials. This allows for the volatile acid to be quenched and cooled prior to headspace analysis. Separate bottles correspond to different time points along the course of the reaction (between 10-50% reaction time, initially determined by the UV-visible kinetic rate data). These are compared to dedicated endpoint bottles in order to confirm the reaction progression. Endpoint bottles are allowed to react for at least ten half-lives to ensure the isotopic ratio reflects that of the completed reaction.

1.5 Purpose of thesis

An unchanging magnitude of the CKIE for decarboxylation can be explained when the reaction takes place by a single-step where carbon-carbon bond-breaking is fully rate-determining. In this scenario, the observed value is close to the intrinsic value due to the lack of steps surrounding carbon-carbon bond-breaking that can attenuate the observed KIE. When carbon-carbon bond-breaking is subject to reversion, pre-association catalysis can describe the changes of the observed CKIE with reaction conditions. Here, localization of charge and competing rates of diffusion and recombination processes play key roles.

One such system that has yet to be studied is how the effect of carboxyl substitution influences the observed CKIE for decarboxylation. Under the same conditions, it is expected that a common intrinsic CKIE would operate. However, the importance of the energetics of pre-decarboxylation intermediates remains unknown. Integral to this study is choosing a system that is robust and can be modified for differential substitution. By examining the effects on the observed CKIEs and rates of reaction, fundamental insights can be obtained for decarboxylation reactions with applications to studies of chemical, environmental and biological systems.

The intention of the study is not to change the perception of decarboxylation from a reaction that irreversibly produces carbon dioxide to one where this is unlikely. Rather, the goal is to show that decarboxylation reactions fall on a spectrum of changing mechanisms that depend not only on the
reaction conditions but also on the structure, energetics and reactivity of the intermediates before and after the carbon-carbon bond is cleaved.
Chapter 2
Decarboxylation of positional isomers: indole- and pyrrole-carboxylic acids

Aromatic compounds undergo acid-catalyzed decarboxylation reactions but the mechanism had been puzzling as reports in the literature directly or indirectly implicated the formation of protonated carbon dioxide, which is an energetically inaccessible species ($pK_a < -30$). Yet, the reported reactions are certainly subject to specific acid catalysis in highly acidic solutions. When Scott Mundle in our group was evaluating the magnitude of carbon kinetic isotope effects (CKIEs), he compared his measured CKIE against the reported CKIE for the acid-catalyzed decarboxylation of pyrrole-2-carboxylic acid as his validation standard. Since the acid catalysis was indeed a correct observation and protonated carbonic acid (PCA) had been observed and is much less acidic than protonated carbon dioxide, it was proposed that the reaction of pyrrole-2-carboxylic acid in acid produces PCA. This requires that water as well as a proton combine with the reactant prior to carbon-carbon bond cleavage. The mechanism proposed by Mundle and Kluger produces the ring-protonated hydrated intermediate that releases PCA and pyrrole (Scheme 2.1).

Scheme 2.1. Pyrrole-2-carboxylic acid undergoes acid-catalyzed decarboxylation

This is an interesting mechanism with important general implications for reactions that effectively promote decarboxylation and for their microscopic reverse where PCA would serve as an electrophile. My work extends these initial efforts to a set of isomeric compounds (Figure 2.1) whose differential reactivity and variation in observed CKIE provide the key to understanding the nature of the intermediates and rate-controlling features.


2.1 Kinetics of decarboxylation

2.1.1 Indole-carboxylic acids

The following publication presents my kinetic study for the isomeric pair of indole-2-carboxylic acid and indole-3-carboxylic acid. The reactions are characterized by rate law analysis and insights into the different reactivity between isomers are presented. “Protonated Carbonic Acid and Reactive Intermediates in the Acidic Decarboxylation of Indolecarboxylic Acids” is reproduced with permission from Adelle A. Vandersteen et al., J. Org. Chem. 2012, 77, 6505–6509. Copyright 2012 American Chemical Society.

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Figure 2.1. Positional isomers indole-3-carboxylic acid (1), indole-2-carboxylic acid (2), pyrrole-3-carboxylic acid (3), and pyrrole-2-carboxylic (4)
**Protonated Carbonic Acid and Reactive Intermediates in the Acidic Decarboxylation of Indolecarboxylic Acids**

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### ABSTRACT:

Elucidation of the mechanism for decarboxylation of indolecarboxylic acids over a wide range of solution acidity reveals the importance of protonated carbonic acid (PCA) as a reaction intermediate. In concentrated acid, the initial addition of water to the carboxyl group of the indolecarboxylic acid leads to a hydrated species that is capable of releasing PCA upon rate-determining carbon—carbon bond cleavage. The overall process is catalytic in water and acid, implicating PCA as a potential carboxylating reagent in the microscopic reverse reaction.

### INTRODUCTION

Decarboxylation reactions normally involve the direct formation of CO₂ by a stepwise electrophilic substitution mechanism in which a proton replaces a carboxyl group after formation of CO₂. The accelerated decarboxylation of certain aromatic compounds in concentrated acid solutions has been a puzzling exception to this pattern: the undissociated carboxyl group appears to eliminate CO₂ with the assistance of an additional proton. The very low proton affinity of CO₂ creates an insurmountable energy barrier to the formation of protonated CO₂ (CO₂H⁺) as a reaction intermediate. However, acid-catalyzed decarboxylation reactions are well-known, and mechanistic proposals have nonetheless assumed the formation of CO₂H⁺ along with theoretical calculations. Our recent kinetic analysis of the decarboxylation of pyrrole-2-carboxylic acid, along with theoretical calculations, has established a reasonable alternative in which protonated carbonic acid (PCA), first observed by Olah and White in 1968, is the protonated product. Determination of the proton affinity of carbonic acid and the gas-phase structure of PCA have been achieved in recent years, further supporting its feasibility as a reaction intermediate. Modification of the acid-catalyzed decarboxylation mechanism to involve a hydrolytic route leads to the conclusion that the reaction proceeds via the formation of a hydrate followed by the release of PCA (rather than CO₂H⁺) in the step that cleaves the carbon—carbon bond. In such a reaction, water serves a true catalytic function by altering the path without affecting stoichiometry.

Based on the importance of PCA as a reaction intermediate and the recent applications of this reaction pathway in a variety of areas, a complete understanding of the mechanism is of considerable importance. In order to resolve many outstanding questions associated with hydrolytic decarboxylation, we have investigated the decarboxylation reactions of indole-2-carboxylic acid and indole-3-carboxylic acid. These substrates are sufficiently similar to pyrrole-2-carboxylic acid to involve a hydrolytic pathway as a likely mechanism; however, the addition of the fused aromatic ring has a significant effect on their overall reactivity compared to that of pyrrole-2-carboxylic acid.

We have determined the rates of decarboxylation of indole-2-carboxylic acid and indole-3-carboxylic acid over a wide range of solution acidity, including reactions in deuterated media that permit determination of solvent kinetic isotope effects. Previous work by Challis and Rzepa reported the rates of decarboxylation of indole-3-carboxylic acid over a limited range of acidity without consideration of the acid-catalyzed hydrolytic process. The results of the present study provide a complete reaction profile for hydrolytic decarboxylation across both pH and acidity function (Hₐ) ranges.

### RESULTS AND DISCUSSION

Rate constants for the conversion of indole-2-carboxylic acid and indole-3-carboxylic acid to indole and carbon dioxide as a function of Brønsted acidity of the medium (measured as Hₐ) and pH as appropriate) are presented in Figure 1 and Table 1. Indole-3-carboxylic acid undergoes decarboxylation more rapidly than indole-2-carboxylic acid.

In the case of indole-3-carboxylic acid, the data reflect two plateaus and two apparent dissociation constants based on the equation for titration of a dibasic acid. The observations are consistent with both the neutral and protonated forms being converted to products by different mechanisms (Scheme 1).
The rate equation for Scheme 1 is given below:

\[
\frac{d[P]}{dt} = k_1[H^+] + k_2[I] = k_{obs}[S_T]
\]

The quantity \([S_T]\) is the total concentration of all protonation states of indole-3-carboxylic acid. A simplified expression for the observed rate coefficient is obtained based on the necessity that \(K_i \ll K_f\):

\[
k_{obs} = \frac{K_i k_1 + k_2[H^+]}{(K_i + [H^+])(1 + K_i)}
\]

Equation 2 was fit to the data for indole-3-carboxylic acid in the \(H_0/pH\) rate coefficient profile (Figure 1). The value of the maximum rate coefficient, \(k_1\), was taken as the observed plateau value \((k_{obs} = 5.0 \times 10^{-3} \text{ s}^{-1} \text{ at } H_0 = -3.22)\) and \(K_i\) as the macroscopic dissociation constant of indole-3-carboxylic acid (\(pK_i = 5.0\), see \(K_f\) in Scheme 1), which was determined by titration of a solution at 60 °C. Values for \(k_2\) and \(K_2\) were calculated by iterative least-squares analysis of the data using the equation for \(k_{obs}\) \((k_2 = 2.5 \times 10^{-3}; pK_i = 0.4)\). Indole-2-carboxylic acid is presumably involved in an equilibrium similar to \(K_f\) shown in Scheme 1. Fitting of the data points leads to a value of \(pK_{i1} = -1.5\) for indole-2-carboxylic acid.

The rate plateau for the decarboxylation reaction of indole-3-carboxylic acid near \(pH = 4\) fits expectations for consequences of the established mechanism of dissociative decarboxylation via the tautomeric zwitterion (1*), Scheme 2. In that mechanism, the carboxyl group of indole-3-carboxylic acid is predominantly present in its neutral form (1), which is converted to (1*) by steps involving rate-determining transfer of a proton, consistent with the substantial solvent kinetic isotope effect at dilute acid concentrations. Conversion of the carbonyl group of (1*) to carbon dioxide provides residual electrons required to achieve aromaticity in the indole product. In more dilute acid solutions (\(pH > 5\)), the conjugate base of indole-3-carboxylic acid (1-\(H^+\)) predominates. With the decrease in concentration of proton donors, the rate of decarboxylation decreases. Direct reaction from the conjugate base (1-\(H^+\)) would require the loss of carbon.
dioxide to occur along with formation of a highly energetic residual anion, which creates a very high barrier to this route.

If the dissociative mechanism for decarboxylation were operating in concentrated acid solutions, we would expect that as the acidity of the medium is increased, the observed rate coefficient would decrease (the conjugate acid (1) is produced, making the zwitterionic form (1*) less available). However, the observed first-order rate constant increases with pH < 4 (Figure 1). Interestingly, when the acidity is between pH = 3 and H0 = 0, the rate is proportional to proton concentration (but not to additional buffer), which is consistent with a mechanism involving specific acid catalysis. As acidity increases beyond this region, the most basic site of indole-3-carboxylic acid, the carboxyl group oxygen, will become protonated (1H+). This structure is stabilized by delocalization of the electron pair from nitrogen. The protonated intermediate is subject to rapid addition of water to form the tetrahedral intermediate (2) with an expected apparent first-order rate constant of 300 s⁻¹ at 25 °C (which is expected to increase to roughly 1 × 10⁴ s⁻¹ at 60 °C). Upon conversion to the reactive tautomer (2H⁺), carbon–carbon bond cleavage produces PCA and aromaticity is restored to the indole ring system.

The reaction of indole-2-carboxylic acid follows a similar mechanism (Scheme 3) with the additional complication that the reactive tautomer (2H⁺) is stabilized by delocalization of the lone pair of electrons from nitrogen, leading to a loss of aromaticity. In addition, the preferential protonation at the C-3 position of the indole ring likely results in a higher concentration of the unreactive tautomer (2H⁺). This is not the case with indole-3-carboxylic acid decarboxylation, as the preferential protonation at the C-3 position produces the reactive predecarboxylation intermediate (2H⁺, Scheme 2). The approximate 50-fold decrease in the observed rate between indole-3- and indole-2-carboxylic acid, shown in the concentrated acid region of Figure 1, is consistent with this consideration.

Following the release of PCA, its in situ decomposition can occur via a series of reasonable steps (loss of a proton, concerted decomposition of carbonic acid) leading to release of carbon dioxide, water, and a proton (Scheme 4). The overall reaction is kinetically equivalent to a process leading to protonated carbon dioxide but avoids that high energy species by producing PCA.
Further insight into the hydrolytic decarboxylation mechanism comes from the solvent kinetic isotope effect (SKIE) $k_d/k_D = 1.7$ at $H_0 = 0.98$ for the decarboxylation of indole-3-carboxylic acid. In concentrated acid solutions, the SKIE decreases to $k_d/k_D = 1$ at $H_0 = −2.30$. This pattern of SKIE values is consistent with the presented mechanisms. In dilute acid solutions, the mechanism of decarboxylation is dissociative, leading to formation of CO$_2$ from the neutral tautomer of the reactant. In this mechanism, formation of the reactive tautomer ($1^*$) is rate-determining via proton transfer (where SKIE is significant). In more acidic solutions the hydrolytic mechanism becomes dominant, with rate-limiting carbon–carbon bond-breaking of the protonated hydrate ($2H^+$) (and therefore a reduced SKIE).

Once in the hydrated form, the electronic effects of the carbonyl group on the protonation of the aromatic ring are eliminated and replaced by that of an ortho acid (−C(OH)$_2$) (2). This substituent should have a small effect on the pK$_a$ for C-protonation of the indole ring based on the effects of similar orthoesters ($\sigma_{C(OH)}$)$_{meta} = −0.03$ and $\sigma_{C(OH)}$)$_{para} = −0.04$).

This suggests that the pK$_a$ of the reactive intermediate (2) at equilibrium would be similar to that of unsubstituted indole (pK$_a = −2.4$). Therefore, the reaction must proceed via initial protonation of the carbonyl group to promote hydration. Once hydrated, protonation of the aromatic ring is dramatically facilitated, unlocking the pathway for the subsequent release of PCA.

A simplified rate expression (eq 3) can be used to represent the overall hydrolytic decarboxylation pathway where the conjugate acid of indole-3-carboxylic acid ($1H^+$) is the initial substrate. Since the solvent isotope effect indicates that proton transfer steps are not rate-determining, we can estimate the rate constant for cleavage of the carbon–carbon bond ($k_2$) in concentrated acid solutions for indole-3-carboxylic acid (eq 4).

$$v = k_{obs}[1H^+] = k_2[2H^+]$$

$$k_2 = k_{obs}[1H^+]/[2H^+]$$

The value of the equilibrium constant for $[1H^+]/[2H^+]$ can be estimated from a thermodynamic cycle between ($1H^+$) and ($2H^+$) that follows $K_1$, $K_2$, and $K_3$ as shown in Scheme 2. As noted above, the pK$_a$ is 0.4 for O-protonated indole-3-carboxylic acid. An estimate of the extent of hydration of the carboxylic acid is possible by extension of previously calculated values. The equilibrium constant for addition of water to the carbonyl group of methyl glycinic (N-protonated) was estimated by Guthrie and Cullimore to be about $10^{-6}$, which is a good model for indolecarboxylic acids based on the location of the nitrogen substituent. Therefore, the log of the equilibrium constant ($K_v$) for hydration is ca. −6 while the pK$_a$ of the C-protonated indole derivative is approximately −2.4. Therefore, the overall equilibrium $[1H^+]/[2H^+]$ is approximately equal to 0.4−6−2.4 = −8.

This leads to an estimate for the carbon−carbon bond-breaking step of $k_2 \approx k_{obs} \times 10^8 = 10^5$ s$^{-1}$ at 60 °C and between $10^3$ and $10^5$ s$^{-1}$ at 25 °C.

The hydrolytic decarboxylation pathway permits a more rapid reaction in acid solution than does the neutral dissociative reaction mechanism because the latter requires rate-determining formation of the minor tautomer by protonation of the conjugate base on carbon at low acid concentrations. An important consequence is that the microscopic reverse reaction in acidic solution is carboxylation of indole. This should occur via a Friedel–Crafts reaction of PCA, a process in which water and a proton would be catalytic in the overall addition of CO$_2$.

We propose, by extension, that it is also likely that a Lewis acid could provide the necessary activation to produce a complex of carboxylic acid analogous to PCA. Interestingly, Lewis acid promoted carboxylation reactions were demonstrated by Olah and co-workers and recent studies show that regioselective carboxylations of derivatives of both pyrrole and indole are possible. Since protonated CO$_2$ is too high in energy to exist as a reasonable intermediate in decarboxylation (and hence, in carboxylation), it is possible that similar Lewis acid complexes of CO$_2$ would be equally high in energy. On the other hand, reactions involving Lewis acid complexes of carboxylic acid would be analogous to the more energetically feasible PCA.

**CONCLUSION**

We have reported kinetic analysis for the decarboxylation of indolecarboxylic acids over a wide range of solution acidities. In dilute acid solutions, the rate-determining step involves formation of the zwitterionic intermediate that is capable of losing CO$_2$ directly. In concentrated acid solutions, a route for acid catalysis leads to the addition of water to the carboxyl group, resulting in expulsion of the energetically feasible PCA. In this hydrolytic mechanism, the rate-determining step is carbon–carbon bond cleavage. By investigating the hydrolytic decarboxylation pathway for indolecarboxylic acids, we have been able to show that the expulsion of water from the addition intermediate has a lower barrier than that of carbon–carbon bond cleavage to form PCA.

**EXPERIMENTAL SECTION**

Commercial indole-2-carboxylic acid, indole-3-carboxylic acid, and potassium chloride were used as purchased. Buffers and acid solutions were made from reagent-grade chemicals with distilled water or deuterium oxide.

**Kinetics of Decarboxylation.** The rates of decarboxylation of indole-2-carboxylic acid and indole-3-carboxylic acid were measured for reactions in hydrochloric acid. The rate of decarboxylation of indole-3-carboxylic acid was also measured in 0.1 M buffers of chloroacetic acid, acetic acid, and monobasic phosphate where the ionic strength (I) of all buffered solutions was maintained at I = 1.0 M by addition of potassium chloride. All measurements were carried out in solutions maintained at 60 °C. The reaction was monitored by the decrease in absorbance at 300 nm (indole-2-carboxylic acid) or 291 nm (indole-3-carboxylic acid) with a UV–vis spectrometer, whose cell compartment was controlled within ±0.1 °C. Data were collected with an interfaced computer, and the observed first-order rate constants (Table 1) were calculated from nonlinear regression fitting to the integrated first-order rate expression. For slow reactions, the method of initial rates was used to calculate rate constants. For determination of solvent kinetic isotope effects, reactions were conducted using comparable concentrations of hydrochloric acid (in water) and deuterium chloride (in deuterium oxide).
**ASSOCIATED CONTENT**

2 Supporting Information
UV spectra and proton NMR spectra of reactants and UV spectrum of the common product from decarboxylation reactions. This material is available free of charge via the Internet at http://pubs.acs.org.

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**Notes**
The authors declare no competing financial interest.

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**REFERENCES**

2.1.2 Pyrrole-carboxylic acids

Since our laboratory has published the kinetic analysis for the substrate pyrrole-2-carboxylic acid,\textsuperscript{25,26} I completed the positional isomer series by measuring the rate constants for acid-catalyzed decarboxylation of pyrrole-3-carboxylic acid. The decrease in concentration of starting material over time was monitored by UV-vis kinetics and first-order rate constants were calculated. The data for solutions of varying concentration of perchloric acid are presented as the lower curve in Figure 2.2. Published data\textsuperscript{25,26} for the decarboxylation of pyrrole-2-carboxylic acid are also shown to compare the relative difference in rates for the decarboxylation reaction of each positional isomer. The rate constant values for the decarboxylation of pyrrole-3-carboxylic acid are presented in Table 2.1 along with the calculated values from titration curve fitting.

![Figure 2.2](image)

**Figure 2.2.** Logarithm of the observed first-order rate coefficients ($k_{obs}$) for the decarboxylation of pyrrole-2-carboxylic acid (▽) and pyrrole-3-carboxylic acid (▽) as a function of solution acidity, measured as $H_0$ at 25 °C. Symbols represent perchloric acid solutions. Data are fit to titration curves.
Table 2.1. Observed and calculated rates for the decarboxylation of pyrrole-3-carboxylic acid

<table>
<thead>
<tr>
<th>$H_0$ HClO$_4$</th>
<th>$k_{obs}$</th>
<th>log $k_{obs}$</th>
<th>log $k_{calc}^{a}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>-2.62</td>
<td>$1.9 \times 10^{-6}$</td>
<td>-5.7</td>
<td>-5.7</td>
</tr>
<tr>
<td>-1.83</td>
<td>$1.3 \times 10^{-6}$</td>
<td>-5.9</td>
<td>-5.8</td>
</tr>
<tr>
<td>-1.31</td>
<td>$8.8 \times 10^{-7}$</td>
<td>-6.1</td>
<td>-6.0</td>
</tr>
<tr>
<td>-0.67</td>
<td>$6.9 \times 10^{-7}$</td>
<td>-6.2</td>
<td>-6.2</td>
</tr>
<tr>
<td>-0.36</td>
<td>$5.0 \times 10^{-7}$</td>
<td>-6.3</td>
<td>-6.3</td>
</tr>
<tr>
<td>0.02</td>
<td>$4.4 \times 10^{-7}$</td>
<td>-6.4</td>
<td>-6.3</td>
</tr>
</tbody>
</table>

$^{a}k_{obs}$ from fitting the data to titration curve

Figure 2.2. shows that pyrrole-3-carboxylic acid follows the same characteristics as that of the acid-catalyzed decarboxylation of pyrrole-2-carboxylic acid. Both positional isomers track the titration curve for protonation of the carboxyl group. Since the same dependence of loss of carbon dioxide on solution acidity is observed, it can be assumed that the mechanism of decarboxylation for pyrrole-3-carboxylic acid is also hydrolytic in nature. Previous studies have confirmed that general acid catalysis is not involved, as solutions of different acids show the same titration curve.$^{25}$ The observed catalysis is a result of specific acid catalysis, where the concentration of hydronium ions promotes the reaction.

2.1.3 Comparing positional isomers of indole- and pyrrole-carboxylic acids

All of the indole- and pyrrole-carboxylic acids follow similar rate profiles for acid-catalyzed decarboxylation, with data fitting to the titration curves for macroscopic protonation of the starting material (Figure 2.3).
Figure 2.3. Logarithm of the first-order rate constants for the decarboxylation of indole-3-carboxylic acid (○), indole-2-carboxylic acid (●), pyrrole-2-carboxylic acid (▼), pyrrole-3-carboxylic acid (▽), as a function of pH and Hammett acidity constants. HClO₄ solutions (▼/▽) at 25 °C, HCl solutions (●/○) at 60 °C. For indole-3-carboxylic acid, data points higher than pH=1.0 are buffered solutions.

The shift of equivalence points for the titration curves are the result of pKₐ values and available resonance structures upon macroscopic protonation of the carboxylic acid. The resonance structures of the indole-carboxylic acids resulting from mono-protonation are shown in Scheme 2.2.
Once protonation occurs at the most basic site (carboxyl oxygen),\textsuperscript{61,62} the positive charge character can be dispersed and stabilized by the electron-rich indole moiety. However, only indole-3-carboxylic acid can stabilize this nascent charge and maintain aromaticity of the fused 6-membered ring. The C-3 carboxyl substitution allows for the enamine character of the indole core to be utilized. In order for the same charge delocalization to take place in indole-2-carboxylic acid, aromaticity of the fused ring must be disrupted, carrying the energetic penalty of a lower observable pK$_a$, as determined by fitting of the data to titration curves (pK$_a$(Indole-2-COOH) = -1.5; pK$_a$(Indole-3-COOH) = 0.4). The pyrrole-carboxylic acids undergo similar resonance stabilization upon mono-protonation. However, in these cases the lack of a fused aromatic ring results in negligible discrepancy between the pK$_a$ values of the two isomers (pK$_a$(Pyrrole-2-COOH) = pK$_a$(Pyrrole-3-COOH) = -1.3). The fitted pK$_a$ values for each substrate coincide with examples from the literature.\textsuperscript{63-65}

**2.2 Mechanistic insights from carbon kinetic isotope effects**

Since decarboxylation reactions involve carbon-carbon bond-breaking, isotopic analysis can lead to meaningful mechanistic insights. When carbon-carbon bond cleavage is rate-determining, changes in the ratio of $^{12}$C to $^{13}$C in the product will result in an observable carbon kinetic isotope
effect (CKIE). Since the carbon dioxide carbon atom is involved in the bond-breaking process, mass spectrometric detection of the ratio of $^{12}$C to $^{13}$C is entirely reflective of the rate-determining step under study. As a gas, the carbon dioxide product can be sampled directly, eliminating the need to isolate any liquid phase components and allowing for small scale reactions to be undertaken.

The accompanying publication presents an in-depth study on the carbon kinetic isotope effects for the acid-catalyzed decarboxylation of indole-carboxylic acids and pyrrole-carboxylic acids. Since there are a variety of substrates, differing only in their substitution pattern of the carboxyl group, we are able to compare directly the observed magnitudes of the CKIEs. By comparing these values, we gain insights into the dynamics of the steps leading to and from the rate-determining transition state. “Carbon Kinetic Isotope Effects Reveal Variations in Reactivity of Intermediates in the Formation of Protonated Carbonic Acid” is reproduced with permission from Adelle A. Vandersteen et al., J. Org. Chem. 2013, 78, 12176–12181. Copyright 2013 American Chemical Society.
Carbon Kinetic Isotope Effects Reveal Variations in Reactivity of Intermediates in the Formation of Protonated Carbonic Acid

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ABSTRACT: Kinetic evidence suggests that acid-catalyzed decarboxylation reactions of aromatic carboxylic acids can occur by a hydrolytic process that generates protonated carbonic acid (PCA) as the precursor of CO₂. Measurements of reaction rates and carbon kinetic isotope effects (CKIE) for decarboxylation of isomeric sets of heterocyclic carboxylic acids in acidic solutions reveal that C−C cleavage to form PCA is rate-determining with significant variation in the magnitude of the observed CKIE (1.018−1.043). Larger values are associated with the more reactive member in each isomeric pair. This variation is consistent with stepwise mechanisms in which C−C cleavage is competitive with C−O cleavage, leading to reversion to the protonated reactant to varying degrees with an invariant intrinsic CKIE for C−C cleavage. Thus, the relative barriers to reversion and formation of PCA control the magnitude of the observed CKIE in a predictable manner that correlates with reactivity. Application of the proposed overall mechanism reveals that carboxylation reactions in acidic solutions will proceed by way of initial formation of PCA.

INTRODUCTION

The structure and properties of protonated carbonic acid (C(OH)₃⁺, PCA) have been the subjects of spectroscopic and theoretical analysis.¹⁻⁵ We have recently reported that kinetic analysis of acid-catalyzed decarboxylation reactions of heterocyclic carboxylic acids implicates PCA as an obligatory reaction intermediate whose role is predicted to be similar in a wide range of reactions.⁶⁻⁹ The overall process involves addition of the equivalent of a water molecule and a proton, in analogy to ester hydrolysis, hence the designation “hydrolytic decarboxylation”.

The rate law for specific-acid-catalyzed decarboxylation of a carboxylic acid requires that the rate-determining transition state arises from a species with the equivalent of one more proton than the neutral carboxylic acid. This is clearly inconsistent with C−C bond cleavage occurring via a simple dissociative process that produces CO₂. Furthermore, while the formation of the conjugate acid of CO₂ (HOCO⁺) would be consistent with such a rate law, the exceedingly low proton affinity of CO₂ makes its conjugate acid an inaccessible reaction intermediate (Scheme 1).¹⁰

These results are instead consistent with an alternative pathway that produces PCA in the step that cleaves the C−C bond. This is achieved by the initial addition of water to the carboxyl group and protonation of the ring α to the carboxyl, leading to a highly reactive precursor of PCA (Scheme 2).⁶⁻⁹

Scheme 1. Prohibitive Decarboxylation Reaction of Pyrrole-2-carboxylic Acid Leading to Protonated Carbon Dioxide

Scheme 2. Acid-Catalyzed Decarboxylation of Pyrrole-2-carboxylic Acid via Addition of H⁺ and H₂O

The overall process has been assessed and supported by independent computational studies that also tested various alternatives.¹¹,¹²

In order to specify the role of PCA in the context of reaction intermediates, we evaluated the kinetic properties of two sets of isomeric heterocyclic carboxylic acids derived from pyrrole and indole. While they are expected to undergo hydration with similar energetics, the reactive intermediates that produce PCA are energetically distinct, leading to observable effects that define the nature of the transition states that produce PCA in competition with those that revert to the reactants.

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On the basis of the overall reaction patterns of these sets of reactants, we find that the transition state for the step that produces PCA is partially rate-determining to varying extents. This information is accessed via measurements that reveal the magnitudes of the observed carbon kinetic isotope effects ($k^{12}/k^{13}$, CKIE) for the reactions in the sets of related compounds under conditions where a defined single reaction mechanism is required by the observed rate law (plateau regions of the acidity-rate profiles). Comparing the results of observed rate constants and CKIE measurements in the acid-catalyzed decarboxylation reactions of compounds 1–4 reveals variations that affect the competing forward and reverse steps from the hydrated intermediates that are C-protonated (protonated at the position α to the hydrated carboxyl). The cations differ in energy depending on the extent to which stabilization by formation of iminium character is accessible. The range of observed CKIEs can be understood from considering a common intrinsic value for the isotope effect that is then attenuated by the extent to which the formation of PCA is competitive with reversion to the reactants from the steady-state reactive intermediate species.

■ RESULTS

As was reported for the acid-catalyzed decarboxylation reactions of pyrrole-2-carboxylic acid$^9$ and the indole-carboxylic acids,$^7$ the observed first-order rate constant for the decarboxylation of pyrrole-3-carboxylic acid increases with increasing acidity, reaching a plateau higher than that for the uncatalyzed reaction. The resulting dependence of the observed first-order rate constants on acidity ($H_0$) for all heterocyclic carboxylic acids fit the calculated titration curves for forming the conjugate acids of the reactants (Figure 1). In the plateau regions, there is necessarily a common mechanism for each species and for which the observed CKIEs were obtained. While the equation-generated plots of acidity vs first-order rate constants are similar in shape for the various reactants, there are significant differences in the values of the high plateaus as well as for the lowest acidities at which the maximum values occur (presumably due to the $pK_a$ of the conjugate acid). The fitted maximum values for the observed first-order rate constants are summarized in Table 1 along with the apparent macroscopic $pK_a$ values that were used to fit the data to the Hammett acidity function, $H_o$. For the indole-carboxylic acids, the rate of decarboxylation is greater where the carboxyl group is positioned at C-3 of the indole ring, while carboxyl substitution at the C-2 position leads to a faster observed rate in the pyrrole-carboxylic acid series.

The variations of the observed maximum rate constants demonstrate a pattern that applies to the step leading to formation of PCA, with additional information provided by the differential magnitudes of the observed carbon kinetic isotope effects (CKIEs) (Table 2).

■ DISCUSSION

Previous studies with pyrrole-2-carboxylic acid$^7$ revealed a large CKIE for the acid-catalyzed decarboxylation which indicates that the rate-determining transition state involves cleavage of the C–C bond (forming PCA). However, the locations of the carboxyl group within a series of indole- and pyrrole-carboxylic acid positional isomers lead to differences in reactivity that affect the magnitudes of the observed CKIEs. It is likely that the step involving C–C cleavage is subject to a common intrinsic isotope effect, while the significant variations in the values of CKIEs arise from the extent to which the C–C cleavage step is rate-determining in competition with reversion to reactants. This is consistent with expectations from theoretical analysis of

![Figure 1. Logarithm of the first-order rate constants for the decarboxylation of (●) indole-3-carboxylic acid, (▲) indole-2-carboxylic acid, (▼) pyrrole-2-carboxylic acid, (▽) pyrrole-3-carboxylic acid, as a function of pH and Hammett acidity constants. (▼/▽) HClO₄ solutions at 25 °C and (●/▲) HCl solutions at 60 °C. For indole-3-carboxylic acid, data points higher than pH 1.0 are buffered solutions.]

| Table 1. Maximum Observed First-Order Rate Constants for Decarboxylation |
|-----------------------------|-----------------------------|-----------------------------|
| aromatic carboxylic acid    | $k_{ab}$(max) ($s^{-1}$)    | $pK_a$ (±0.002)              |
| pyrrole-2-carboxylic acid$^a$ | $6.2 \times 10^{-4}$        | −1.3                        |
| pyrrole-3-carboxylic acid   | $1.9 \times 10^{-6}$        | −1.3                        |
| indole-2-carboxylic acid$^b$ | $1.2 \times 10^{-4}$        | −1.5                        |
| indole-3-carboxylic acid$^b$ | $5.0 \times 10^{-3}$        | 0.4                         |

$^a$Reference 6. $^b$Reference 9. For monoprotonation of the carboxylic acids from titration using $H_o$-defined media.

| Table 2. Carbon Kinetic Isotope Effects Observed for Acid-Catalyzed Decarboxylation Reactions |
|---------------------------------------------|-----------------------------|-----------------------------|
| solution acidity ($H_o$ or pH)              | CKIE (±0.002)               |
| Pyrrole-2-carboxylic Acid$^b$               |                             |
| $H_o = −2.6$                                | 1.043                       |
| $H_o = −0.7$                                | 1.027                       |
| Pyrrole-3-carboxylic Acid$^b$               |                             |
| $H_o = −2.6$                                | 1.036                       |
| $H_o = −0.4$                                | 1.028                       |
| Indole-2-carboxylic Acid$^b$                |                             |
| $H_o = −3.8$                                | 1.018                       |
| $H_o = −2.7$                                | 1.017                       |
| $H_o = −1.3$                                | 1.002                       |
| Indole-3-carboxylic Acid$^b$                |                             |
| $H_o = −3.2$                                | 1.030                       |
| $H_o = 0.2$                                 | 1.027                       |
| pH 4.4                                     | 1.003                       |

carbon kinetic isotope effects from $S_n2$ and E2 reactions which
reveal that, despite significant changes in the transition state (or bond order), intrinsic CKIEs are essentially invariant.$^{13−17}$

An instructive example can be found in the heavy-atom isotope effects for decomposition of substituted benzenediazonium ions.$^{18}$ The electrons from the cleaved bond are transferred to nitrogen (forming N$_2$) rather than to the residual organic species as in decarboxylation reactions. While the electron transfer is in the opposite sense to that observed in the acid-catalyzed decarboxylation reactions, substituents also affect the bond-breaking event. This is apparent in the 100–1000-fold range in the observed rates for dediazoniation with various substituents.$^{19}$ This variation in reactivity might suggest that there are changes in the position of the transition state for the carbon–nitrogen cleavage step that lead to the variation in observed isotope effects; however, the measured nitrogen kinetic isotope effects are invariant for all derivatives.$^{19,20}$ Thus, variations in the observed kinetic isotope effects for breaking bonds to heavy atoms are not associated with the extent of bond breaking in the transition state for C–N bond cleavage.

An important study from Hilvert, O’Leary, and co-workers$^{21}$ determined the CKIEs for the decarboxylation of 5-nitro-3-carboxybenzisoxazole under a variety of conditions. In this one-step reaction coupled to decomposition, reversion to reactants is not possible. Therefore, the magnitude of the observed CKIE should be identical with the magnitude of the intrinsic isotope effect. The polarity of the reaction medium was varied (including the presence of a catalytic antibody), which produced a large variation in rate; the observed first-order rate constants vary by a factor as large as $2 \times 10^4$. However, a nearly constant magnitude for the CKIE was observed ($\sim 1.045$). This value is a reasonable expectation for the intrinsic CKIE. In the present study there are significant changes in the magnitudes of CKIEs. This is clear evidence that variations in the CKIEs result from effects of variability in competition among partially rate determining steps in a multistep pathway. The invariance of the intrinsic CKIEs is the result of their arising only from differences in the ground state vibrational energies where the C–C bond is in place, whereas in the transition states that bond is broken and no new bond to either position is in the process of being formed (in contrast to the case for proton transfers between Bronsted acids and bases).

On the basis of the preceding discussion, we assume that the magnitudes of the observed CKIEs arise from a common intrinsic CKIE in the C–C bond-breaking steps. The observed CKIE for reaction of the conjugate acid of indole-3-carboxylic acid (1-SH; Scheme 3) is significantly larger than that for the conjugate acid of indole-2-carboxylic acid (2-SH) (1.030 vs 1.018). The extent to which the step competes with the reversion to the reactants controls the magnitude of the observed CKIE.

In Scheme 3 the magnitude of $k_2/k_{-1}$ must be smaller than that for $k_{-2}/k_{-1}^\prime$ to give the observed differential CKIE values, as expressed in eqs 1–3. The magnitudes of the observed CKIEs (eq 3) depend on the relative values of $k_2/k_{-1}$ for each reactant. The smaller the ratio, the larger the value of the observed CKIE, approaching the intrinsic CKIE for the unimolecular process as a limit. This follows the analysis from which Northrop identifies the nature of the effects of “commitment factors”.$^{22,23}$

$$k_{obs} = \frac{k_j k_2}{k_{-1} + k_2}$$  
(1)

$$\text{CKIE}_{obs} = \frac{k_{12}}{k_2^\prime}  \frac{1 + \frac{k_{13}}{k_2}}{1 + \frac{k_{13}}{k_2}}$$  
(3)

$$\frac{\text{CKIE}_{obs}}{\text{CKIE}_{obs}} = \frac{k_{12}}{k_{12}}  \frac{k_{13}}{k_{13}}  \frac{k_{-1}}{k_{-1}}  \frac{k_{-1} + k_{-1}^\prime}{k_{-1} + k_{-1}^\prime}$$  
(2)

In general, where the magnitudes of $k_{-1}$ and $k_2$ are comparable, the CKIE that is observed depends on the ratio of the values of those rate constants. Where the rate constant for C–C cleavage is larger than that for the reversion process (loss of water and a proton), the observed CKIE will be smaller. For the less reactive substrate in each set (i.e., indole-2-carboxylic and pyrrole-3-carboxylic acids), protonation at a site that is more highly energetic than is the case for their paired isomers leads to a smaller observed rate constant within the pair of isomers. Therefore, reactions of the less reactive isomer occur from intermediates that are closer in energy to the transition state for formation of PCA than in the cases for the more reactive member. As a result, the larger observed CKIE is associated with the more reactive substrates, since the barrier...
The sources of these observations can be understood from mathematical models for these processes. The curves in Figure 2 were generated to illustrate the relationship between commitment factors and observed CKIEs as derived from the rate law for the mechanism in Scheme 2. The x axis is the value of the rate constant subject to the intrinsic CKIE divided by the effective rate constant for conversion of the same intermediate to the protonated reactant. The y axis is the resulting observed CKIE. The value of the observed CKIE is reduced from the intrinsic CKIE (on the basis of a range of illustrative intrinsic values for a CKIE) as the rate constant for the C−C breaking step becomes larger relative to the rate constant for reversion (the latter increases and the former remains constant). As the barrier for reversion becomes larger relative to that for the C−C bond-breaking step, the value for the observed CKIE approaches a limiting value of 1.0.

Pathways for the decarboxylation of the isomeric pyrrole-carboxylic acids are presented in Scheme 4. These provide the necessary intermediates to understand the basis of the variation in CKIEs. The rapid decomposition of PCA leads to formation of CO₂, which is the detected product other than pyrrole, as the proton and water are catalytically cycled.

In all of the reactions that we have presented here, the species that precedes the cleavage of the C=C bond (to release PCA) contains a hydrated carboxyl group where protonation at the position α to the (hydrated) carboxyl carbon has taken place. These intermediates have a barrier to the transition state for decarboxylation lower than those in which the proton is added to the β position, although the latter in some cases are thermodynamically favored.²⁴–²⁶

The relative energies of the cationic intermediates depend on the effects of protonation on aromatic stabilization, as the positive charge is dispersed onto the nitrogen of the heterocycle, as shown in Scheme 5. In the case of the indole-carboxylic acids, the pathway for the less reactive isomer (indole-2-carboxylic acid) proceeds through the structure shown in Scheme 5b. However, protonation to produce the structure in Scheme 5c gives a more stable intermediate.²⁵,²⁶ In other words, protonation of the aromatic ring must occur at the less basic site in order for the system to be able to lose PCA in the next step. On the other hand, disruption of aromaticity is less significant for the pyrrole-carboxylic acid, as there is no benzenoid moiety as in the indole derivatives. As a result, this leads to a smaller distinction in the magnitudes observed for the CKIEs for the pyrrole-carboxylic acid isomers.

The difference between the pKₐ values for protonation at the lower energy position vs that which is required for the reaction pathway of decarboxylation of pyrrole-3-carboxylic acid is about 2.0 pKₐ units.²⁴ The observed rate constant for decarboxylation of pyrrole-3-carboxylic acid is about 300 times smaller than that for pyrrole-2-carboxylic acid (Table 1), suggesting that the rate differences arise principally from differences in the energies of the sites for protonation. In pyrrole-2-carboxylic acid, the required site of protonation for decarboxylation is also the lower energy site of protonation under the reaction conditions. The reaction patterns of the indole-carboxylic acids follow the same trend, leading to differences in the values for the rate maxima. However, in contrast to the pyrrole derivatives, it is the isomer with the carboxyl at the 3-position that is preferentially protonated and that also leads to C−C bond cleavage.
In typical decarboxylation reactions in neutral solutions, formation of the residual carbanion is the key rate-controlling feature and the reaction falls within the realm of carbanion chemistry. We see from the present study that in acid-catalyzed decarboxylation reactions which produce PCA, the leaving group is derived from a cation that becomes neutral and aromatic upon passing through the transition state involving C–C cleavage. Since the other product, PCA, is a cation, we consider the step that produces it will be subject to factors that parallel the typical rate-determining step in substitution reactions that proceed by an $S_n1$ mechanism. Where the intermediate preceding PCA is higher in energy than in the case of its isomer, the leaving group is thereby activated, reducing the barrier to C–C cleavage. Although the barrier to that step is reduced, the overall reaction is slower than in the case where the pre-PCA intermediate is subject to greater stabilization. Thus, $k_f$ for the higher energy species will be greater (with a lower barrier) than $k_f$ for the lower energy species. On the other hand, in the competing process, loss of water to re-form the carboxyl group ($k_{-1}$) should be independent of the nature of the specific intermediate. As a result, $k_f/k_{-1}$ is larger for the less reactive species, resulting in a lower value for the observed CKIE (see eq 3).

Our results show that the protonated carboxylic acid is more reactive toward decarboxylation than any other protonation state of the substrate. However, it does not directly produce CO$_2$ in the C–C cleavage step. This is not because PCA is formed more easily than CO$_2$; rather, it is due to the ring-protonated carboxylation leaving group being formed to a much greater extent in acid, as compared to the zwitterion that forms in neutral solutions to produce CO$_2$.

Thus, we see the importance of PCA in defining the key intermediates in a major class of readily accessible decarboxylation reactions. Its formation occurs where an aromatic species acquires a proton to form a carboxation at the position $\beta$ to a carboxyl group, even if the site of protonation is not the site that gives the intermediate that is lowest in energy. Rather, the key factor is that protonation must occur on the site that leads directly to the production of PCA. Significantly, the pattern of observed CKIEs provides the necessary context for arriving at this understanding and the results also provide insights into the factors leading to the observed value of a CKIE.

### CONCLUSIONS

The mechanisms of acid-catalyzed decarboxylation reactions implicate the formation of PCA from a carboxation intermediate that is generated by addition of water to the carboxyl group and a proton to the $\beta$ position of the adjoining unsaturated species, regardless of the relative energy of protonation at that site. The variation in the observed CKIE is consistent with a common intrinsic value that depends on the extent to which hydration is also partially rate-determining. The key reactive intermediate is one that leads to the formation of PCA. The principle of microscopic reversibility suggests that electrophilic aromatic substitution based on PCA should be an accessible route to carboxylation of aromatic heterocycles.

### EXPERIMENTAL SECTION

Pyrrrole-2-carboxylic acid, pyrrole-3-carboxylic acid, indole-2-carboxylic acid, and indole-3-carboxylic acid were obtained from commercial sources. All structures were verified spectroscopically, and the compounds were used without further purification. Acidic solutions were prepared from combinations of reagent-grade hydrochloric acid or perchloric acid with purified water.

**Kinetics of Decarboxylation.** The rates of decarboxylation for pyrrole-2-carboxylic acid, pyrrole-3-carboxylic acid, and indole-3-carboxylic acid in acidic solutions have been previously reported.\(^{6,7,9}\) The rate of decarboxylation of pyrrole-3-carboxylic acid was measured in solutions of perchloric acid of H$_2$-defined acidity. The reaction was followed by the decrease in absorbance at 255 nm with a UV–vis spectrometer at 25 °C, with the cell compartment kept within ±0.1 °C of the reported temperature. Data were collected with an interfaced computer, and the observed first-order rate constants were calculated by regression to the apparent first-order rate expression using the method of initial rates.

**Measurement of Carbon Kinetic Isotope Effects.** Reactions were carried out in 125 mL bottles sealed with butyl-blue stoppers. The acidic reaction solution (50 mL) was placed in the bottle, and the headspace was purged with helium to remove atmospheric CO$_2$. The carboxylic acid reactant (16 mg) was dissolved in degassed dimethyl sulfoxide (0.5 mL) and injected into the vessel to initiate the reaction. The reactions were maintained at 60.0 °C (indo-carboxylic acids) or 25.0 °C (pyrrole-carboxylic acids) in a circulating water bath. At specific reaction progress intervals, the bottle was cooled in ice and the reaction was quenched with 25 mL of degassed acetate buffer (1 M, pH 5) for reactions taking place in dilute acid solutions or by addition of 60 mL of degassed acetate buffer (5 M, pH 4.5) for reactions taking place in concentrated acid solutions in order to produce a dilute acid solution (~0.01 M) appropriate for headspace analysis. Solutions were kept at 0 °C prior to analysis. The headspace was sampled with a pressure-lock analytical syringe with a side-port taper needle.\(^{7,28,29}\) Different reaction progress intervals were sampled from the headspace up to 50% conversion. Reaction progress was approximated by reaction time and by comparison with the peak area obtained from mass intensity scans on an isotope-ratio mass spectrometer coupled to a combustion oven and gas chromatograph (GC-C-IRMS). Samples of CO$_2$ from complete conversion of the reactants were taken after 10 half-lives for each reaction. As a control, the sequence was repeated without reactant. In these cases, CO$_2$ was not detected in the headspace.

The CKIEs were calculated from the measured data using an equation adapted from Bothner-By and Bigeleisen:\(^{7,30}\)

$$k^{12}/k^{13} = \log(1 - f)/\log[1 - (N_f/N_o)] \quad (4)$$

In eq 4, $k^{12}$ and $k^{13}$ are the observed first-order rate coefficients for reaction of the corresponding carbon isotopes and $f$ denotes the fractional extent of the decarboxylation process, which varies from 0 at the start to 1 at completion. The originally defined terms $R'$ and $R''$ have been replaced with $N_f$ and $N_o$ (where the ratio of abundance of $^{13}$CO$_2$/$^{12}$CO$_2$ from the IRMS has been converted to relative abundances).

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#### Notes

The authors declare no competing financial interest.

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### ABBREVIATIONS

CKIE, carbon kinetic isotope effect; PCA, protonated carbonic acid
REFERENCES

2.3 Experimental

2.3.1 Kinetics of the decarboxylation of indole- and pyrrole-carboxylic acids

Indole-carboxylic acids and pyrrole-carboxylic acids were obtained from commercial sources. All structures were verified and used without further purification. Acid and buffered solutions were prepared using reagent-grade hydrochloric acid, perchloric acid, chloroacetic acid, acetic acid or monobasic phosphate with purified water.

The rates of decarboxylation of indole-2-carboxylic acid and indole-3-carboxylic acid were measured for reactions in hydrochloric acid. The rate of decarboxylation of indole-3-carboxylic acid was also measured in 0.1 M buffers of chloroacetic acid, acetic acid and monobasic phosphate where the ionic strength (I) of all buffered solutions was maintained at I = 1.0 M by addition of potassium chloride. All measurements were carried out in solutions maintained at 60 °C. The rate of decarboxylation of pyrrole-3-carboxylic acid was measured in solutions of perchloric acid with the temperature monitored at 25 °C. The reactions were monitored by the decrease in absorbance at 300 nm (indole-2-carboxylic acid), 291 nm (indole-3-carboxylic acid) or 255 nm (pyrrole-3-carboxylic acid) with a UV-vis spectrometer, whose cell compartment was controlled within ±0.1 °C. Data were collected with an interfaced computer and the observed first-order rate constants were calculated from non-linear regression fitting to the integrated first-order rate expression. For slow reactions, the method of initial rates was used to calculate rate constants (GraFit, Erithacus Software Limited).

2.3.2 Carbon kinetic isotope effects for the decarboxylation of indole- and pyrrole-carboxylic acids

Reactions were carried out in 125 mL bottles sealed with butyl-blue stoppers. A solution of acid or buffer (50 mL, degassed) was added to the bottle and the headspace was purged with helium to remove atmospheric CO₂. The carboxylic acid substrate (0.1 mmol) was dissolved in dimethyl sulfoxide (500 μL, degassed) and injected into the bottle to initiate the reaction. All reactions were maintained at 60 °C (indole-carboxylic acids) or 25 °C (pyrrole-carboxylic acids) in a circulating
water bath. Concentrated acid solutions of appropriate identity and concentration (see tables below) were cooled to 0 °C (for HClO₄ solutions) and quenched with degassed acetate buffer for a final solution pH of approximately 2 (for HCl solutions) prior to analysis. Buffered solutions were rapidly cooled to 0 °C prior to analysis. The headspace was sampled by use of a pressure-lock analytical syringe with a side-port taper needle. Reaction progress was approximated by reaction time and by comparison with the peak area obtained from mass intensity scans on an isotope-ratio mass spectrometer coupled to a gas chromatograph and combustion oven (GC-IRMS). Measurements of materials from complete-conversion of the reactants were taken after 24-96hrs based on the half-life of the reaction. The sequence was repeated without substrate as a controlled experiment. In these cases, CO₂ was not detected in the headspace.

¹²C/¹³C kinetic isotope effects (CKIE) were calculated based on the Bothner-By and Bigeleisen equation for monitoring kinetic isotope effects in decarboxylation reactions:

\[
k_{12}/k_{13} = \log (1 - f)/\log [1 - f(N_x/N_{x0})]
\]

where \(k_{13}\) and \(k_{12}\) are the observed first-order rate coefficients for reaction of the heavy and light isotopes. \(R\) and \(R_0\) are replaced with \(N_x\) and \(N_{x0}\) (the \(^{13}\text{C}/^{12}\text{C}\) ratio reported from the IRMS are converted into abundances).

Data for the CKIEs of indole-2-carboxylic acid, indole-3-carboxylic acid and pyrrole-3-carboxylic acid can be found in Table 2.4., Table 2.5., and Table 2.6., respectively.
Table 2.2. $^{12}$C/$^{13}$C kinetic isotope effects for the acid-catalyzed decarboxylation of indole-2-carboxylic acid

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<td></td>
<td>0.51</td>
<td>0.011213</td>
<td>0.011223</td>
<td>1.0013</td>
</tr>
<tr>
<td></td>
<td>0.52</td>
<td>0.011215</td>
<td>0.011223</td>
<td>1.0010</td>
</tr>
<tr>
<td>Av.</td>
<td></td>
<td></td>
<td></td>
<td>1.002 ±0.002</td>
</tr>
</tbody>
</table>

Table 2.3. $^{12}$C/$^{13}$C kinetic isotope effects for the acid-catalyzed decarboxylation of indole-3-carboxylic acid

<table>
<thead>
<tr>
<th>[HCl], wt % / pH</th>
<th>$f$</th>
<th>$N_x$</th>
<th>$N_{x0}$</th>
<th>CKIE</th>
</tr>
</thead>
<tbody>
<tr>
<td>24%</td>
<td>0.15</td>
<td>0.010007</td>
<td>0.010277</td>
<td>1.0283</td>
</tr>
<tr>
<td></td>
<td>0.21</td>
<td>0.010007</td>
<td>0.010277</td>
<td>1.0307</td>
</tr>
<tr>
<td></td>
<td>0.29</td>
<td>0.010015</td>
<td>0.010277</td>
<td>1.0318</td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>0.010020</td>
<td>0.010277</td>
<td>1.0310</td>
</tr>
<tr>
<td>Av.</td>
<td></td>
<td></td>
<td></td>
<td>1.030 ±0.002</td>
</tr>
<tr>
<td>2%</td>
<td>0.10</td>
<td>0.010007</td>
<td>0.010246</td>
<td>1.0251</td>
</tr>
<tr>
<td></td>
<td>0.13</td>
<td>0.009987</td>
<td>0.010246</td>
<td>1.0277</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td>0.009987</td>
<td>0.010246</td>
<td>1.0281</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>0.010007</td>
<td>0.010246</td>
<td>1.0267</td>
</tr>
<tr>
<td>Av.</td>
<td></td>
<td></td>
<td></td>
<td>1.027 ±0.002</td>
</tr>
<tr>
<td>pH = 4.4 0.1M Acetate</td>
<td>0.41</td>
<td>0.010258</td>
<td>0.010272</td>
<td>1.0019</td>
</tr>
<tr>
<td></td>
<td>0.41</td>
<td>0.010252</td>
<td>0.010278</td>
<td>1.0033</td>
</tr>
<tr>
<td></td>
<td>0.42</td>
<td>0.010248</td>
<td>0.010268</td>
<td>1.0026</td>
</tr>
<tr>
<td></td>
<td>0.42</td>
<td>0.010242</td>
<td>0.010273</td>
<td>1.0041</td>
</tr>
<tr>
<td>Av.</td>
<td></td>
<td></td>
<td></td>
<td>1.003 ±0.002</td>
</tr>
</tbody>
</table>
Table 2.4. $^{12}\text{C}/^{13}\text{C}$ kinetic isotope effects for the acid-catalyzed decarboxylation of pyrrole-3-carboxylic acid

<table>
<thead>
<tr>
<th>[HClO$_4$], wt %</th>
<th>$f$</th>
<th>$N_x$</th>
<th>$N_{x_0}$</th>
<th>CKIE</th>
</tr>
</thead>
<tbody>
<tr>
<td>42%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.14</td>
<td>0.010327</td>
<td>0.010665</td>
<td>1.0353</td>
<td></td>
</tr>
<tr>
<td>0.14</td>
<td>0.010331</td>
<td>0.010665</td>
<td>1.0349</td>
<td></td>
</tr>
<tr>
<td>0.27</td>
<td>0.010336</td>
<td>0.010665</td>
<td>1.0374</td>
<td></td>
</tr>
<tr>
<td>0.27</td>
<td>0.010337</td>
<td>0.010665</td>
<td>1.0372</td>
<td></td>
</tr>
<tr>
<td>Av.</td>
<td></td>
<td></td>
<td></td>
<td>1.036 ±0.002</td>
</tr>
<tr>
<td>10%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.12</td>
<td>0.010402</td>
<td>0.010665</td>
<td>1.0269</td>
<td></td>
</tr>
<tr>
<td>0.12</td>
<td>0.010398</td>
<td>0.010665</td>
<td>1.0274</td>
<td></td>
</tr>
<tr>
<td>0.23</td>
<td>0.010415</td>
<td>0.010665</td>
<td>1.0274</td>
<td></td>
</tr>
<tr>
<td>0.26</td>
<td>0.010410</td>
<td>0.010665</td>
<td>1.0286</td>
<td></td>
</tr>
<tr>
<td>Av.</td>
<td></td>
<td></td>
<td></td>
<td>1.028 ±0.002</td>
</tr>
</tbody>
</table>
Chapter 3
Acid-catalyzed decarboxylation of substituted benzoic acids

Reports from our laboratory have provided insights into the operative mechanism for the acid catalysis observed in the decarboxylation reactions of pyrrole- and indole-carboxylic acids in dilute and concentrated acid solutions.\textsuperscript{25,26,66,67} For these substrates, decarboxylation takes place by way of a mechanism that parallels ester hydrolysis. In this alternative ‘hydrolytic route’, the neutral substrate undergoes hydration and protonation of the aromatic ring to produce a pre-decarboxylation intermediate that is subject to rate-determining carbon-carbon bond cleavage. The immediate product resulting from this bond cleavage is protonated carbonic acid (PCA), a more feasible reaction product as compared to the highly energetic protonated carbon dioxide. PCA subsequently hydrolyzes in solution to form the observed carbon dioxide product.

I sought to determine whether or not this mechanism required an electron-rich heteroaromatic substrate in order to promote the necessary protonation of the ring. Using the nitrogen-containing pyrrole- and indole-carboxylic acids is practically feasible as the presence of the heteroatom usually increases the $pK_a$, leading to protonation under slightly acid conditions. My further work has focused on expanding the scope of the aromatic substrates to include homoatomic aromatic compounds, requiring acid solutions of elevated concentration. The acid-catalyzed hydrolytic route presented in previous chapters is now implicated for the decarboxylation of derivatives of benzoic acid. The substrate dimethoxybenzoic acid undergoes acid-catalyzed decarboxylation, implicating a more universal application of the hydrolytic decarboxylation mechanism that is inclusive of non-heteroaromatic systems.

An early report by Hay and Taylor presents findings of the acid-catalyzed decarboxylation of 2,4-dimethoxybenzoic acid.\textsuperscript{13} The authors monitored the decarboxylation at various concentrations of sulfuric acid and demonstrated specific acid catalysis. Although their data are correct, the mechanistic interpretations do not correlate with experimental observations. Their mechanism proposes that the carbon-carbon bond-breaking step for the decarboxylation of 2,4-dimethoxybenzoic acid yields protonated carbon dioxide. As discussed in Chapter 2, protonated carbon dioxide is not a feasible species in the reaction pathway due to its extremely high acidity.\textsuperscript{36} The authors suggest that a water molecule plays a role in the decarboxylation step, but this secondary mechanism implies that the leaving group has carboxylate character in the carbon-
carbon bond-breaking step. This is unlikely due to the strongly acidic reaction medium. Instead, it is likely that the mechanism is taking place by way of hydrolytic decarboxylation. Under acidic conditions, the carboxyl group becomes hydrated, followed by ring protonation of the aromatic core. Upon carbon-carbon bond-breaking, the immediate products are PCA and neutral benzene. By measuring the solvent kinetic isotope effects (SKIEs) and carbon kinetic isotope effects (CKIEs), I characterized the mechanism as hydrolytic decarboxylation.

This extension to a non-heteroatom aromatic reactant broadens the scope of applicability for this mechanism. The more universal nature of the mechanism allows for insights into potentially unexplained biological and environmental processes involving carboxylic acid decomposition and carbon dioxide trapping mechanisms.

### 3.1 Acid-catalyzed decarboxylation of 2,4-dimethoxybenzoic acid

The substrate 2,4-dimethoxybenzoic acid (DMBA) decarboxylates in the presence of acid to form 1,3-dimethoxybenzene and carbon dioxide (Scheme 3.1).

![Scheme 3.1. Acid-mediated decarboxylation of 2,4-dimethoxybenzoic acid to form 1,3-dimethoxybenzene and carbon dioxide](image)

The logarithm of the observed first order rate constant increases with increasing acid concentration (more negative $H_0$ value, Figure 3.1). The reaction was monitored by UV-vis as the absorbance of the starting material 2,4-dimethoxybenzoic acid decreases over time. The decarboxylation reaction
was tested over a range of acidities and types of acid. Hydrochloric (HCl), perchloric (HClO₄) and sulfuric (H₂SO₄) acids were used to make the acid solutions of varying concentrations. From the plot of the acidity-rate profile (Figure 3.1) it can be seen that the identity of the acid does not affect the rate of decarboxylation. The rates with all acids follow the same titration curves, demonstrating that we are observing specific acid catalysis to form the conjugate acid of the reactant in acidic hydrolytic decarboxylation.

The data in Figure 3.1 are fit to the titration curve for the first protonation state of 2,4-dimethoxybenzoic acid (pKₐ~ -0.8). The most likely site of protonation is at the electron-rich oxygen atom of the carboxylic acid group.⁶¹,⁶²

**Figure 3.1.** Logarithm of the first order rate constant for the decarboxylation of 2,4-dimethoxybenzoic acid at 60°C as a function of Hammett acidity functions (HClO₄ (○), H₂SO₄ (□), HCl (Δ)). Data points are fit to titration curve for protonation of the benzoic acid.

Data for the observed rates and corresponding acid solution are presented in Table 3.1.
Table 3.1. Observed rates for the decarboxylation of 2,4-dimethoxybenzoic acid

<table>
<thead>
<tr>
<th>$H_0$</th>
<th>$k_{obs}$</th>
<th>$\log k_{obs}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HClO$_4$</td>
<td></td>
</tr>
<tr>
<td>-2.96</td>
<td>$2.1 \times 10^{-4}$</td>
<td>-3.7</td>
</tr>
<tr>
<td>-2.62</td>
<td>$1.7 \times 10^{-4}$</td>
<td>-3.8</td>
</tr>
<tr>
<td>-2.35</td>
<td>$1.5 \times 10^{-4}$</td>
<td>-3.8</td>
</tr>
<tr>
<td>-1.83</td>
<td>$8.5 \times 10^{-5}$</td>
<td>-4.1</td>
</tr>
<tr>
<td>-1.31</td>
<td>$3.9 \times 10^{-5}$</td>
<td>-4.4</td>
</tr>
<tr>
<td>-1.01</td>
<td>$2.0 \times 10^{-5}$</td>
<td>-4.7</td>
</tr>
<tr>
<td>-0.67</td>
<td>$1.1 \times 10^{-5}$</td>
<td>-5.0</td>
</tr>
<tr>
<td>-0.36</td>
<td>$5.6 \times 10^{-6}$</td>
<td>-5.3</td>
</tr>
<tr>
<td>0.02</td>
<td>$2.3 \times 10^{-6}$</td>
<td>-5.7</td>
</tr>
<tr>
<td></td>
<td>HCl</td>
<td></td>
</tr>
<tr>
<td>-2.86</td>
<td>$2.4 \times 10^{-4}$</td>
<td>-3.6</td>
</tr>
<tr>
<td>-1.93</td>
<td>$1.3 \times 10^{-4}$</td>
<td>-3.9</td>
</tr>
<tr>
<td>-1.05</td>
<td>$4.4 \times 10^{-5}$</td>
<td>-4.5</td>
</tr>
<tr>
<td>-0.03</td>
<td>$3.6 \times 10^{-6}$</td>
<td>-5.4</td>
</tr>
<tr>
<td>0.55</td>
<td>$1.1 \times 10^{-6}$</td>
<td>-5.9</td>
</tr>
<tr>
<td></td>
<td>H$_2$SO$_4$</td>
<td></td>
</tr>
<tr>
<td>-2.82</td>
<td>$2.0 \times 10^{-4}$</td>
<td>-3.7</td>
</tr>
<tr>
<td>-2.71</td>
<td>$1.9 \times 10^{-4}$</td>
<td>-3.7</td>
</tr>
<tr>
<td>-1.97</td>
<td>$9.6 \times 10^{-5}$</td>
<td>-4.0</td>
</tr>
<tr>
<td>-1.59</td>
<td>$5.3 \times 10^{-5}$</td>
<td>-4.3</td>
</tr>
<tr>
<td>-1.05</td>
<td>$2.5 \times 10^{-5}$</td>
<td>-4.6</td>
</tr>
</tbody>
</table>
3.1.1 Solvent kinetic isotope effects for the decarboxylation of 2,4-dimethoxybenzoic acid

In order to gain insights on the rate-determining steps of the reaction, solvent kinetic isotope effect (SKIE) studies were performed. The decarboxylation reactions for 2,4-dimethoxybenzoic acid were measured in solutions of sulfuric acid (H_2SO_4) and deuterated sulfuric acid (D_2SO_4) at various concentrations. Figure 3.2 shows that the logarithm of the observed first order rate constant for decarboxylation is smaller for reactions performed in D_2SO_4 compared to those in H_2SO_4. Although the SKIE does not reach unity under the most acidic conditions, it is significantly closer to unity than for reactions in less concentrated solutions. That is, as the acidity of the medium is increased, a proton-transfer step becomes less significant for the rate of the overall transformation.

![Figure 3.2. Solvent kinetic isotope effect for the acid-catalyzed decarboxylation of 2,4-dimethoxybenzoic acid (H_2SO_4 (○) and D_2SO_4 (■)).](image)

The observation that the SKIE does not reach unity likely results from the barrier to the ring-protonation step due to the low pK_a of protonation of the benzoic acid ring. The pre-decarboxylation intermediate requires that the substrate bears a positive charge at the position β to the hydrated carboxyl group. Although the aromatic core of the benzoic acid receives electron
donation through the methoxy substituents, it is not as electron-rich as the heteroatomic indole-and pyrrole-carboxylic acids previously studied. It is evident then that the SKIE of 1 could be observed for the decarboxylation of 2,4-dimethoxybenzoic acid while using even more concentrated solutions of acid.

Presumably, benzoic acid substrates bearing electron-withdrawing substituents (i.e. 2,4-dinitrobenzoic acid) would require an even further increase in acid concentration to effect the decarboxylation reaction. The nascent positive charge of the pre-decarboxylation intermediate would be destabilized by the electron-withdrawing groups, resulting in a decreased $pK_a$ for ring-protonation.

### 3.1.2 Carbon kinetic isotope effects for the decarboxylation of 2,4-dimethoxybenzoic acid

In addition to the information gained from solvent isotope effects, carbon kinetic isotope effects (CKIEs) play an important role in the characterization of the operative mechanisms for decarboxylation. Since the carbon-carbon bond is broken during the course of the reaction, measuring the carbon dioxide produced gives insights into the rate-determining step. By quantifying the natural abundance ratio of carbon-12 to carbon-13 in the substrate, the CKIE can be calculated. For the decarboxylation of 2,4-dimethoxybenzoic acid, the CKIE values for the reaction at dilute and concentrated acidities are provided in Table 3.2.

<table>
<thead>
<tr>
<th>$H_0$ (HClO₄)</th>
<th>CKIE ($±0.002$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-2.62</td>
<td>1.022</td>
</tr>
<tr>
<td>-0.36</td>
<td>1.002</td>
</tr>
</tbody>
</table>

**Table 3.2.** Observed CKIEs for the acid-catalyzed decarboxylation of 2,4-dimethoxybenzoic acid
Upon changing the acidity from less concentrated ($H_0 = -0.36$) to more concentrated ($H_0 = -2.62$), the CKIE value increases significantly ($\Delta$CKIE = 0.020). As the acidity increases, the carbon-carbon bond-breaking step becomes rate-determining. Following the trend of the previously outlined mechanisms of acid-catalyzed decarboxylation for indole- and pyrrole-carboxylic acids, the CKIEs for 2,4-dimethoxybenzoic acid have the same dependency on acidity.

The magnitude of the CKIE is a function of the effects of protonation as well as carbon-carbon bond-breaking. Since protonation of the benzoic acid ring requires concentrated acid solutions (more concentrated than for protonation of indole- and pyrrole-carboxylic acids), it is likely that the amount of reactive protonated species is limited. If not fully protonated, the reactant is a mixture of protonation states and this extent of protonation is a function of the solvent isotope. As a result, the observed CKIE is attenuated, leading to a smaller observed magnitude as compared to the intrinsic value for the carbon-carbon bond-breaking step. This is consistent with specific acid catalysis.

### 3.1.3 Mechanism for the acid-catalyzed decarboxylation of 2,4-dimethoxybenzoic acid

A mechanism for the acid-catalyzed decarboxylation of 2,4-dimethoxybenzoic acid is presented in Scheme 3.2.
Scheme 3.2. Mechanism for the acid-catalyzed decarboxylation of 2,4-dimethoxybenzoic acid

Similar to the mechanism of decarboxylation of indole- and pyrrole-carboxylic acids, 2,4-dimethoxybenzoic acid undergoes protonation of the electron-rich carboxyl group followed by nucleophilic attack of water to form the hydrated intermediate (\(5\text{H}_2\text{O}\)). Subsequent protonation at the position α to the carboxyl group produces the pre-decarboxylation intermediate (\(5\text{I}\)). This positive charge is resonance stabilized by the methoxy groups present at the ortho- and para-positions. Cleavage of the carbon-carbon bond provides the necessary pair of electrons to quench the positive charge, driving the reaction forward. The resulting products from this bond-breaking step are 1,3-dimethoxybenzene and protonated carbonic acid (PCA). PCA is then hydrolyzed \textit{in situ}, yielding the observed carbon dioxide from the reaction.

By demonstrating that this substituted benzoic acid undergoes decarboxylation via the same mechanism as the previously studied indole- and pyrrole-carboxylic acids, the general nature of hydrolytic decarboxylation is apparent.
3.2 Experimental

3.2.1 Kinetics of the decarboxylation of 2,4-dimethoxybenzoic acid

2,4-dimethoxybenzoic acid and 1,3-dimethoxybenzene were obtained from commercial sources. All structures were verified and used without further purification. Acid solutions were prepared using reagent-grade hydrochloric acid, sulfuric acid or perchloric acid with purified water.

The rate of decarboxylation of 2,4-dimethoxybenzoic acid was measured in solutions of hydrochloric acid, sulfuric acid and perchloric acid of $H_0$-defined acidity. The reaction was followed by the decrease in absorbance at 260 nm with a UV-vis spectrometer at 60°C whose cell compartment was maintained within ± 0.1 °C of the reported temperature. Data were collected with an interfaced computer and the observed first-order rate constants were calculated by regression to the apparent first order rate expression using the method of initial rates (GraFit, Erithacus Software Limited).

3.2.2 Carbon kinetic isotope effects for the decarboxylation of 2,4-dimethoxybenzoic acid

Reactions were carried out in 125 mL bottles sealed with butyl-blue stoppers. A solution of acid (50 mL) was added to the bottle and the headspace was purged with helium to remove atmospheric CO$_2$. The carboxylic acid substrate (0.1 mmol) was dissolved in dimethyl sulfoxide (500 μL, degassed) and injected into the bottle to initiate the reaction. All reactions were maintained at 60 °C in a circulating water bath. Concentrated perchloric acid solutions of appropriate concentration (see table below) were cooled to 0 °C prior to analysis. The headspace was sampled by use of a pressure-lock analytical syringe with a side-port taper needle. Reaction progress was approximated by reaction time and by comparison with the peak area obtained from mass intensity scans on an isotope-ratio mass spectrometer coupled to a gas chromatograph and combustion oven (GC-IRMS). Measurements of materials from complete-conversion of the reactants were taken after 24-96hrs based on the half-life of the reaction. The sequence was repeated without substrate as a controlled experiment. In these cases, CO$_2$ was not detected in the headspace.
$^{12}$C/$^{13}$C kinetic isotope effects (CKIE) were calculated based on the Bothner-By and Bigeleisen\textsuperscript{42} equation for monitoring kinetic isotope effects in decarboxylation reactions:

$$k_{12}/k_{13} = \log (1 - f)/\log [1 - f(N_x/N_{x0})]$$

where $k_{13}$ and $k_{12}$ are the observed first-order rate coefficients for reaction of the heavy and light isotopes. $R$ and $R_0$ are replaced with $N_x$ and $N_{x0}$ (the $^{13}$C/$^{12}$C ratio reported from the IRMS are converted into abundances).

Data for the CKIEs of 2,4-dimethoxybenzoic acid can be found in Table 3.3.

Table 3.3. $^{12}$C/$^{13}$C kinetic isotope effects for the acid-catalyzed decarboxylation of 2,4-dimethoxybenzoic acid

<table>
<thead>
<tr>
<th>[HClO$_4$], wt %</th>
<th>$f$</th>
<th>$N_x$</th>
<th>$N_{x0}$</th>
<th>CKIE</th>
</tr>
</thead>
<tbody>
<tr>
<td>42%</td>
<td>0.13</td>
<td>0.010498</td>
<td>0.010712</td>
<td>1.0213</td>
</tr>
<tr>
<td></td>
<td>0.13</td>
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<tr>
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<td>0.010497</td>
<td>0.010712</td>
<td>1.0225</td>
</tr>
<tr>
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<td>0.25</td>
<td>0.010500</td>
<td>0.010712</td>
<td>1.0221</td>
</tr>
<tr>
<td>Av.</td>
<td></td>
<td></td>
<td></td>
<td>1.022 ±0.002</td>
</tr>
<tr>
<td>10%</td>
<td>0.13</td>
<td>0.010693</td>
<td>0.010710</td>
<td>1.0018</td>
</tr>
<tr>
<td></td>
<td>0.13</td>
<td>0.010692</td>
<td>0.010710</td>
<td>1.0019</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0.010694</td>
<td>0.010710</td>
<td>1.0018</td>
</tr>
<tr>
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<td>0.25</td>
<td>0.010693</td>
<td>0.010710</td>
<td>1.0019</td>
</tr>
<tr>
<td>Av.</td>
<td></td>
<td></td>
<td></td>
<td>1.002 ±0.002</td>
</tr>
</tbody>
</table>
4.1 Acidic hydrolytic decarboxylation in general

Though the acid-catalyzed decarboxylation of carboxylic acids has been known for many years, only recent work has helped to clarify the operative mechanism. The advances of instrumentation with improved resolution and methodologies that allow for simultaneous detection of both rate and stable isotope ratio have led to insights regarding the importance of intermediates involved in the decarboxylation reaction. In addition, mathematical modeling with extrapolation has enabled the calculation of the energy and feasibility of these intermediates.

The energy of intermediates in the decarboxylation of positional isomers of pyrrole- and indole-carboxylic acids is reflected in the observed carbon kinetic isotope effect (CKIE) and the observed rate of reaction. The difference among isomers results from the preferential site of protonation of the respective aromatic ring structures that furnish the necessary intermediate for decarboxylation to take place. Between isomeric pairs, where protonation is less favoured, the observed rates of reaction and CKIEs are suppressed. This is a result of the requirement of production of protonated carbonic acid (PCA) upon carbon-carbon bond-breaking. Thus, we see that PCA serving as a leaving group is the key to defining the intermediates of acid-catalyzed decarboxylation reactions.

By expanding the substrate scope, I have outlined the importance of PCA as the only viable product in acid-catalyzed decarboxylation mechanisms. Measurement of the CKIEs along with kinetic analysis and energetic considerations have provided evidence that leads to the conclusion that hydrolytic decarboxylation is the most likely mechanism. Further expansion to reaction of 2,4-dimethoxybenzoic acid illustrates that this mechanism is not confined to electron-rich heterocycles. Further extension to aliphatic carboxylic acids is likely. Our studies establish the reality that wherever acid-catalyzed decarboxylation has been reported, it is likely that the hydrolytic mechanism is operating.

Studying the isomeric series of heterocyclic carboxylic acids has also led to the development of useful experimental methods. The combination of pressure-monitored headspace analysis and compound-specific isotope analysis (CSIA) has provided an accurate and robust system for
determining stable isotope fractionation in reactions that produce gaseous products. This methodology has the potential to be extended to any gas-producing reactions where measurement of isotope effects is also desired.

4.2. Lewis acid-catalyzed reactions

Investigations into electrophilic aromatic substitution reactions involving carbon dioxide that proceed in the presence of Lewis acids have been shown.\textsuperscript{68-70} These reactions are typically performed under Friedel-Crafts conditions. Olah and co-workers suggest that the increased electrophilicity of the Lewis acid-carbon dioxide complex is well-suited for forming carbon-carbon bonds with aromatic substrates. However, since the energy of the Lewis acid complex should parallel that of protonated carbon dioxide, it is likely that these intermediates are also prohibitively high in energy. Therefore, a possible alternative is that carboxylation in the presence of Lewis acids proceeds via a complex with PCA. Such a complex still provides the necessary electrophilic activation and reaction products as the kinetically equivalent carbon dioxide route (considering that water concentrations would not be detected). Interestingly, the primary report from Olah and co-workers for the detection of PCA has illustrated its viability as a superelectrophile\textsuperscript{71} and carboxylating agent in the formation of carbamates.\textsuperscript{39}

4.3. Biological (de)carboxylation mechanisms

Many researchers have turned to biology for inspiration when it comes to capturing carbon dioxide.\textsuperscript{72} The most abundant enzyme,\textsuperscript{73} Rubisco, enables plants and photosynthetic organisms to meet their requirements for organic carbon. Carboxylase enzymes involved in catabolic processes attach carbon dioxide to non-polar substrates to increase solubility and excretion. This also minimizes their interaction with sensitive lipophilic biological components. These carboxylases have been exploited to limited extents\textsuperscript{73} and often suffer from poor turnover rates and a greater propensity to proceed in the direction of decarboxylation. However, this thermodynamically favoured decarboxylation allows for facile biochemical assays and fast turnovers. The principle of microscopic reversibility states that if the mechanism in one direction
is known, then the mechanism in the opposite direction is also known. Therefore, carboxylation pathways can be elucidated from decarboxylation studies. To this end, researchers have been able to use decarboxylase enzymes for the reverse process to carboxylate electron rich aromatic substrates, as summarized below.

Pyrrole-2-carboxylate decarboxylase catalyzes the nonoxidative decarboxylation of pyrrole-2-carboxylate to yield pyrrole and carbon dioxide. This enzyme has been shown to catalyze the reverse carboxylation at appreciable rates and with high regioselectivity (Scheme 4.1).74

Scheme 4.1. Regioselective carboxylation of pyrrole to form pyrrole-2-carboxylic acid

These reactions are typically forced in the direction of carboxylation by increased concentration of carbonate. This allows for a more accessible form in place of carbon dioxide, which decreases the entropic barrier of the reaction. Presumably, carbonate could interact with Brønsted acid residues within the enzyme to further increase the electrophilicity of the carbon atom to which the new bond will form. The local pH environment in an enzyme active site can be changed from that of the surrounding solution to facilitate this protonation to form a PCA-like intermediate. The requirement of an organic acid for this reaction supports this idea.74

Yoshida and coworkers75 isolated the thermophilic γ-resorcylate decarboxylase (γ-RDC) from *Rhizobium* sp. strain MTP-10005 that catalyzes the reversible decarboxylation of the substrate 2,6-dihydroxybenzoic acid (commonly named γ-resorcylate) (Scheme 4.2). The enzyme is specific for the decarboxylation of γ-resorcylate along with the substrate 2,3-dihydroxybenzoic acid. Unlike other decarboxylases, γ-RDC is coenzyme-independent and relies on hydroxyl group substitution for binding specificity.
Recent crystal structure elucidation by the same research group has shown that the catalytic activity of the $\gamma$-RDC enzyme is Zn-dependent.\textsuperscript{76} Wuensch and co-workers have used this enzyme for carboxylation studies to mimic the synthetic Kolbe-Schmitt reaction.\textsuperscript{77} The authors were able to carboxylate a variety of substituted phenol derivatives, leading to a powerful method for enzymatic carbon capture. The proposed carboxylation reaction for $\gamma$-RDC is shown in Scheme 4.3.

\begin{scheme}
\begin{center}
\includegraphics[width=\textwidth]{scheme4.3.png}
\end{center}
\end{scheme}

\textbf{Scheme 4.3.} Carboxylation of 1,3-dihydroxybenzene by the enzyme $\gamma$-RDC\textsuperscript{77}

The active site zinc ion coordinates carbonate and facilitates attack of the aromatic ring. This electrophilic activation mimics PCA. It can be envisaged then that PCA could be used as a carbon source for carbon-carbon bond-forming processes like these. The microscopic reverse
Decarboxylation reaction implicates water addition into the carboxyl group in order to achieve carbon-carbon bond-breaking. Via hydrolytic decarboxylation, the enzyme has circumvented the high energy protonated carbon dioxide and has effectively prevented any reversion by trapping carbon dioxide as zinc-coordinated carbonate.

4.4 Microscopic reversibility and carbon capture

Future work includes the testing of PCA and PCA-like substrates as activated carbon dioxide analogues for the formation of carbon-carbon bonds. This would provide a means to increase the electrophilicity of carbon dioxide in situ. The acid or activating metal could be added exogenously and would be recycled over the course of the reaction. As carbon dioxide represents a largely unused feedstock of carbon,\textsuperscript{78} any means for its direct use in carbon-carbon bond-making processes is desirable. Though it is electrophilic, its reactivity is attenuated by entropic factors. By hydrating and protonating carbon dioxide, it becomes a solution-phase reagent of increased concentration and a potentially powerful carboxylating reagent.
Appendix A
Data for Chapter 2

UV-vis spectrum of commercial indole-2-carboxylic acid (15109 Aldrich) ($H_0 = -3.84$).

UV-vis spectrum of commercial indole-3-carboxylic acid (284734 Aldrich) ($H_0 = -2.30$).
Comparison of the UV-vis spectrum of (solid) commercial indole (I3408 Aldrich) in water and the indole product formed upon decarboxylation of indole-2-carboxylic acid (dashed) and indole-3-carboxylic acid (dotted) in acidic media.

End product of Indole-carboxylic acid
decarboxylation and indole

![UV-vis spectrum graph](image-url)
Appendix B

Data for Chapter 3

UV-vis spectrum of commercial 2,4-dimethoxybenzoic acid (DMBA) (D131504 Aldrich).

Comparison of the UV-vis spectrum of commercial 1,3-dimethoxybenzene (solid) (126306 Aldrich) and the product formed upon decarboxylation of 2,4-dimethoxybenzoic acid (dashed) and in acidic media.
References


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