A Pulse Radiolysis Study of Some Dicarboxylic Acids of the Citric Acid Cycle. The Kinetics and Spectral Properties of the Free Radicals Formed by Reaction with the OH Radical

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Dedicated to Professor Schulte-Frohlinde on the occasion of his 60th birthday

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Pulse Radiolysis, Dicarboxylic Acid Radicals

The reactions between the OH radical and fumaric acid, maleic acid, malic acid and succinic acid were studied using pulse radiolysis techniques. The rates of reaction, the absorption spectra of the resultant free radicals and the rates of disappearance of those radicals were determined as a function of pH over virtually the entire pH range (pH 2–14).

Introduction

The free radicals formed by H-atom abstraction from many hydroxy-, mono- and polycarboxylic acids have been characterized by a variety of fast-kinetic techniques [1–4]. These studies led to some general conclusions, formulated by Hayon et al. [2], concerning the spectra and pK's of the radicals.

i) The absorption spectra of α-carboxyalkyl radicals have maxima in the spectral range of 320 to 350 nm with molar absorbances less than 1000 M⁻¹ cm⁻¹.

The absorption spectra of α-carboxy-α-hydroxyalkyl radicals have maxima near 250 nm with molar absorbances ranging from 5000 to 9000 M⁻¹ cm⁻¹.

The absorption spectra of polycarboxylic acid radicals are in general combinations of the characteristics of the corresponding monocarboxylic acid radicals.

ii) The dissociation constants for the carboxylic groups of polycarboxylic radicals are generally too close to be resolved but are usually higher than those of the parent compounds.

iii) The pK of the hydroxyl group on the same carbon as the radical site is generally 3–4 pH units lower than that of the parent compound while that on a carbon adjacent to the radical site is relatively unchanged.

The dicarboxylic acids studied here are fumaric and maleic acids (HO₂CCH=CHCO₂H, the trans and cis isomers, respectively), malic acid (HO₂CCH(OH)CH₂CO₂H) and succinic acid (HO₂CCH₂CH₂CO₂H). We undertook to determine the rates of reaction of these acids with the OH radical, the absorption spectra of the resultant free radicals and the rates of disappearance of these free radicals, all as a function of pH. Some of these radicals have been characterized by ESR methods [4] and as intermediates in photolytic pathways [5–7]. However, as we are interested in future studies of these acids with radiolytically generated oxidants [8, 9], the optical characteristics and rates of disappearance of these radicals are of particular importance. The extent to which each of these acids was studied makes it practical to discuss them individually.

Experimental

All solutions were prepared using water which, after distillation, had been passed through a Millipore ultrapurification system. Fumaric acid, maleic acid, d- and l-malic acids, succinic acid and oxaloacetic acid are all commercially available and were used without further purification. The pH was adjusted by the addition of potassium hydroxide (Apache Chemicals Inc., 99.999%) or perchloric acid (G. F. Smith Chemical Co., double distilled from Vycor); no buffers were used. Nitrous oxide (Matheson Co.) was used to saturate all solutions.

Routine spectrophotometric measurements were recorded on a Cary 210 UV/VIS spectrophotometer. All ⁶⁰Co gamma ray irradiations involved using sources with dose rates of 410 rads/minute and 700 rads/minute. Pulse radiolysis experiments were carried out using a 2 MeV van de Graaff generator with 0.4 to 0.9 μsec pulses and doses ranging from 100 to 700 rads; as described previously [10]. All reported rates are averages of 3–5 runs. The studies were carried out in an optical cell with a 6.1 cm light path. Both ferrous dosimetry, taking G(Fe³⁺) = 15.8, and thiocyanate dosimetry, taking G(−(SCN)₂⁻) = 6.13 and ε₄₇₂ mm = 7500 M⁻¹ cm⁻¹, were used as necessary.

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Results

Upon radiolysis of water the following radicals and molecules are produced [11]:

\[ 
\text{H}_2\text{O} \rightarrow \text{OH} (2.74), \epsilon_{\text{OH}} (2.76), \text{H} (0.55), \]
\[ \text{H}_2\text{O}_2 (0.72), \text{H}_2 (0.45) \]  
(1)

where the values in parentheses are G values, that is the number of molecules formed or transformed per 100 eV of energy dissipated in water. The saturation of an aqueous solution with \( \text{N}_2\text{O} \) effectively converts all hydrated electrons to OH radicals. A report of \( G(\text{OH}) = 3.2 \) for reaction (2) [12] suggests that at \( \text{N}_2\text{O} + e^- + \text{H}_2\text{O} \rightarrow \text{H}_2 + \text{OH}^- + \text{OH} \)  
(2)

\[ 
\epsilon_{\text{OH}} = 8.7 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1} 
\]

1 mM scavenger or less the total \( G(\text{OH}) \sim 6.0 \). All molar absorbances of radicals produced from the interaction of the dicarboxylic acid with OH are calculated assuming \( G(\text{OH}) = G(\text{radical}) = 6.0 \). At low pH, (pH < 3.5), \( \text{N}_2\text{O} \) begins to compete with \( \text{H}^+ \) for the \( e^- \) and \( \text{H} \) atoms are generated at the expense of OH radicals. Therefore, in systems where the transient radical is produced by addition of OH to a double bond, all pH studies were carried out at pH \( > 3.5 \).

Malic acid

The rates of reaction of both d- and l-malic acids with OH radicals were determined under pseudo first-order conditions \( (10^{-4} - 10^{-3} \text{ M malic acid, } 2.2 \times 10^{-2} \text{ M N}_2\text{O}, (2-8)\times 10^{-6} \text{ M } \text{OH}/\text{O}^- \text{ radical, } 25.1 \text{ °C, pH 1.5—14}) \) by monitoring the formation of the absorbance immediately following the pulse in the spectral range 235—330 nm (see Fig. 1). The rate, \( k_3 = 8.2 \pm 1.6 \times 10^8 \text{ M}^{-1} \text{ sec}^{-1} \), was found to be identical for both \( \alpha \)- and \( \epsilon \)-forms of the acid and independent of wavelength and pH (1.5—10.0). This is in reasonable agreement with the value obtained by Scholes et al. [13] from a competition study with thymine \( (k_3' = 5.4 \times 10^8 \text{ M}^{-1} \text{ sec}^{-1} \text{ at pH 2.0—2.2}). \)

A complete description of the rate of reaction of OH' with malic acid as a function of pH (range studied, 1.5—14.0) must take into consideration the \( \text{O}^- \) species:

\[ \text{HOOC}^-\text{CH(OH)}^-\text{CH}_2\text{COOH} + \text{OH} \rightarrow \text{a-malate} \]  
(3)

\[ \text{"OOC}^-\text{CH(OH)}^-\text{CH}_2\text{COO}^- + \text{O}^- \rightarrow \text{a-malate} \]  
(4)

\[ \text{OH} + \text{OH}^- \rightleftharpoons \text{O}^- + \text{H}_2\text{O} \]  
(5)

\[ k_{\text{OH}} = 1.26 \times 10^{-12} \text{ M} \]  
(14)

where

\[ k_{\text{obs}} \text{ M}^{-1} \text{ sec}^{-1} = \left[ \frac{k_3 + k_4(K_{\text{OH}}/\text{H}^+)}{1 + (K_{\text{OH}}/\text{H}^+)} \right] \]  
(1)

The solid line in Fig. 2A was calculated using equation (1) and taking \( k_3 = 8.2 \times 10^8 \text{ M}^{-1} \text{ sec}^{-1}, k_4 = 1.2 \times 10^8 \text{ M}^{-1} \text{ sec}^{-1} \), both rates determined from the respective plateau regions, and \( K_{\text{OH}} \) as given above. The calculated rate curve is in good agreement with the experimentally determined values except at very high pH. As is apparent, the dissociation constants for malic acid (pK_1 = 3.4, pK_2 = 5.1) do not affect the rates of reactions (3) and (4), in which the acid is described for convenience as fully protonated. Any perturbations with pH of the OH/O" radical reaction with malic acid as a result of the ionization of the acid is negligible.

The changes in absorbance of the radical as a function of pH yield the various pK's of the radical

\[ \text{OH} + \text{OH}^- \rightleftharpoons \text{O}^- + \text{H}_2\text{O} \]  
(5)

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(14)

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The changes in absorbance of the radical as a function of pH yield the various pK's of the radical
Fig. 2. Observed rates vs pH for (A) the reaction between malic acid and OH/O⁻ radicals, (B) the disappearance of the malic free radical (see text for conditions); the three curves were obtained assuming (-----) \( k_{10} = k_{12} = k_{14} = 0 \), ( ) \( k_{10} = k_{11}, k_{12} = k_{13}, k_{14} = k_{15} \) and (----) \( k_{10} = 1/2 (k_9+k_{11}), k_{12} = 1/2 (k_{11}+k_{13}), k_{14} = 1/2 (k_{13}+k_{15}) \).

(Fig. 3). Here, \( pK_6 = 4.0 \) and \( pK_7 = 5.7 \) refer to the two carboxylic groups while \( pK_8 = 11.0 \) represents the dissociation constant of the \( \alpha \)-hydroxyl group. As the carboxylic acid groups are sterically different, it will be assumed for the sake of convenience that \( pK_6 \) is associated with the dissociation constant of the \( C_1 \)-carboxylic group. The acid-base equilibria of the malate free radical are, therefore:

\[
\begin{align*}
\text{HOOC-CH}_2-\dot{\text{C(OH)}}-\text{COOH} & \rightleftharpoons \text{HOOC-CH}_2-\dot{\text{C(OH)}}-\text{COO}^- + \text{H}^+ \quad (6, -6) \\
\text{HOOC-CH}_2-\dot{\text{C(OH)}}-\text{COO}^- & \rightleftharpoons \text{HOOC-CH}_2-\dot{\text{C(OH)}}-\text{COO}^- + \text{H}^+ \quad (7, -7) \\
\text{"OOC-CH}_2-\dot{\text{C(OH)}}-\text{COO}^- & \rightleftharpoons \text{"OOC-CH}_2-\dot{\text{C(OH)}}-\text{COO}^- + \text{H}^+ \quad (8, -8)
\end{align*}
\]

The rate of disappearance of the malate free radical as a function of pH can be described by Equation (II), which involves the aforementioned three equilibria [(6, -6), (7, -7) and (8, -8)] and the following reactions:

\[
\begin{align*}
\text{HOOC-CH}_2-\dot{\text{C(OH)}}-\text{COOH} + \\
\text{HOOC-CH}_2-\dot{\text{C(OH)}}-\text{COO}^- \rightarrow \text{Product(s)} \\
\text{HOOC-CH}_2-\dot{\text{C(OH)}}-\text{COO}^- + \\
\text{HOOC-CH}_2-\dot{\text{C(OH)}}-\text{COO}^- \rightarrow \text{Product(s)} \\
\text{HOOC-CH}_2-\dot{\text{C(OH)}}-\text{COO}^- + \\
\text{HOOC-CH}_2-\dot{\text{C(OH)}}-\text{COO}^- \rightarrow \text{Product(s)} \\
\text{"OOC-CH}_2-\dot{\text{C(OH)}}-\text{COO}^- + \\
\text{"OOC-CH}_2-\dot{\text{C(OH)}}-\text{COO}^- \rightarrow \text{Product(s)} \\
\text{"OOC-CH}_2-\dot{\text{C(OH)}}-\text{COO}^- + \\
\text{"OOC-CH}_2-\dot{\text{C(OH)}}-\text{COO}^- \rightarrow \text{Product(s)} \\
\text{"OOC-CH}_2-\dot{\text{C(OH)}}-\text{COO}^- + \\
\text{"OOC-CH}_2-\dot{\text{C(OH)}}-\text{COO}^- \rightarrow \text{Product(s)}
\end{align*}
\]

Fig. 3. Change in O.D. as a function of pH for the transient formed in the reaction between OH/O⁻ and malic acid, determined at 280 nm (see text for conditions).
\[ k_{\text{obs}}, \text{M}^{-1}\ \text{sec}^{-1} = \frac{k_9 + k_{10}Y_1 + k_{11}Y_1^2 + k_{12}Y_1^3 + k_{13}Y_2 + k_{14}Y_3}{(1 + Y_1 + Y_2 + Y_3)^2} \]

(II)

where \( Y_1 = (K_6/H^+) \), \( Y_2 = (K_7/H^+) \) and \( Y_3 = (K_8/H^+) \).

The values for \( k_9 = 2.5 \times 10^6 \text{M}^{-1}\ \text{sec}^{-1}, k_{13} = 2.0 \times 10^6 \text{M}^{-1}\ \text{sec}^{-1} \) and \( k_{15} = 2.0 \times 10^6 \text{M}^{-1}\ \text{sec}^{-1} \) were determined experimentally from the respective plateau regions shown in Fig. 2B (pH range 1.3–2.3 yields \( k_9 \), pH 7–9 yields \( k_{13} \) and pH 12–13 yields \( k_{15} \)). An approximate value for \( k_{11} = 1.3 \times 10^6 \text{M}^{-1}\ \text{sec}^{-1} \) can be estimated from the slight plateau between pH 4.5 and 5.0.

As \( k_{10}, k_{12} \) and \( k_{14} \) cannot be directly determined from the experimental data, three sets of arbitrary rate constants for these reactions were chosen and corresponding curves of \( k_{\text{obs}} \) vs pH were calculated. As is apparent from Fig. 2B, all lines fit reasonably well and a kinetic fit to the experimental data is hence not meaningful. The experimentally determined rates of disappearance of the malate free radical were calculated using the molar absorbances of the free radical that are listed in Table 1.

**Fumaric/maleic acids**

The rates of reaction of OH radicals with fumaric and maleic acids were measured under pseudo first-order conditions \( [(1–5) \times 10^{-4}\text{M} \text{ac}id, 2.2 \times 10^{-2}\text{M} \text{N}_2\text{O}, (2–8) \times 10^{-6}\text{M} \text{OH/O}^- \text{radical, 25.1 °C, pH 4–10.5} \) by monitoring the appearance of the transient formed immediately following a 0.7 \( \mu \text{sec} \) pulse in the spectral region between 280–400 nm (see Fig. 4). Since the rate, \( k_{16} = (6.0 \pm 0.8) \times 10^6 \text{M}^{-1}\ \text{sec}^{-1} \), was found to be identical for both fumaric and maleic acid and is invariant with wavelength and pH (4–10.5), both acids/radicals will be discussed in terms of fumaric acid only. Analogous rates measured by Walling et al. [15] using Fenton's reagent \( (k_{16}' = 1.1 \times 10^9 \text{M}^{-1}\ \text{sec}^{-1} \) and \( k_{16}'' = 4.6 \times 10^8 \text{M}^{-1}\ \text{sec}^{-1} \) for fumaric acid and maleic acids respectively at pH 1) differ from \( k_{16} \); the differences may be due to the presence of metal cations and/or the lower pH in the former work. Our own work could not be carried out below pH 4 for reasons discussed previously concerning the H atom addition to the double bond but, as illustrated with malic acid, pH effect between pH 1 and pH 4 should be negligible.

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**Table 1. Spectral data and dissociation constants for some dicarboxylic acids and derived radicals formed from OH/O^- interaction with the acids.**

<table>
<thead>
<tr>
<th>Acid</th>
<th>( pK_{\text{Acid}} )</th>
<th>( pK_{\text{Radical}} )</th>
<th>( e \ \text{[M}^{-1}\ \text{cm}^{-1} )</th>
<th>( \lambda_{\text{max}} \ \text{[nm]} )</th>
<th>pH range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malic</td>
<td>3.44</td>
<td>4.0</td>
<td>3150</td>
<td>235</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>5.11</td>
<td>5.7</td>
<td>2850</td>
<td>240</td>
<td>7–10</td>
</tr>
<tr>
<td></td>
<td>11.0</td>
<td>2600</td>
<td>260</td>
<td>12.5–13.5</td>
<td></td>
</tr>
<tr>
<td>Fumaric</td>
<td>3.03</td>
<td>1160</td>
<td>320</td>
<td>3.8–5.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.44</td>
<td>850</td>
<td>320</td>
<td>6.0–10.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt; 12.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Succinic</td>
<td>4.16</td>
<td>4.0</td>
<td>330</td>
<td>290</td>
<td>1.5–3.0</td>
</tr>
<tr>
<td></td>
<td>5.61</td>
<td>780</td>
<td>280</td>
<td>6.0–10.0</td>
<td></td>
</tr>
</tbody>
</table>

\( a \) Not a maximum, see Fig. 1; \( b \) measurements not made below pH 3.8, see text and Fig. 6; \( c \) both pKs are indistinguishable, see text and Fig. 9.
A kinetic description of the overall mechanism for the reaction between OH/O⁻ and fumaric acid,

\[
\text{HOOC–CH=CH–COOH + OH →} \\
\beta\text{-malate radical}
\]

\[
^\cdot\text{OOO}=\text{CH}=\text{CH}=\text{COO}^− + O^− → \\
^\cdot\text{O}=\text{C}−\text{CH}=\text{CH}=\text{CO}_2^− \tag{17}
\]

analogous to that for malic acid, using equation (I), \(k_{16} = 6 \times 10^9 \text{M}^{-1}\text{sec}^{-1}\) and \(k_{17} = 4 \times 10^7 \text{M}^{-1}\text{sec}^{-1}\), leads to a curve of \(k_{\text{calc}}\) vs pH (see Fig. 5, upper curve). The value of \(k_{17}′ = 1 \times 10^7 \text{M}^{-1}\text{sec}^{-1}\) suggested by Neta et al. [16] is in reasonable agreement with these results.

The change in absorbance of the β-malate radical with pH (see Fig. 6) indicates that in this system either the pK’s of both carboxylic groups cannot be separated and are approximately 5.2 or that pK, lies below pH 3.8. The pK of the hydroxyl group is much higher than pH 11 but cannot be determined accurately by this method as the reaction between OH and fumaric acid leads to OH addition while O⁻ is known to abstract an H atom.

The observed rate of disappearance of the β-malate free radical as a function of pH is illustrated in Fig. 5. The molar absorbances used to calculate these second-order rates are given in Table I. Since a calculated \(k_{\text{disappearance}}\) vs pH curve would involve an arbitrary choice of individual rate constants (see discussion involving malic acid) it is omitted. The increase in rate at pH > 10.0 is most likely a result of two different mechanisms, that is OH addition and H abstraction. The radical resulting from H abstraction should be more reactive and is generated in increasing quantity with increasing pH.

**Succinic acid**

The rate of reaction of succinic acid with OH radicals was determined under pseudo first-order conditions (\(10^{-3}−10^{-4}\) M succinic acid, \(2.2 \times 10^{-2}\) M N₂O, \((2−8) \times 10^{-6}\) M radical, 25.1 °C, pH 1.5−10.0) by monitoring the absorbance immediately following the pulse in the spectral region of 250−350 nm (see Fig. 7). The rate was found to be independent of wavelength and pH under these conditions; \(k_{18} = (3.1 \pm 0.3) \times 10^8 \text{M}^{-1}\text{sec}^{-1}\). This is in reasonable agreement to a value

\[
\text{HOOC–CH₂–CH₂–COOH + OH →} \\
succinate radical \tag{18}
\]

reported previously, \(k_{18} = 1.4 \times 10^9 \text{M}^{-1}\text{sec}^{-1}\) at pH 2.0−2.2 [15]. The rate of reaction of succinate with
O\(^-\) is reported as \(k_{19} = 1.35 \times 10^8 \text{M}^{-1}\text{sec}^{-1}\) [10] and as that was so similar to the analogous OH

\[
\text{\textsuperscript{-}OOC-CH}_2-\text{CH}_2-\text{COO}^- + \text{O}^- \rightarrow \text{succinate radical}
\]

reaction, we did not pursue this system.

The change in absorbance of the succinate radical as a function of pH (Fig. 8) indicates a pK near 4. Actually, the shape of the O.D. vs pH curve suggests that there may be two pKs in that pH range (3–5), at 3.3 and 4.3, but the intermediate plateau is too slight to be definitive.

The observed disappearance of the transient occurred by a second-order process (0.1–0.001 M acid, otherwise same) and rates were calculated using the molar absorbances given in Fig. 7. The rate of disappearance as a function of pH (Fig. 9) shows that the fully protonated species disappears at \(k_{20} = 4 \times 10^8 \text{M}^{-1}\text{sec}^{-1}\) while the fully

\[
\text{HOOC-CH}_2-\text{CH}-\text{COOH} + \\
\text{HOOC-CH}_2-\text{CH}-\text{COOH} \rightarrow \\
\text{Product}
\]

ionized species disappears at \(k_{21} = 5 \times 10^6 \text{M}^{-1}\text{sec}^{-1}\). As the pKs are

\[
\text{\textsuperscript{-}OOC-CH}_2-\text{CH}-\text{COO}^- + \\
\text{\textsuperscript{-}OOC-CH}_2-\text{CH}-\text{COO}^- \rightarrow \\
\text{Product}
\]

uncertain, a theoretical curve would involve an arbitrary choice of rate constants and pKs (see discussion for malic acid) and was therefore not calculated.
Discussion

The spectral characteristics and dissociation constants of the various dicarboxylic acid radicals (Table I), in conjunction with previously published work on \( \alpha \) and \( \beta \) hydroxy radicals [3], indicate the following structures:

- Malic acid + OH →\n  \[ \text{HOOC-CH}_2-\text{CH(OH)-COOH} + \text{H}_2\text{O} \]
  (\( \alpha \)-malate radical)
- Fumaric/maleic acid + OH →\n  \[ \text{HOOC-CH-CH(OH)-COOH} \]
  (\( \beta \)-malate radical)
- Succinic acid + OH →\n  \[ \text{HOOC-CH}_2-\text{CH(COOH)} + \text{H}_2\text{O} \]
  (succinate free radical)

The product of the reaction between maleic/fumaric acid and OH\(^-\) was shown, in the presence of iron (Fenton system) [17], to yield malic acid. If, in our system, malic acid was one product, the other product should be oxalacetic acid

\[
\text{Oxalacetic acid} = 2[\text{HOOC-CH-CH(OH)-COOH}] \rightarrow \text{HOOC-CH}_2-\text{CH(OH)-COOH} + \text{HOOC-CH}_2-C(=O)-\text{COOH} \quad (22)
\]

No spectral evidence was found for oxalacetic acid which suggests that iron forms some intermediate complex with the transient that favors reaction (22), a pathway which is not favored in the absence of iron. Further product analysis on our system was not carried out at this time. It is the aspect of the project that will be pursued in future work.

Oxalacetic acid was generated as a product of the reaction between malic acid and OH\(^-\), as indicated by the growth of a permanent absorbance at 260 nm. Preliminary data indicates that the quantity of oxalacetic acid produced increased with increasing pH above 9, suggesting that the pathway is favored as the hydroxy group in the radical is deprotonated (pK = 11 for the hydroxy group). Again, further product analysis on these systems was beyond the scope of this project.

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