Zinc(II) Catalysed Pathways for the Enolization of Oxalacetate

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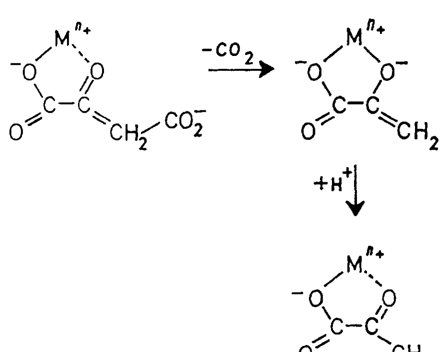
Summary Keto–enol tautomerization rates are reported for oxalacetate and zinc(II) oxalacetate complexes in acetate buffer solutions at 25°.

The decarboxylation and enolization of β-keto-acids are catalysed by certain enzymes that have a metal ion requirement. Metal ions in the absence of enzyme also catalyse these reactions, albeit at slower rates. An extensively studied enzyme-free model system for an important biological reaction is the metal-ion catalysed decarboxylation of oxalacetate (oxac^{2-}).[1] Experimental evidence indicates that the sequence shown in reaction (1) takes place.



(1)

The keto–enol isomerization, (I) $\rightleftharpoons$ (II), is coupled to decarboxylation,[1d–g] and when the rates of these two reactions become comparable, an interpretation of the rate data of one reaction is complicated by the presence of the other. Furthermore, decarboxylation proceeds *via* the keto form[1b,d] and when the enol is strongly stabilized by co-ordination to a metal ion, the rate of the reaction enol → keto may represent the limiting step in CO_2 release. To avoid these difficulties some authors[1b,2] have used 3,3-dimethyloxalacetate to study the effects of different metal ions on decarboxylation rates. Thus, knowledge of the effects of metal ions on the enolization rates of ligands such as oxac^{2-} is important not only in its own right but also for a proper understanding of the metal-ion catalysed decarboxylation rates. We present here the first quantitative rate law for metal-ion catalysed keto–enol interconversion of oxac.$^{2-}$

A Durrum–Gibson stopped flow apparatus was used in which acetate-buffered Zn^{II} solutions were mixed with oxac^{2-} solution similarly buffered (pH 3·0—4·9). The total initial oxac concentration was maintained at 1—3 × 10^{-4}M in the mixed solutions, while total initial zinc(II) chloride concentration was 1—20 × 10^{-2} M. The reaction mixtures were maintained at 25°, with I being kept at 0·1 by addition of KCl. Two relaxations were observed, after 30 s and after several minutes. The slower correlates with the rate of CO_2 evolution and therefore, the faster arises mainly from the keto–enol interconversion.

The relaxation data were analysed with an improved version of the computer routine CORNEK.[3] This includes the Marquardt CURVFIT routine[4] to obtain optimum values of rate constants for trial rate laws, and also includes a routine to fit complex stability constants and rate constants simultaneously.

The pathways important under our reaction conditions are given in the Table. Both Zn^{II} independent and dependent paths were indicated for keto–enol interconversion of oxalacetate. The sum of the forward and the backward rate constants, obtained from the relaxation data, is given in the Table for each reaction path. The forward and the backward rates were resolved from the sums

TABLE

Ketonization–enolization rates of oxalacetate and zinc(II) oxalacetate

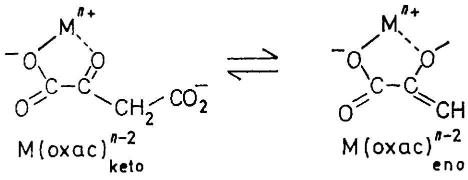
$$\mathrm{oxac}^{2-}_{\mathrm{keto}} \underset{k_b}{\overset{k_f}{\rightleftharpoons}} \mathrm{oxac}^{2-}_{\mathrm{enol}} \qquad K_{eq} = [\mathrm{enol}]/[\mathrm{keto}]$$

Reaction path	$(k_f + k_b)/\mathrm{M}^{-1}\,\mathrm{s}^{-1}$	K_{eq}	$k_f/\mathrm{M}^{-1}\,\mathrm{s}^{-1}$	$k_b/\mathrm{M}^{-1}\,\mathrm{s}^{-1}$	Ref.
$\mathrm{oxac}^{2-}_{\mathrm{keto}} + \mathrm{H}^+$	$1{\cdot}16 \times 10^3$	0·18	$1{\cdot}8 \times 10^2$	$9{\cdot}8 \times 10^2$	(1·5°)
	$(9{\cdot}1 \pm 0{\cdot}4) \times 10^3$	0·164[a]	$1{\cdot}28 \times 10^3$	$7{\cdot}8 \times 10^3$	this work (25°)
$\mathrm{oxac}^{2-}_{\mathrm{keto}} + \mathrm{HOAc}$	1·44	0·18	0·22	1·22	7 (1·5°)
.. .. ..	$10{\cdot}8_5 \pm 0{\cdot}50$	0·164	1·53	9·3	this work (25°)
$\mathrm{oxac}^{2-}_{\mathrm{keto}} + \mathrm{OAc}^-$	0·043	0·18	0·0066	0·036	7 (1·5°)
$\mathrm{H(oxac)}^{2-}_{\mathrm{keto}} + \mathrm{HOAc}$	$0{\cdot}23 \pm 0{\cdot}05$	0·12[a]	0·025	0·21	this work (25°)
$\mathrm{Zn(oxac)}_{\mathrm{keto}} + \mathrm{H}^+$ [b]	$(9{\cdot}2 \pm 0{\cdot}6) \times 10^2$	9	8×10^2	$0{\cdot}9 \times 10^2$	this work (25°)
$\mathrm{Zn(oxac)}_{\mathrm{keto}} + \mathrm{HOAc}$[b] ..	$2{\cdot}6 \pm 0{\cdot}8$	9	2	0·3	this work (25°)
$\mathrm{Zn(oxac)}_{\mathrm{keto}} + \mathrm{OAc}^-$ [b] ..	28 ± 4	9	25	3	this work (25°)
$\mathrm{Zn(oxac)}_{\mathrm{keto}}\mathrm{(OAc)}^- + \mathrm{HOAc}$[b]	$2{\cdot}8 \pm 2{\cdot}0$	9	2·5	0·3	this work (25°

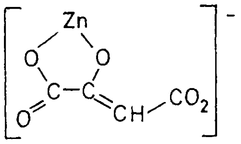
[a] Ref. 5. [b] log β [Zn(oxac)] = 2·22; log β [Zn(OAc)(oxac)$^-$] = 4·05. Ref. 6.

using the values for the keto–enol equilibrium constants of uncomplexed oxac^{2-} reported by Tate *et al.*,[5] and for Zn(oxac) a value found in our laboratory.[6]

Earlier, Banks[7] had investigated the rates of uncomplexed oxac^{2-} keto–enol interconversion at 1·5° at pH 5—10, and found the reactions to be proton and general acid catalysed with unobservable water solvent catalysis. Our results at 25° for the metal independent paths agree with these findings. We did not observe the slow acetate catalysed path reported by Banks,[7] probably because of our different experimental conditions. We did uncover, however, a path arising from the acetic acid catalysed isomerization of H(oxac)$^-$. This path is slightly slower than that for the corresponding reaction of oxac^{2-}.



(I) (II)



(III)

In the Zn$^{\mathrm{II}}$ complex the prototropic shift proceeds *via* analogous proton and general acid catalysed pathways. The forward rate constant for the reaction,

$$\mathrm{H}^+ + \mathrm{Zn(oxac)}_{\mathrm{keto}} \rightleftharpoons \mathrm{Zn(oxac)}_{\mathrm{enol}} + \mathrm{H}^+$$

is about 45% smaller than the rate constant for the corresponding reaction

$$\mathrm{oxac}^{2-}_{\mathrm{keto}} + \mathrm{H}^+ \rightleftharpoons \mathrm{oxac}^{2-}_{\mathrm{enol}} + \mathrm{H}^+$$

Electrostatic repulsion by Zn$^{\mathrm{II}}$ seems to account for this difference.

Zn(oxac) is considerably more susceptible to attack by acetate than is oxac^{2-} itself. The rate constant for the reaction,

$$\mathrm{Zn(oxac)}_{\mathrm{keto}} + \mathrm{OAc}^- \rightleftharpoons \mathrm{Zn(oxac)}_{\mathrm{enol}} + \mathrm{OAc}^-$$

is 1—2 times larger than the expected after allowing for temperature effects as reported by Banks.[7] The rate enhancement could arise from the ability of Zn$^{\mathrm{II}}$ to stabilize the intermediate enolate (III).

Reaction *via* a mixed complex, Zn(OAc)(oxac)$^-$, was indicated to be present. The rate constants for this species are not well defined by the data, but its acetic acid catalysed isomerization rate appears to be similar to that of Zn(oxac).

The proportion of Zn(oxac) which is present in the enol form (II), 90%, is greater than that reported by Tate for any of the uncomplexed forms, oxac^{2-}, H(oxac)$^-$, and H$_2$(oxac).[5] A comparison of the rate constants in the Table shows that the increase in the enol–keto ratio on complexing has its origins primarily in a decrease in the rate of the back reaction (enol → keto) rather than in an increase in the rate of the forward reaction. This seems to imply that the activated complex in this reaction resembles the keto form more than the enol.

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