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Mechanism of Base Catalyzed Oxidation of 2-(1-Hydroxyalkyl)thiazolium Ion with 3-Methyllumiflavin

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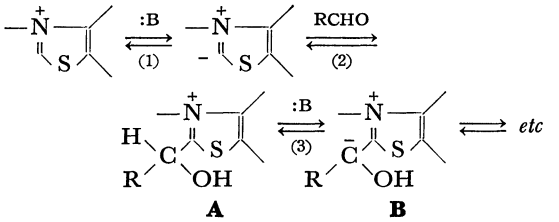
(Received January 30, 1980)

The oxidation of 2-(α-hydroxybenzyl)- and 2-(1-hydroxyethyl)thiazolium ions with 3-methyllumiflavin was studied kinetically in an aqueous buffer solution. The results indicate that the rate-determining step is carbanion formation followed by rapid oxidation. The carbanion formation was subjected to general base catalysis.

The mechanism of catalysis of thiamine coenzyme involves the formation of active aldehyde as a key intermediate[1] as shown in the following equation.

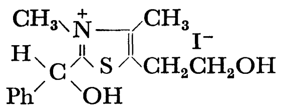


(1)

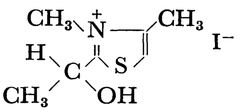
The first step (1) of ionization of 2-hydrogen of thiazolium ring was thoroughly investigated.[2] However, the mechanism of the subsequent deprotonation of **A** (step 3) has remained unclarified, whether it is subjected to a general base or a specific base catalysis, although α-hydrogen exchange of 2-(1-hydroxyethyl)thiazolium ion (**A**, R=CH_3) had been confirmed by NMR.[3] Recently, it has been shown by NMR that carbanion formation from 2-(1-hydroxyethyl)- and 2-(α-hydroxybenzyl)thiazolium ions is subjected to general base catalysis in aqueous buffer solutions.[4]

Flavins are reduced by carbanions such as those generated from α-hydroxy ketones. Usually, the reduction is so fast that the rate limiting step is the formation of carbanion.[5] Namely, the rate of carbanion formation can be measured spectrophotometrically by observing oxidation-reduction of flavin. The method allows the use of a much lower concentration of substrate than that for NMR. In addition to such a merit, the use of flavin is of interest as a model reaction of flavin pyruvate oxidase. This enzyme catalyzes the oxidative decarboxylation of pyruvate to give acetate and CO_2,[6] the flavoprotein being reduced by 2-(1-hydroxyethyl)-thiamine diphosphate.[7]

As a part of studies on the model thiamine catalysis,[8] a kinetic study was carried out on the reaction of 2-(α-hydroxybenzyl)- and 2-(1-hydroxyethyl)thiazolium ions (**1** and **2**) with flavin (**3**), together with the H-D exchange reaction of **1** for comparison.



1



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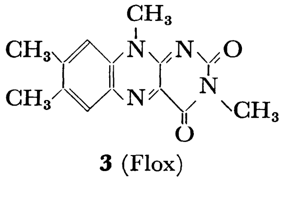
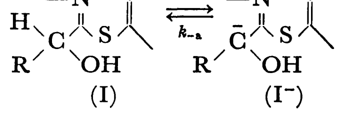


3 (Flox)

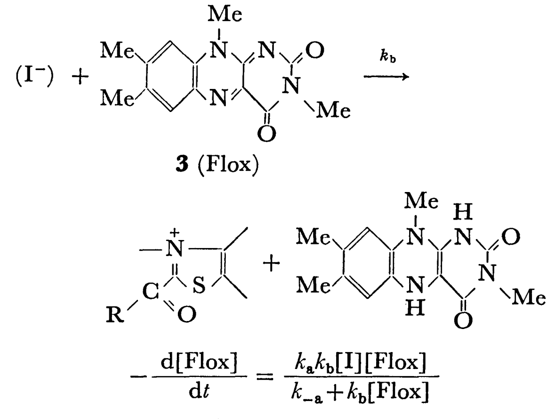
Results and Discussion

Carbanion Oxidation by Flavin. The reaction was followed by monitoring the decrease in absorption of **3** (Flox) at 443 nm under anaerobic conditions. The disappearance of Flox was found to be linear with the reaction time to 75% reaction. The reaction is of zero order with respect to flavin concentration as observed in the other α-hydroxy carbanion oxidations by flavins.[5] The bubbling of O_2 after the reaction regenerated more than 95% Flox in all the runs.

The results are in line with the mechanism of Eqs. 2 and 3, *i.e.* the rate limiting formation of carbanion (I^-) followed by a rapid oxidation. Thus, on the assumption of steady state with respect to (I^-), Eq. 4 can be derived resulting in the zero order dependency in [Flox] (Eq. 6) under the conditions of Eq. 5. The rates were also confirmed to be of first order with respect to (I) to give first order rate constant, k_a (Fig. 1).



(2)



(3)[9]

$$-\frac{d[\text{Flox}]}{dt} = \frac{k_a k_b[\text{I}][\text{Flox}]}{k_{-a}+k_b[\text{Flox}]} \tag{4}$$

$$k_b[\text{Flox}] \gg k_{-a} \tag{5}$$

$$-\frac{d[\text{Flox}]}{dt} = k_a[\text{I}] \tag{6}$$

$$k_a = k_{\text{OH}^-}[\text{OH}^-] + k_\text{B}[\text{B}] \tag{7}$$

The k_a values obtained under various conditions are summarized in Table 1. The pH-log k_a plots for compound **1** give a straight line of slope=0.93 (Fig. 2). The buffer concentrations were changed for Tris and carbonate buffers. The rate increase is linear with the

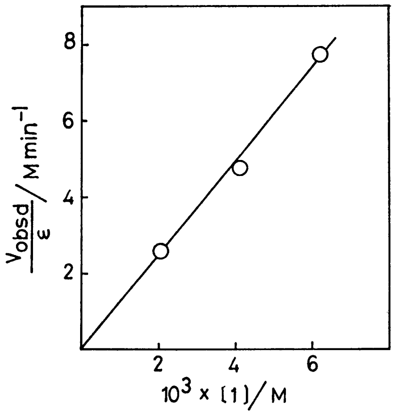


Fig. 1. Plots of V_{obsd} vs. [I].
pH=9.19 (0.1 M Borate), μ=0.5 (NaCl) at 30 °C, [Flox]=6.9×10^{-5} M.

TABLE 1. THE FIRST ORDER RATE CONSTANTS OF **3**(Flox) REDUCTION AT 30 °C

Compd	pH	[Buffer]/M	$10^4\times k_a$/min^{-1}
1	8.23$^{b)}$	0.1	1.69
	8.64$^{b)}$	0.1	3.86
	9.01$^{c)}$	0.1	9.63
		0.25	12.6
		0.4	13.8
		0.5	15.9
	9.23$^{b)}$	0.1	13.9
	9.45$^{d)}$	0.1	22.5
		0.2	24.5
		0.4	30.5
	9.53$^{b)}$	0.1	25.6
	9.78$^{b)}$	0.1	44.3
2	9.46$^{d)}$	0.1	0.392
		0.25	0.751
		0.5	1.14

a) [Flox]=6.9×10^{-5}M, [**1**]=[**2**]=2.07×10^{-3}M, μ=0.5 (NaCl). b) Borate buffer. c) Tris buffer. d) Carbonate buffer.

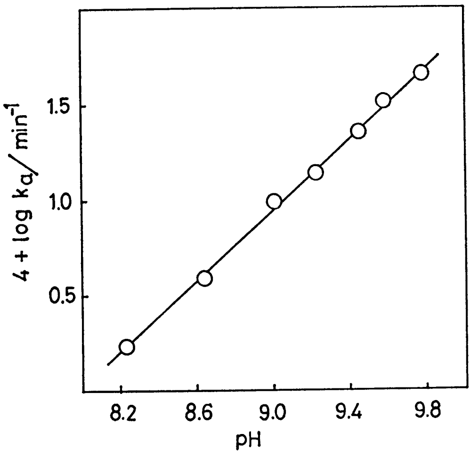


Fig. 2. pH-log k_a plots for **1** (see Table 1).

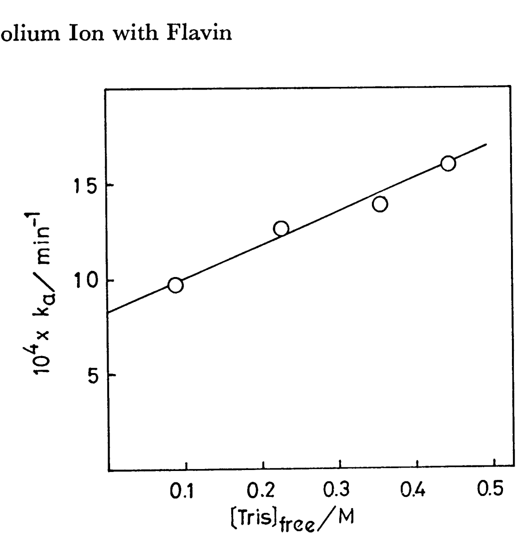


Fig. 3. Plots of k_a vs. Tris buffer concentration for **1** (pH=9.01, see Table 1).

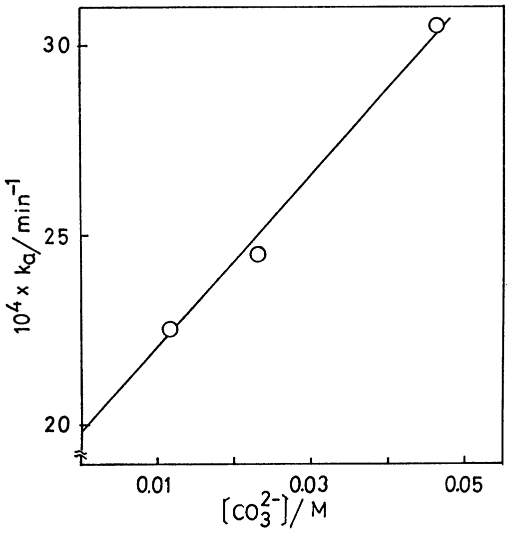


Fig. 4. Plots of k_a vs. carbonate buffer concentration for **1** (pH=9.45, see Table 1).

increase of buffer concentration in both cases (Figs. 3 and 4). Thus, k_a is represented by Eq. 7, where k_{OH^-} and k_B are the hydroxide ion and buffer (general base) catalyzed second order rate constants, respectively. The k_{OH^-} and k_{Tris} values for **1** are 91.3 and 1.66×10^{-3} M^{-1} min^{-1}, respectively in the Tris buffer (Fig. 3). For the carbonate buffer, k_{OH^-} and $k_{CO_3^{2-}}$ are 72 and 2.3×10^{-2} M^{-1} min^{-1}, respectively (Fig. 4). Since k_{OH^-} values should be the same, the average value, 82 M^{-1} min^{-1} can be used as the k_{OH^-} for **1**. In a similar way, k_{OH^-} and $k_{CO_3^{2-}}$ for **2** can be calculated to be 0.80 and 1.68×10^{-3} M^{-1} min^{-1}, respectively. The reactivities of **1** and **2** can then be compared by the ratio of k_{OH^-} (**1**)/k_{OH^-} (**2**)=10^2 and $k_{CO_3^{2-}}$ (**1**)/$k_{CO_3^{2-}}$ (**2**)=14, respectively. The ratios seem to be reasonable, since the carbanion formation should be much easier from **1** than from **2**.

The β value of the Brönsted plots could be regarded as a criterion to distinguish a general base from a specific base catalysis; the β value is close to unity in the case of specific base catalysis and *ca.* 0.5 for general base catalysis.[10] As shown in Fig. 5, the Brönsted plots of log k_a vs. pK_a of bases for **1** give a straigh line of slope=0.63 (β values). The β value for **2** can be calculated to be 0.5, from the available two data of k_{OH^-}

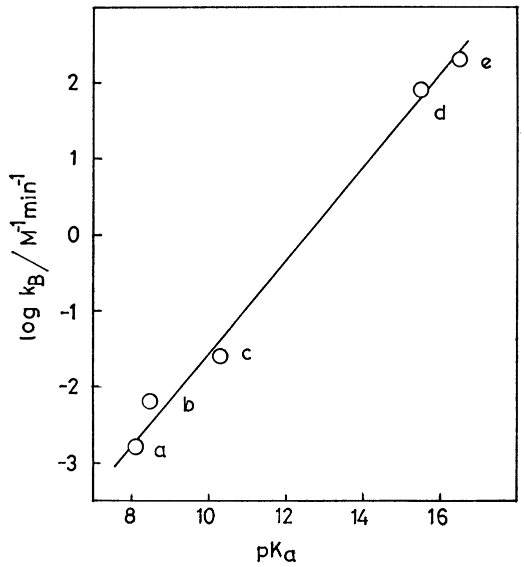


Fig. 5. The Brönsted plots for carbanion formation of **1**. a) k_{Tris}, pK_a=8.10,[11] b) k_{Tris} (NMR), pK_a=8.50,[12] c) $k_{\mathrm{CO_3^{2-}}}$, pK_a=10.3,[13] d) $k_{\mathrm{OH^-}}$, pk_a=15.6,[14] e) $k_{\mathrm{OD^-}}$ (NMR), pK_a=16.5.[14]

and $k_{\mathrm{CO_3^{2-}}}$. The β values indicate that the carbanion formation is a process of general base catalysis for both **1** and **2**.

H-D Exchange of 1. Since the carbanion formation is rate limiting in the above flavin reduction, the rates should be the same as those of H-D exchange of α-hydrogen of **1** and **2**. Thus, H-D exchange of **1** was also examined by means of NMR. The rate was followed by determining the relative intensities of the α-hydrogen and unexchangeable hydrogens of phenyl ring of **1**. First order kinetics was observed, although measurement of 1 : 5 proton ratio involves large experimental error. The rate constants (k_{obsd}) thus obtained, however, were found to involve the rate term of decomposition of **1** to the corresponding thiazolium ion and benzaldehyde as shown by $k_{\mathrm{obsd}}=k_{\mathrm{ex}}+k_{\mathrm{dec}}$. Therefore, in order to obtain k_{ex}, the rate of decomposition of **1** (k_{dec}) was determined spectrophotometrically by following the decrease of **1** at λ_{max} 267 nm (ε=10,000 M^{-1} cm^{-1}) under the same conditions as those of H-D exchange reaction. The results are given in Table 2. Although we have only two data, the k_{ex} values give $k_{\mathrm{OD^-}}=2.0\times10^2$ M^{-1} min^{-1} and $k_{\mathrm{Tris}}=6.0\times10^{-3}$ M^{-1} min^{-1} on the basis of Eq. 7. These rate constants should involve considerable error. Nevertheless, they fit reasonably on the straight line of Fig. 5, when we consider the relationship, pK_a(D_2O)=pK_a(H_2O)+0.4.[12]

The results indicate that the deprotonation of active aldehyde such as **1** and **2** proceeds *via* general base catalysis. It should be noted that the present method is superior to the usual NMR method. The rate of carbanion formation can easily be determined by the spectrophotometric method. Substrate concentration required (2×10^{-3} M) is much lower than that (0.2 M) for NMR method. Furthermore, the time necessary for rate measurement can be reduced a great deal. For example, if the rate constant (k_a or k_{ex}) is 4×10^{-3} min^{-1}, the half-life is 170 min in the H-D exchange reaction, whereas, in the flavin reduction, zero order rate is $V_{\mathrm{obsd}}=\Delta\mathrm{OD}/\Delta t=k_a[\mathrm{I}]\varepsilon=(4\times10^{-3})\times(2.07\times10^{-3})\times(1.2\times10^4)=0.099$, and when [Flox] is 6.9×10^{-5} M (OD=0.83), the half-life is only 4.6 min (=0.415/0.099). This makes the decomposition of **1** negligible during the course of rate determination.

TABLE 2. RATE CONSTANTS OF H-D EXCHANGE OF **1**

pD[a)]	[Tris]/M	$10^3\times k_{\mathrm{ex}}$/min^{-1} [b)]
9.20	0.1	1.4
9.20	0.4	2.9

a) pD=$\mathrm{pH_{obsd}}$+0.4. b) $k_{\mathrm{ex}}=k_{\mathrm{obsd}}-k_{\mathrm{dec}}$; $k_{\mathrm{dec}}=4.76\times10^{-4}$ min^{-}, [**1**]=0.2 M, μ=0.5(NaCl) at 30 °C.

Experimental

Materials and Methods. All melting points were uncorrected. UV and visible spectra were recorded with a Shimadzu UV 200 spectrophotometer, and NMR spectra with a Varian A-60 spectrometer. pH was measured with a Hitachi-Horiba F-7DE pH meter. Water used for kinetics was purified by treating deionized water with potassium permanganate and by distillation twice. Buffer reagents were of commercial special grade.

3,4-Dimethyl-2-(α-hydroxybenzyl)-5-(2-hydroxyethyl)thiazolium iodide (**1**) was prepared by the method reported. Mp 113—115 °C (from ethanol–acetone) (lit,[15] mp 111—113 °C).

2-(1-Hydroxyethyl)-4-methylthiazolium iodide (**2**) was prepared from the corresponding thiazole and methyl iodide in ethanol. Mp 149—151 °C (from methanol–ether) (lit,[2a)] mp 149—150 °C).

3-Methyllumiflavin (**3**) was prepared by the method reported. Mp 315 °C (from DMF) (lit,[16] mp 318 °C).

Kinetics. All kinetic measurements were carried out in an aqueous buffer solution containing 1% acetonitrile at 30 °C. 30 μl of the substrate (**1** or **2**) solution (1×10^{-1} M) was placed in the upper compartment of a Thunberg cuvette, and 3 ml of an aqueous buffer solution and 30 μl of Flox (6.9×10^{-3} M in acetonitrile) in the lower cell. Each solution was bubbled for 30 min with nitrogen which had been scrubbed with vanadous ion solution[17] and prehumidified with water. In the case of Tris buffer, nitrogen bubbling was performed in the dark to avoid the light-reduction of flavin. After equilibration at 30 °C for 15 min, the reaction was initiated by mixing the two solutions, the reaction being followed by observing the decrease of optical density at 443 nm. The slopes of optical density *vs.* time plots (ΔOD/Δt) are plotted against substrate concentration (Fig. 1, 1 M=1 mol dm^{-3}).

Kinetics for H-D Exchange. The reaction mixtures for the kinetics were prepared as follows. The thiazolium salt (0.1 mmol) was weighed accurately in a microtube, and 0.5 ml of buffer solution added, the tube being capped with septum-rubber. Nitrogen was bubbled for 5 min through an injection needle. The solution was transfered to the NMR tube with syringe and capped quickly. The rate constants were determined by following the relative intensities of α-proton and phenyl ring protons in a similar manner to that reported.[18]

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