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REMARKABLE CATALYTIC ACTIVITY OF 2-HYDROXYMETHYLIMIDAZOLE-Zn(II) COMPLEXES IN THE HYDROLYSIS OF p-NITROPHENYL PICOLINATE

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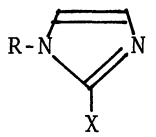
The zinc complexes of 2-hydroxymethylimidazole derivatives were found to be remarkably active catalysts for the hydrölysis of p-nitrophenyl picolinate. For example, 2-hydroxymethylimidazole is much more active than the 4-hydroxymethyl isomer, and N-benzyl-2-hydroxymethylimidazole is more active than pyridine-2-carboxaldoxime. These results are discussed in relation to the catalyses of zinc containing metalloenzymes.

The role of Zn(II) ion at the active site of zinc containing metalloenzymes has been the subject of intensive studies in recent years.1) The Zn(II) ion may function to activate water in carbonic anhydrase2) or to activate serine hydroxyl group in alkaline phosphatase.3) In the former enzyme, an imidazole group of histidyl residue is known to act as the ligand of Zn(II) ion. Therefore, it is important to device a model system in which imidazole and hydroxyl groups act cooperatively in the presence of Zn(II) ion for better understanding of the mechanism of enzymic catalysis. Surprisingly, however, such a model is very few in the literature. We now wish to report a simple, good example of such a model system, i.e. we have found that 2-hydroxymethylimidazole derivatives(**2** and **3**) are remarkably active catalysts in the presence of Zn(II) ion toward the hydrolysis of p-nitrophenyl picolinate(**8**). Closely related catalysts known in the literature are the Zn(II) ion complexes of pyridine-2-carboxaldoxime(**6**)4) and N-(β-hydroxyethyl)ethylenediamine(**7**).5) However, they are less active than **3**.

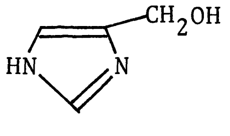
The rates of release of p-nitrophenol from **8** were measured spectrophotometrically.5) The kinetics were carried out in most cases under the conditions of excess



1 R=$PhCH_2$, X=H

2 R=H, X=CH_2OH

3 R=$PhCH_2$, X=CH_2OH



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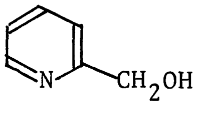

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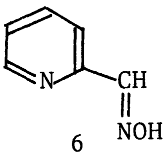

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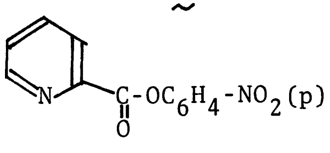

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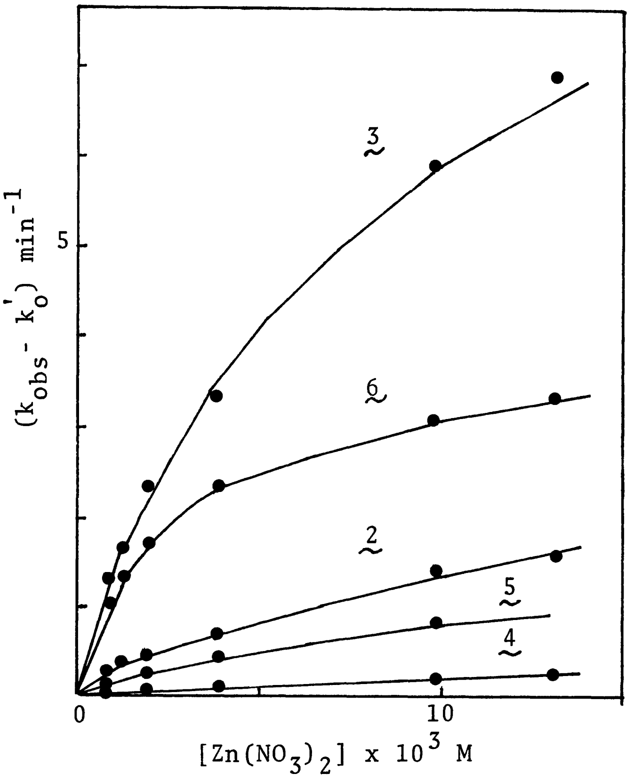

Fig. 1. Plots of rate constants for p-nitrophenol release from p-nitrophenyl picolinate(8) vs. Zn(II) ion concentration: [ligand]=$1x10^{-3}$M; see Table 1 for other experimental conditions.

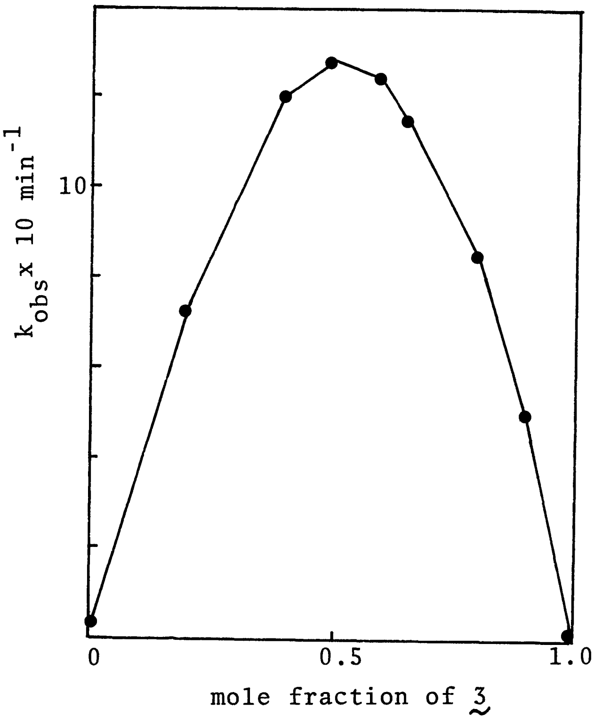

Fig. 2. The change in k_{obs} as a function of the mole fraction of 3. Total concentration of Zn(II) plus 3 is $2.0x10^{-3}$M; see Table 1 for other conditions.

catalysts (or reagents) over the substrate(8). In the case of N-benzyl-2-hydroxymethylimidazole(3), however, the reaction was also examined by using excess substrate, and it was confirmed that the reaction proceeded catalytically, i.e. the release of p-nitrophenol continued until completion of reaction in the first-order manner in the presence of ten molar excess of 8 over 3. The observed pseudo-first-order rate constants (k_{obs}) increased by increasing Zn(II) ion concentration to give a saturation kinetics as shown in Fig. 1. These saturation curves were then found to be nicely analyzed by assuming a 1:1 complex of Zn(II) ion and the ligand according to the scheme of Eqs. 1-3 and the rate equations of Eqs. 4-5.[6] This 1:1 stoichiometry of the complex was also confirmed for **3** with a kinetic version of the Job plot,[5] i.e. as shown in Fig. 2, the plots of k_{obs} values as a function of mole fraction of **3** gave a maximum at the mole fraction of 0.5 which corresponds to the stoichiometry of the kinetically active species. The results of analyses using Eq. 5 are shown in Table 1.

$$M + L \overset{K}{\rightleftharpoons} C \qquad (1)$$

$$S + C \xrightarrow{k_c} P \qquad (2)$$

$$S \xrightarrow{k_o'} P \qquad (3)$$

$$k_{obs} = k_o' + \frac{k_c K [L]_T [M]_T}{1 + K([M]_T + [L]_T)} \qquad (4)$$

$$\frac{1}{(k_{obs} - k_o')} = \frac{1}{k_c [L]_T} + \frac{1 + K[L]_T}{k_c K [L]_T} \cdot \frac{1}{[M]_T} \qquad (5)$$

Table 1. Rate constants for p-nitrophenol release from p-nitrophenyl picolinate(**8**) in the presence of Zn(II) and ligand[a)]

Ligand	pK_{1a} b)	K (M^{-1})	k_c ($M^{-1}min^{-1}$)
1	6.67 c)	-	($k_{M.L} = 9.7 \times 10^3\ M^{-2}min^{-1}$)
2	6.73 c)	142	2.56×10^3
3	6.24 c)	162	10.0×10^3
4	6.50 c)	95	0.31×10^3
5	5.55 c)	178	1.26×10^3
6	10.04 d)	557	4.00×10^3

a) Observed at 25°C, pH 7.06 (0.05 M 2,6-lutidine-HNO_3 buffer), $\mu = 0.1$ (KNO_3), [**8**]= 1×10^{-4}M.
b) These are the values of uncomplexed pyridinium ions except for that of **6**.
c) Obtained by titration, 25°C, $\mu = 0.01$(KNO_3).
d) For the hydroxyl group: see ref. 4a.

The rate law for **1** which lacks hydroxyl group was $k_{obs} = (k_o' + k_{M.L}[L]_T[M]_T)$ and any saturation phenomenon as in Fig. 1 was not observed. Presumably, this is due to a much smaller K value for **1**. At any rate, the $k_{M.L}$ value which corresponds to the k_cK values for other ligands is very small and suggests the importance of internal hydroxyl group for an effective catalysis. Among the ligands of **2**-**5**, all of which have a hydroxyl group, a comparison of **2** and **4** is particularly interesting. Construction of CPK molecular models indicates essentially no difference between these two isomers with respect to the spacial arrangements of imidazole nitrogen and hydroxyl oxygen atoms, and Zn(II) ion to form a complex. Therefore, a geometry of complexation alone appears to be difficult to explain why **2** is much more active than **4**. For the answer, it may be necessary to determine the pK_a of the hydroxyl groups of Zn(II)-ligand complexes, although difficult to do at present. Another comparison of **3** and **6** is also interesting. As already known, **6** is a good chelating agent toward Zn(II) ion.[4] Furthermore, an oxime hydroxyl group generally has a lower pK_a(10.04 for **6**) and is much better nucleophile than primary alcohols. Nevertheless, **3** is twice more active than **6**.

It is reasonable to assume that the hydroxyl group of **3** participates in the catalysis as a nucleophile. The role of Zn(II) is then to activate the hydroxyl group by complexation. Positioning of the substrate at proper orientation for the reaction to occur may also be an important role of Zn(II) ion. Further studies to modify **3** into more powerful catalysts are in progress in our laboratories.

References and Notes

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6) M=Zn(II), L=ligand, C=complex, S=substrate(**8**), K=association constant, k_c=catalytic rate constant, and $k_o' = k_o + k_L[L]_T + k_M[M]_T$. The k_o' values were less than 3% of k_{obs} values in the case of **3**: $k_o = 7.90 \times 10^{-4} min^{-1}$, k_L(**3**)$= 1.78\ M^{-1}min^{-1}$, and $k_M = 15.3\ M^{-1}min^{-1}$. The k_L values for other ligands were also much smaller than k_M value. Eq. 5 gives three linear plots by changing $[M]_T$ and $[L]_T$, i.e. $1/(k_{obs} - k_o')$ vs. $1/[M]_T$, 1/(intercept) vs. $[L]_T$, and slope vs. $1/[L]_T$, and these plots allow to calculate k_c and K values.

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