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Metal Ion-Assisted Intramolecular Nucleophilic Attack of the Amide Oxygen at Ester Linkages in 2-Pyridineoxime Esters of (*Z*)-α-(Acetylamino)cinnamic Acid

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Kinetics of the cyclization of 2-pyridinecarboxaldoxime ester (1a) or 2-acetylpyridineketoxime ester (1b) of (*Z*)-α-(acetylamino)cinnamic acid were measured in the presence and absence of divalent metal ions (Zn(II), Ni(II), and Cu(II)). Rate data for the hydrolysis of 2-acetylpyridineketoxime (*E*)-cinnamate were obtained in the presence and absence of Ni(II) or Cu(II) ion. The kinetic data measured in the presence of a divalent metal ion were analyzed in terms of complexation of the substrate with the metal ion and the subsequent conversion of the complex into products. Comparison of the rate data indicated that the metal ions catalyze the cyclization of 1a and 1b by enhancing the leaving ability of the oximate anions. The degree of acceleration of the expulsion of 2-acetylpyridineketoxime achieved by the bifunctional participation of Ni(II) ion and the amide group was much greater than the multiplication product of those achieved individually by Ni(II) ion and the amide group. The efficient cooperation between the metal ion and the amide group may also operate in the action of enzymes.

INTRODUCTION

The amide group is the polar functional group that is most abundant in enzymes. In this regard, cooperative catalytic action on organic reactions by the amide group together with other functional groups is an important subject in mechanistic organic chemistry as well as in enzymology. Previously, we demonstrated that the intramolecular nucleophilicity of the amide group can be as high as or even higher than the carboxyl group (*1*). In the preceding article, we reported that collaboration by the amide group and imidazole can lead to the effective nucleophilic attack of the amide oxygen atom at ester linkages (*2*).

Elucidation of catalytic features of metal ions as Lewis acids in organic reactions has been subject to intensive investigation in order to gain insights into the catalytic roles of metal ions in metalloenzymes (*3*). Several novel catalytic features of metal ion catalysis in organic reactions have been disclosed by previous studies performed in this laboratory (*4*). In particular, studies on the catalytic action of metal ions combined with other catalytic factors have been performed as more elaborate models of metalloenzymes.

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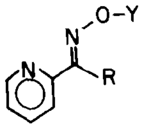
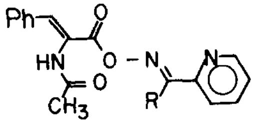
The mechanism of the metal ion catalysis in the hydrolysis of the acetyl esters (APA and APK)[2] containing 2-pyridinecarboxaldoxime (PA) or 2-acetylpyridineketoxime (PK) has been elucidated (*4*). In the present study, esters **1a**, **1b**, and **2** which also contain PA or PK as leaving groups were employed as substrates and kinetics of the cyclization reactions of **1a** and **1b** leading to **3** (Eq. [1]) and the hydrolysis of **2** were measured in the presence and absence of divalent metal ions:



a



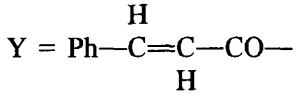
b

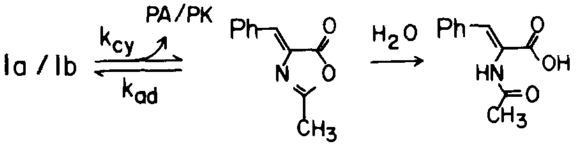
APA: R = H, Y = CH_3CO— 1a: R = H

APK: R = CH_3, Y = CH_3CO— 1b: R = CH_3

PA: R = H, Y = H

PK: R = CH_3, Y = H

2: R = CH_3,

$$Y = \mathrm{Ph{-}}\overset{\mathrm{H}}{\mathrm{C}}{=}\underset{\mathrm{H}}{\mathrm{C}}\mathrm{{-}CO{-}}$$



[1]

3 Ic

Thus, the intramolecular nucleophilic attack of the amide group at ester linkages with cooperative participation of metal ions is investigated.

MATERIALS AND METHODS

Materials. 2-Pyridinecarboxaldoxime (Z)-α-(acetylamino)cinnamate (**1a**) and *2-acetylpyridineketoxime (Z)-α-(acetylamino)cinnamate* (**1b**) were prepared by the reaction of the respective oximes (1.5 g) with **3** (0.1 g) in 1 : 1 mixtures (100 ml) of acetone and water (pH 7.0, 0.1 M *N*-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes)) at room temperature for 5 h. When about two-thirds of acetone was evaporated *in vacuo* and then the mixture was left overnight, white

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[2] Abbreviations used: APA, *O*-acetyl-2-pyridinecarboxaldoxime; APK, *O*-acetyl-2-acetylpyridineketoxime; PA, 2-pyridinecarboxaldoxime; PK, 2-acetylpyridineketoxime; Hepes, *N*-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

crystals were obtained, which were recrystallized from water–ethanol, mp 127–129°C for **1a** and 140–142°C for **1b**. *Anal.* C, H, N.

2-Acetylpyridineketoxime (E)-cinnamate (**2**) was prepared by the reaction of (*E*)-cinnamoyl chloride with PK in tetrahydrofuran and was recrystallized from ethanol, mp 88–90°C. *Anal.* C, H, N.

Acetonitrile, PA, and PK were purified by distillation or recrystallization. Water was distilled, deionized, and then used in kinetic measurements. Pure chloride salts of Zn(II), Ni(II), and Cu(II) ions were prepared as described previously (*4*).

Kinetic measurements. Reaction rates were measured with a Beckman Model 5260 uv/vis spectrophotometer, either by following the formation of **3** at 350–360 nm in the reactions of **1a** and **1b** or by measuring the absorbance changes at 265 nm in the reaction of **2**. Temperature was maintained at 25 ± 0.1°C with a Lauda Brinkman T-2 circulator. pH measurements were performed with a Dongwoo DF-215 pH meter as described previously (*2*). Buffers (0.01 M) used were monochloroacetate (pH 2–3.5), formate (pH 3.6–4.3), acetate (pH 4.4–5.4), 4-morpholineethanesulfonate (pH 5.5–6.7), Hepes (pH 6.8–8.2), borate (pH 8.3–9.7), and bicarbonate (pH 9.8–11). Ionic strength was maintained at 1.0 M with sodium chloride. Initially added concentrations (S_0) of substrates used in the rate measurements were $0.1–1 \times 10^{-4}$ M. When Zn(II), Ni(II), or Cu(II) ion was added to the reaction mixture, its concentration ([M]) was at least 20 times greater than S_0. Kinetic measurements were performed in the presence of 9.1% (v/v) acetonitrile.

RESULTS

Kinetics of the formation and breakdown of **3** during the cyclization of **1a** and **1b** in the presence and absence of divalent metal ions were analyzed with the biphasic absorbance changes corresponding to the changes in the concentration of **3** as described previously (*1, 2*). The rate constant for the first phase of the biphasic absorbance change was not affected by the addition of 2×10^{-4} M PA or PK and, thus, was taken as k_{cy} (Eq. [1]) (*1, 2*). The k_{cy} for **1a** and **1b** measured in the absence of divalent metal ions depended linearly on [OH$^-$], as was observed (*2*) in the reactions of the aryl and alkyl esters of **1c**. The consequent proportionality constant is denoted as k_{cy}^{OH} and summarized in Table 1 and its logarithmic value is plotted against pK_{LG} (pK_a of leaving group)[3] in Fig. 1.

In the presence of Zn(II), Ni(II), or Cu(II) ion, the k_{cy} value manifested saturation kinetic behavior with respect to [M], as observed (*4*) previously for the metal-catalyzed hydrolysis of APA or APK. The kinetic data were analyzed in terms of the scheme of Eq. [2], whose rate expression is Eq. [3]. In Eq. [2], S stands for the ester substrate:

$$\mathrm{SH^+} \xrightleftharpoons{K_a'} \mathrm{S} \xrightleftharpoons{\mathrm{M(II)},\ K_f} \mathrm{M(II)S} \xrightarrow{k_{cat}} \mathbf{3} + \mathrm{M(II)PA\ or\ M(II)PK} \qquad [2]$$

$$k_{cy} = \frac{k_{cat}K_f[\mathrm{M}]/(1 + [\mathrm{H^+}]/K_a')}{1 + K_f[\mathrm{M}]/(1 + [\mathrm{H^+}]/K_a')}. \qquad [3]$$

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[3] The pK_a values of PA, PK, M(II)PA, and M(II)PK are reported in the literature. (*4*(b), *4*(f)) except for Ni(II)PK, whose pK_a is 6.92 (ionic strength 0.3 M adjusted with NaCl, 3.2% (v/v) acetonitrile, at 25°C) as measured by spectral titration in this study.

TABLE 1

Values of Kinetic Parameters for the Cyclization of **1a** and **1b** and Hydrolysis of **2**[a]

Compound	Metal	Parameter	Value
1a	None	k_{cy}^{OH}	1.3×10^4
	Zn(II)	k_{cat}^{OH}	8.3×10^5
		K_f	540
	Ni(II)	k_{cat}^{OH}	4.6×10^6
		K_f	710
	Cu(II)	k_{cat}^{OH}	1.2×10^8
		K_f	410
1b	None	k_{cy}^{OH}	2.0×10^3
	Zn(II)	k_{cat}^{OH}	2.3×10^7
		K_f	620
	Ni(II)	k_{cat}^{OH}	7.9×10^7
		K_f	660
	Cu(II)	k_{cat}^{OH}	4.0×10^9
		K_f	2000
2	None	k_{OH}	81
	Ni(II)	k_{cat}^{OH}	1.4×10^5
		K_f	150
	Cu(II)	k_{cat}^{OH}	4.2×10^8
		K_f	1500

[a] Measured at ionic strength 1.0 M and 25°C in the presence of 9.1% (v/v) acetonitrile. Units are M^{-1} s^{-1} for k's and M^{-1} for K_f. Standard deviations were less than 10% and ca. 20% of the respective parameter values for k's and K_f, respectively.

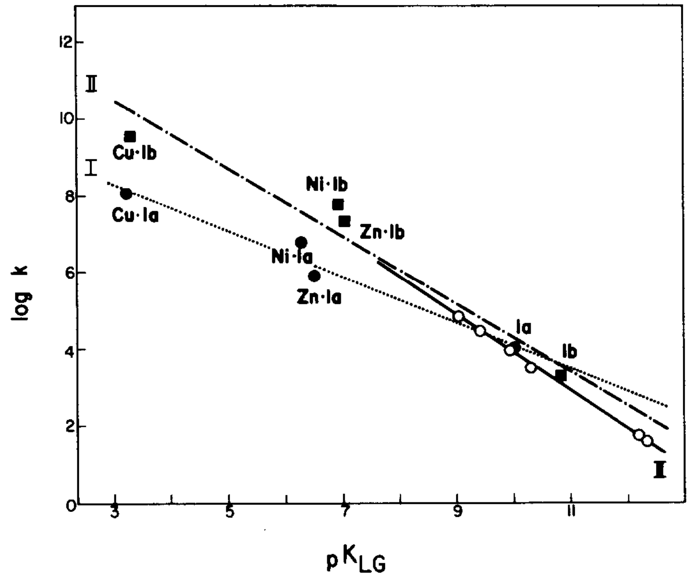

FIG. 1. Plots against pK_{LG} of the logarithmic values of rate constants for the formation of **3** due to the cyclization reactions. Line I, k_{cat}^{OH} for **1a** measured in the presence and absence of divalent metal ions. Line II, k_{cat}^{OH} for **1b** measured in the presence and absence of divalent metal ions. Line III, k_{cy}^{OH} for the aryl and alkyl esters of **1c**. Data for line III are taken from Ref. (2).

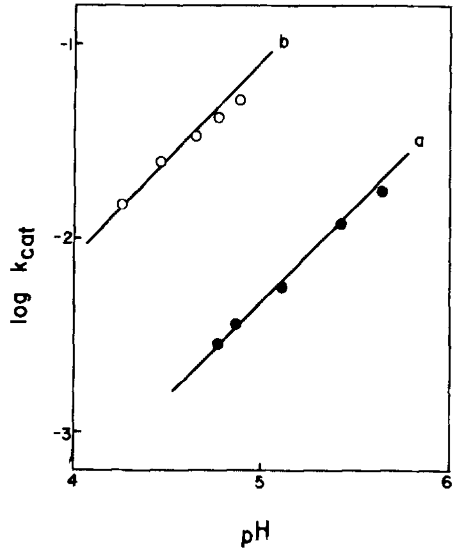

FIG. 2. Typical plots of log k_{cat} against pH. The data illustrated are those for the cyclization of **1a** (line a) and **1b** (line b) measured in the presence of Ni(II) ion. The lines are drawn with slope 1.0.

Except for the Cu(II)-catalyzed cyclization of **1b** ($pK'_a = 2.84$), $[H^+]$ was much smaller than K'_a under the experimental conditions and, thus, Eq. [3] was converted into Eqs. [4] and [5]:

$$k_0 = k_{cat}K_f[M]/(1 + K_f[M]) \quad [4]$$

$$1/k_0 = 1/k_{cat} + (1/k_{cat}K_f)(1/[M]). \quad [5]$$

Kinetic data were analyzed in terms of Eqs. [3]–[5], as reported previously for the metal-catalyzed hydrolysis APA or APK (*4*).

As illustrated in Fig. 2, the k_{cat} values obtained for the metal ion-assisted cyclization of **1a** or **1b** was proportional to $[OH^-]$. The proportionality constant (Eq. [6]) was denoted as k_{cat}^{OH}:

$$k_{cat} = k_{cat}^{OH}[OH^-]. \quad [6]$$

Values of kinetic parameters k_{cat}^{OH} and K_f are summarized in Table 1, and the logarithmic values of k_{cat}^{OH} are plotted against pK_{LG} in Fig. 1.

Kinetics of the hydrolysis of **2** to produce (*E*)-cinnamate and PK were measured in the presence or absence of Ni(II) or Cu(II) ion. Rate data obtained in the presence of the metal ion also conformed to Eqs. [2]–[6], and the value of k_{cat}^{OH} and K_f obtained therefrom are summarized in Table 1.[4] The second-order rate constant

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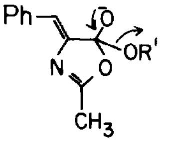
[4] The kinetic data for the Zn(II)-catalyzed hydrolysis of **2** did not deviate considerably from second-order kinetic behavior when [Zn(II)] was raised up to 7 mM at pH 6.6–7.4. The limited solubility of Zn(II) ion at these pH valves hampered further increase in [Zn(II)]. Saturation kinetic behavior, therefore, was not observed apparently due to the small formation constant for the Zn(II) complex of **2**.

(k_{OH}) for the alkaline hydrolysis of **2** measured in the absence of divalent metal ions is also summarized in Table 1.

DISCUSSION

As shown in the preceding paper (*2*), the nucleophilic attack of the amide oxygen in both aryl and alkyl esters of **1c** proceeds through the rate-determining breakdown of intermediate A. The cyclization of **1a** and **1b** in the presence and absence of the divalent metal ions would also involve the same mechanism. This is because the leaving ability of the anions of PA, PK, and their metal complexes is similar to or better than that of phenolate anions.



A

Parameters k_{cat}^{OH} and k_{cy}^{OH} represent the second-order rate constants for the hydroxide-assisted cyclization of the metal-complexed **1a**/**1b** and the metal-free **1a**/**1b**, respectively. The linear free energy relationship illustrated in Fig. 1 reveals β_{LG} values (slopes of the straight lines) of -0.59 ± 0.07 for the cyclization of **1a** or M(II) · **1a** (line I of Fig. 1) and -0.87 ± 0.14 for that of **1b** or M(II) · **1b** (line II of Fig. 1).[5] The β_{LG} obtained for the aryl and alkyl esters of **1c** (line III of Fig. 1) was -0.97 ± 0.01 (*2*).

The β_{LG} value reflects the difference in the effective charge on the leaving atom in the rate-determining transition state and the ground state (*2*). The different β_{LG} values estimated from lines I–III indicate different effective negative charges developed on the leaving oxygen atoms in the transition states for the three reactions. This may be related to the different degrees of expulsion of the leaving groups in the transition state. In the rate-determining transition states (B) for the metal ion-catalyzed hydrolysis of **1a** and **1b**, however, the electrostatic interaction between the oppositely charged atoms (metal and leaving oxygen) can reduce the effective negative charge on the oxygen atom. Thus, a smaller $|\beta_{LG}|$ can be observed even if the bond cleavage occurs to the same extent in the transition state. The reduction of effective charges by the electrostatic interaction between oppositely charged atoms in the transition state has also been observed in the nucleophilic substitution by quinuclidine derivatives on sulfite esters (*5*). The smaller

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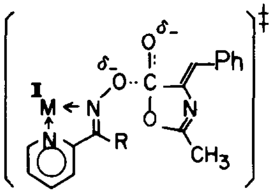
[5] The degree of acceleration by the metal ions is greater for **1b** compared with **1a** (e.g., 2×10^6 and 1×10^4 times, respectively, for **1b** and **1a** by Cu(II) ion). The greater rate enhancement by the metal ions for **1b** is attributable to the greater $|\beta_{LG}|$ value, although it is not easy to explain the effects of the methyl group on the β_{LG} value.



B

$|\beta_{LG}|$ values for the metal ion-catalyzed reactions compared with the spontaneous reaction, therefore, do not necessarily indicate the smaller degree of bond cleavage in the transition states.[6]

Comparison of the rate data for the hydrolysis of **2** (Table 1) indicates that the degrees of rate enhancement in the expulsion of PK caused by the addition of the metal ions are 1.7×10^3 times for Ni(II) ion and 5.2×10^6 times for Cu(II) ion.[7] Comparison of the rate data for the cyclization of **1b** with those for the hydrolysis of **2** in the absence of divalent metal ions reveals that the amide group enhances the rate of the expulsion of PK by 25 times. On the other hand, comparison of the rate data for the metal-catalyzed cyclization of **1b** with those for the spontaneous hydrolysis of **2** shows that the bifunctional participation of the amide group and the metal ion leads to the acceleration of the expulsion of PA by 1×10^6 times for Ni(II) ion and 5×10^7 times for Cu(II) ion.

Catalytic cooperative constant (c_{cat}) has been defined as Eq. [7], where k_c and k_u represent the rate constants for the catalyzed and the uncatalyzed reactions (*6*):

$$(k_c/k_u)_{\text{polycat}} = c_{\text{cat}}(k_c/k_u)_1(k_c/k_u)_2 \cdots (k_c/k_u)_n. \qquad [7]$$

The magnitude of c_{cat} may be referred to as the measure of the degree of cooperativity between the catalytic groups involved in a multifunctional catalytic

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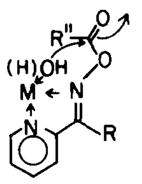
[6] Lines I and II would possess $|\beta_{LG}|$ values similar to that of line III if the data points for Cu · **1a** and Cu · **1b** were moved somewhat upward. The bimolecular rate constants observed for these data points are very large. Thus, the observed rate constants may represent the diffusion-controlled limit or may be underestimated because they are close to the diffusion-controlled limit. The smaller $|\beta_{LG}|$ values for lines I and II, therefore, may be ascribed to the underestimation of $k_{\text{cat}}^{\text{OH}}$ for the Cu(II)-catalyzed reactions.

[7] In the metal ion-catalyzed hydrolysis of APA, APK, or the pyridinecarboxyl esters of PK, the mechanism of C is operative (*4*). Here, the metal ion enhances the leaving ability of oximate anion and the metal-bound nucleophile makes intramolecular attack at the ester linkage:



C

system.[8] In the present reaction, c_{cat} is 23 for the collaboration by Ni(II) ion and the amide group and 0.4 for that by Cu(II) ion and the amide group.

A recent X-ray crystallographic study on human c-H-ras p-21 oncogene protein revealed that the catalytic site does not contain any polar side chains of amino acids (*7, 8*). It is possible, therefore, that the amide group might act as a nucleophilic functional group in the guanosine triphosphatase activity of the oncogene protein. In addition, a metal ion appears to be bound in the catalytic domain and participate in the catalytic process (*8*). Then, the cooperation between the metal ion and the amide group might play an important catalytic role.

ACKNOWLEDGMENT

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[8] A catalytic system containing several catalytic factors has been classified as being "supercooperative," "cooperative," "semicooperative," and "noncooperative" when c_{cat} is >1, 1, <1, and 0, respectively (*6*). According to this classification, the collaboration by Ni(II) ion and the amide group in the present reaction is highly supercooperative and that by Cu(II) ion and the amide group is almost cooperative. The expulsion of PK from **1b** by the Ni(II)-assisted nucleophilic attack of the amide group is one of the rate examples in which supercooperativity is demonstrated by two catalytic groups.