The Metal-ion-promoted Water- and Hydroxide-ion-catalysed Hydrolysis of Amides

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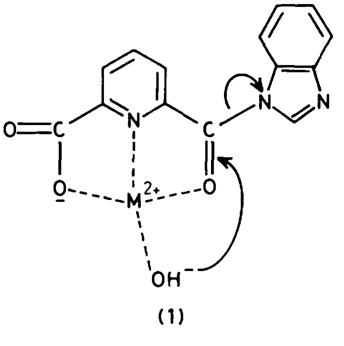
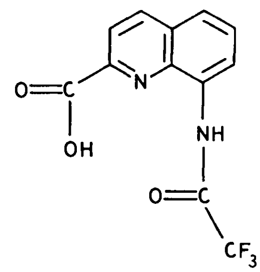
Rate constants have been determined for the hydrolysis of 8-trifluoroacetamidoquinoline-2-carboxylic acid and *N*-(6-carboxypicolinyl)-2,4-dinitroaniline in water at 70 °C. The hydrolysis reactions of these amides in the absence of metal ions are OH^- catalysed at pH values >7. Divalent metal ions (Co^{2+}, Ni^{2+}, and Zn^{2+}) have a significant catalytic effect on the rates of hydrolysis. Binding of the metal ions to the amides is strong, and saturation occurs at low metal ion concentrations (<0.01 mol dm^{-3}). At saturating concentrations of the metal ions k_{obs} is pH independent at pH < 7. Thus, the metal-ion-catalysed reactions are water reactions or a kinetic equivalent. Metal-ion-promoted OH^- catalysed reactions are not observed. In contrast, the Ni^{2+} catalysed hydrolysis of the β-lactam, *N*-(8-quinolyl)azetidin-2-one, proceeds through a metal-ion-promoted OH^- reaction. A 0.01 mol dm^{-3} concentration of Ni^{2+} (non-saturating) provides a rate enhancement of 10^5 over that observed in the absence of metal ion. The type of metal ion catalysis observed in amide hydrolysis, *i.e.*, pH independent or OH^- dependent, must be determined by the nature of the rate-determining step and the ease of C–N bond breaking. A metal-ion-promoted OH^- catalysed reaction will occur at pH values near neutrality when C–N bond breaking is facile and nucleophilic attack is rate determining, whereas when C–N bond breaking is difficult, and therefore part of the rate-determining step, the reaction will be pH independent because of the requirement for protonation of the nitrogen leaving group.

By contrast with the large rate enhancements in ester hydrolysis due to metal-ion-promoted hydroxide ion[1–7] or water[8] catalysis, the effects of metal ions are usually small in the OH^- catalysed hydrolysis reactions of amides,[9,10] and metal-ion-promoted water reactions are not generally observed. However, rate enhancements of 10^3 to 10^6 occur in the hydroxide-ion catalysed hydrolysis of lactams[11] due to the presence of Zn^{2+} or Cu^{2+}, and rapid hydrolysis reactions of substitution-inert Co^{III} complexes of amides have been observed.[12] Gensmantel *et al.*[13,14] have also reported large metal ion effects in the opening of the β-lactam ring of benzyl penicillin.

We have recently found exceedingly large catalytic effects by divalent metal ions in the hydrolysis of *N*-acylimidazoles and *N*-acylbenzimidazoles.[15] The reactions involve metal ion promoted OH^- catalysis (**1**), and provide rate enhancements of 10^9 with Cu^{2+} and 10^6 with Co^{2+}, Ni^{2+}, and Zn^{2+} at saturating metal ion concentrations. The reaction of the Cu^{II} complex must be an intramolecular attack of metal-ion bound OH^- since attack of external OH^- would require a second-order rate constant greater than that for a diffusion-controlled reaction. The transition state in the OH^- catalysed hydrolysis of *N*-acylimidazoles must resemble the reactants, *i.e.*, there is little bond making with the nucleophile and little or no C–N bond breaking.[16] Metal-ion complexation of the carbonyl oxygen should greatly accelerate the reaction in cases where nucleophilic attack is rate limiting. Therefore, it may be the nature of the rate-determining step that has led to the metal-ion-promoted OH^- catalysed reaction and the large rate enhancements observed in the hydrolysis of the *N*-acylimidazoles.



(1)



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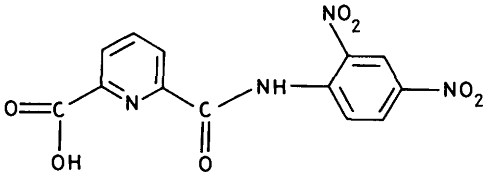
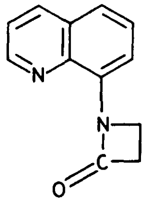


(3)

Increased understanding of the chemical mechanisms of metal-ion-catalysed amide hydrolysis should provide insight into the mechanisms employed by metalloenzymes that catalyse the hydrolysis of peptides, *e.g.*, carboxypeptidase A. To obtain evidence on the general relationship between the mechanism of metal-ion catalysis and the facility of nucleophilic attack and ease of C–N bond breaking, we have in the present work investigated the divalent metal-ion-catalysed hydrolysis of 8-trifluoroacetamidoquinoline-2-carboxylic acid and *N*-(6-

carboxypicolinyl)-2,4-dinitroaniline (**3**). In both cases a metal ion can be strongly chelated, and a nucleophile should be able readily to attack the carbonyl group because of the strong electron withdrawal in the acyl group (**2**) or the leaving group (**3**). However, C–N bond breaking will be much more difficult than in the hydrolysis of *N*-acylimidazoles, and it might reasonably be expected that the rate-determining step will entail breakdown of a tetrahedral intermediate to products. We have also investigated the hydrolysis of *N*-(8-quinolyl)azetidin-2-one (**4**). In this case the ease of C–N bond breaking will be enhanced by the β-lactam structure. We have found significant metal-ion catalysis in the hydrolysis of these compounds, which is pH independent with (**2**) and (**3**), and which involves metal ion-promoted OH^- catalysis in the case of (**4**).



(**4**)

Experimental

Materials.—8-Trifluoroacetamidoquinoline-2-carboxylic acid (**2**) was prepared by mixing 0.02 mol of 8-aminoquinoline-2-carboxylic acid and trifluoroacetic anhydride. After 1 h the product was dissolved in 150 cm^3 of chloroform and extracted with several 50 cm^3 portions of water containing sodium hydrogencarbonate. The chloroform layer was dried with sodium sulphate, and the chloroform was removed by rotary evaporation. The residue was recrystallized from toluene, m.p. 232–235 °C (decomp.) (Found: C, 50.5; H, 2.7; N, 10.1. Calc. for $C_{12}H_7F_3N_2O_3$: C, 50.7; H, 2.5; N, 9.9%).

N-(6-Carboxypicolinyl)-2,4-dinitroaniline (**3**) was prepared by mixing 0.02 mol of 6-carboxypicolinic acid and trifluoroacetic anhydride and allowing the solution to stand for 2 h. An equivalent amount of 2,4-dinitroaniline was added, and the mixture was heated gently and stirred to ensure homogeneity. The mixture was allowed to stand at room temperature for 16 h and was then dissolved in 150 cm^3 of warm chloroform. The chloroform solution was extracted with several 50 cm^3 portions of water. The chloroform layer was dried with sodium sulphate, and the chloroform was then removed by rotary evaporation. The amide product was recrystallized several times from acetonitrile, m.p. 232–235 °C (decomp.) (Found: C, 46.9; H, 2.6; N, 17.0. Calc. for $C_{13}H_8N_4O_7$: C, 47.0; H, 2.4; N, 16.9%). *N*-(6-Carboxypicolinyl)-2-chloro-4-nitroaniline was also prepared by this method, m.p. 256–258 °C (Found: C, 48.5; H, 2.7; N, 12.9. Calc. for $C_{13}H_8ClN_3O_5$: C, 48.5; H, 2.5; N, 13.1%).

N-(8-Quinolyl)azetidin-2-one (**4**) was synthesized by the procedure of Manhas and Jeng[17] using the sodium hydride method. The compound was recrystallized from hexane, m.p. 80–82 °C (Found: C, 72.6; H, 5.2; N, 14.1. Calc. for $C_{12}H_{10}N_2O$: C, 72.7; H, 5.05; N, 14.1%). The hydrolysis product γ-(8-quinolylamino)propionic acid, was prepared in the following manner. 8-Aminoquinoline (5 g) was refluxed with an equivalent amount of methyl γ-iodopropionate in dry acetone for 24 h. The acetone was removed by rotary evaporation, and the solid product was extracted with several 50 cm^3 portions of toluene. The toluene extracts were combined, and the toluene was rotary evaporated to give an oily residue. This residue was dissolved in 50 cm^3 of EtOH–H_2O (80:20) containing 0.5 mol dm^{-3} NaOH. After 2 h, the ethanol was evaporated, and the solid product was dissolved in the minimum amount of water. The pH was adjusted to 4 with HCl, and the water was removed by rotary evaporation. The final product, γ-(8-quinolylamino)-propionic acid, was obtained by extraction and recrystallization from toluene, m.p. 149–150 °C (Found: C, 66.7; H, 5.5; N, 13.0. Calc. for $C_{12}H_{12}N_2O_2$: C, 66.7; H, 5.6; N, 13.0%).

Kinetic Methods.—The rate measurements were carried out with a Pye-Unicam SP8-100 recording spectrophotometer. In the hydrolysis of the amide (**2**), product formation was followed at 260 nm from pH 4 to 12 in the absence of metal ions, and reactant disappearance at 260 nm was monitored in the presence of metal ions at pH values < 7. The hydrolysis of *N*-(6-carboxypicolinyl)-2,4-dinitroaniline (**3**) was followed by observing product formation at 340 nm or reactant decrease at 290 nm. The kinetics of the hydrolysis of *N*-(8-quinolyl)azetidin-2-one (**4**) were monitored by observing product formation at 310 nm at pH 9–12 or disappearance of the reactant in the presence of metal ions at pH values <9. In all cases the spectra of the product solutions quantitatively matched the spectra of the expected amine and carboxylic acid products or their metal-ion complexes.

The ionic strength was 0.1 mol dm^{-3} maintained with KCl, and the solutions used for rate measurements in the absence of metal ions contained 2×10^{-5} mol dm^{-3} EDTA as a precaution against trace metal ions in the buffer or salt. The buffer concentration was maintained at 0.02 mol dm^{-3} except in those cases where buffer catalysis made extrapolation to zero buffer necessary; no corrections were made for buffer–metal ion complexation. Hydrochloric acid solutions were employed in the pH range 1–3. The buffers used were formate (pH 3.2–4.2), acetate (pH 4.2–5.2), 2,6-lutidine (pH 5.5–6.6), *N*-ethylmorpholine (pH 6.6–8.0), morpholine (pH 8.0–9.0), and carbonate (pH 9.4–10.8). The metal-ion-catalysed reactions could not be studied at pH values > 7 because of precipitation of the metal ion.

Kinetic runs were initiated by injecting 15–30 mm^3 of stock solution (0.005–0.02 mol dm^{-3} amide in acetonitrile) into 3 cm^3 of buffered solution maintained at the desired temperature. The reactions followed pseudo first-order kinetics for at least four half-lives, and the kinetic parameters were evaluated with a least-squares computer program. Reaction mixture pH values were obtained at the reaction temperature with a Beckman Model 3500 pH meter. The value of the ion product of water at 70 °C was taken to be 2.04×10^{-13}.[18]

Results

In Figure 1 a plot is shown of log k_{obs} *vs.* pH for the hydrolysis of the amide (**2**) in water at 70 °C ($\mu = 0.1$ mol dm^{-3} with KCl). Hydroxide ion catalysis is observed ($k_{OH} = 2.25$ dm^3 mol^{-1} s^{-1}), and at pH < 6 the profile is bell shaped. This is in accord with a water reaction of a formally neutral species or zwitterion with the quinoline nitrogen protonated and the carboxy group ionized, as in the Scheme; k_{obs} would then decrease with decreasing pH at $a_H > K_1$. Possibilities that are kinetically equivalent to the water reactions governed by k_2 and k_1 involve attack of hydroxide ion on the neutral species (k_{OH}') or protonated species (k_{OH}''), respectively. Equation (1) was

$$k_{obs} = \frac{k_1K_1a_H + k_2K_1K_2 + k_{OH}K_1K_2(OH^-)}{a_H^2 + K_1a_H + K_1K_2} \quad (1)$$

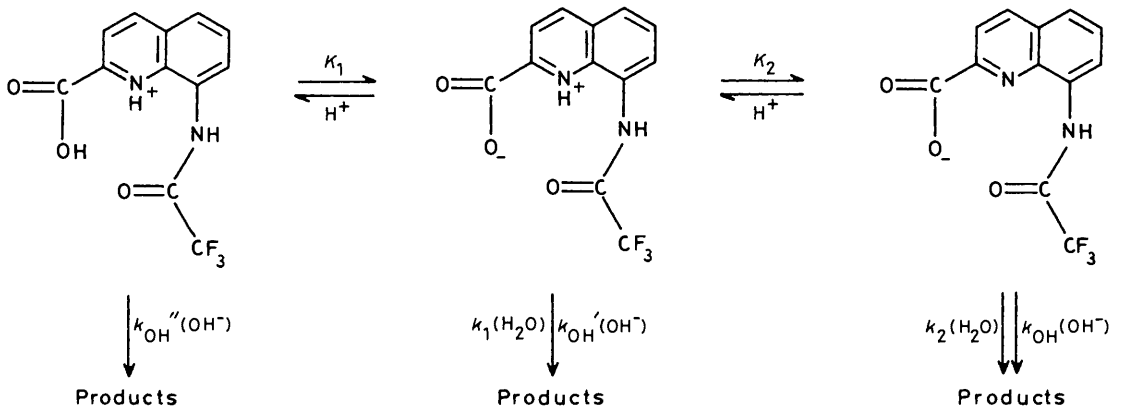
derived from the Scheme and provides a good fit to the experimental points in Figure 1 with $k_1 = 4.0 \times 10^{-4}$ s^{-1}, $k_2 = 5 \times 10^{-5}$ s^{-1}, $k_{OH} = 2.25$ dm^3 mol^{-1} s^{-1}, pK_1 = 1.8, and pK_2 = 5.6. Buffer catalysis occurs at pH > 7.

Divalent metal ions (Ni^{2+}, Co^{2+}, and Zn^{2+}) catalyse the hydrolysis of (**2**). Binding is strong, and saturation occurs at low

Table. Rate constants for the hydrolysis of compounds (2), (3), and (4) at 70 °C or 30 °C in water with μ = 0.1 mol dm^{-3}.

Compound	k_1/s^{-1} [a]	k_2/s^{-1} [a]	$k_{OH}/dm^3\ mol^{-1}\ s^{-1}$ [a]	Metal ion	k_0/s^{-1}	$k_{OH}/dm^3\ mol^{-1}\ s^{-1}$
(2)	4×10^{-4}	5×10^{-5}	2.25	—		
				Zn^{2+}	0.007[b]	
				Co^{2+}	0.006[b]	
				Ni^{2+}	0.0029[b]	
				Cu^{2+}	0.0042[c]	
				Zn^{2+}	0.0006[c]	
				Co^{2+}	0.000 23[c]	
				Ni^{2+}	0.000 15[c]	
(3)			7.4	—		
				Zn^{2+}	0.000 35[b]	
				Co^{2+}	0.000 19[b]	
				Ni^{2+}	0.000 04[b]	
(4)			0.02	—		
				Ni^{2+}		2 100[d]
				Zn^{2+}		1 200[e]

[a] At 70 °C. [b] At a saturating 0.01 mol dm^{-3} concentration of metal ion, 70 °C. [c] At a saturating 0.01 mol dm^{-3} concentration of metal ion, 30 °C. [d] At 0.01 mol dm^{-3} metal ion (nonsaturating), 70 °C. [e] At 0.02 mol dm^{-3} metal ion (nonsaturating), 70 °C.



Scheme.

metal ion concentrations (< 0.01 mol dm^{-3}), as seen in Figure 2 where k_{obs} is plotted *vs.* the concentration of Co^{2+} at pH 6.25. Equation (2) is followed at constant pH, where K_M is the metal

$$k_{obs} = \frac{k_M K_M[M^{2+}]}{1 + K_M[M^{2+}]} \qquad (2)$$

ion association constant and k_M is the limiting rate constant for metal-ion-catalysis. From this equation K_M was calculated to be 5×10^3 dm^3 mol^{-1}, and k_M, the limiting rate constant at pH 6.25, is 5.5×10^{-3} s^{-1}. The association constants for Ni^{2+} and Zn^{2+} are: Ni^{2+}, 10^4 dm^3 mol^{-1} at pH 6.25; and Zn^{2+}, 10^3 dm^3 mol^{-1} at pH 4.51. These constants were calculated at the given pH and are uncorrected for protonation of the substrate. Corrected values are: Co^{2+}, 6×10^3 dm^3 mol^{-1}; Ni^{2+}, 2×10^4 dm^3 mol^{-1}, and Zn^{2+}, 2×10^4 dm^3 mol^{-1}. At the constant saturating metal-ion concentration of 0.01 mol dm^{-3} the plots of k_{obs} *vs.* metal-ion concentration in Figure 1 are pH independent in the pH range 4–7. The average values of the pH-independent rate constants are given in the Table. At 30 °C, k_{obs} in the presence of 0.001 mol dm^{-3} Cu^{2+} is pH-independent in the pH range 3.68–4.73 (k_{obs} = 4.2×10^{-3} s^{-1}). By comparison, the pH-independent value of k_{obs} at 30 °C in the presence of 0.01 mol dm^{-3} Zn^{2+}, Co^{2+}, or Ni^{2+} is 5.8×10^{-4} s^{-1}, 2.3×10^{-4} s^{-1}, and 1.5×10^{-4} s^{-1}, respectively.

The hydrolysis of *N*-(6-carboxypicolinyl)-2,4-dinitroaniline (3) is OH^- catalysed at pH values < 10 (Figure 3). The value of k_{OH} at 70 °C (μ = 0.1 mol dm^{-3}) is 7.4 dm^3 mol^{-1} s^{-1}. At pH > 10 the plot of log k_{obs} *vs.* pH bends downward as can be seen in Figure 3. At pH 11–12, k_{obs} is independent of pH. A pK_{app} of *ca.* 10 is indicated. Divalent metal ions also bind strongly to (3) and saturate at concentrations < 0.01 mol dm^{-3}. The plots of k_{obs} *vs.* pH in the presence of 0.01 mol dm^{-3} Ni^{2+}, Co^{2+}, or Zn^{2+} are pH-independent. The average values of the pH-independent rate constants are given in the Table. Cupric ion is a poor catalyst in the hydrolysis of (3). In the presence of 0.002 mol dm^{-3} Cu^{2+} an upper limit on k_{obs} at pH 4.20 (70 °C) is 1.5×10^{-5} s^{-1}.

The hydrolysis of *N*-(6-carboxypicolinyl)-2-chloro-4-nitroaniline is also OH^- catalysed at 70 °C (k_{OH} = 0.43 dm^3 mol^{-1} s^{-1}) and becomes pH-independent at pH > 10 (pK_{app} = 10.8). The hydrolysis reaction in the presence of 0.01 mol dm^{-3} Zn^{2+} is pH-independent in the pH range 4.4–6.2 with k_0 = 7×10^{-5} s^{-1} at 70 °C (μ = 0.1 mol dm^{-3}). *N*-(6-Carboxypicolinyl)-4-nitroaniline hydrolyses with k_{OH} = 0.044 dm^3 mol^{-1} s^{-1} at 70 °C (μ = 0.1 mol dm^{-3}). A very slow reaction could be detected at pH 5–6 in the presence of 0.01 mol dm^{-3} Zn^{2+}, but the half-life is > 10 h.

Figure 4 shows the plot of log k_{obs} *vs.* pH for the hydrolysis of *N*-(8-quinolyl)azetidin-2-one (4) at 70 °C. The hydrolysis of (4) is OH^- catalysed with k_{OH} = 2×10^{-2} dm^3 mol^{-1} s^{-1}. Divalent metal ions bind very weakly to (4) but nevertheless exert a very large catalytic effect. A plot of k_{obs} for hydrolysis of (4) *vs.* the concentration of Ni^{2+} at pH 6.18 is shown in Figure 5. The plot is still linear at the high metal-ion concentration of 0.01

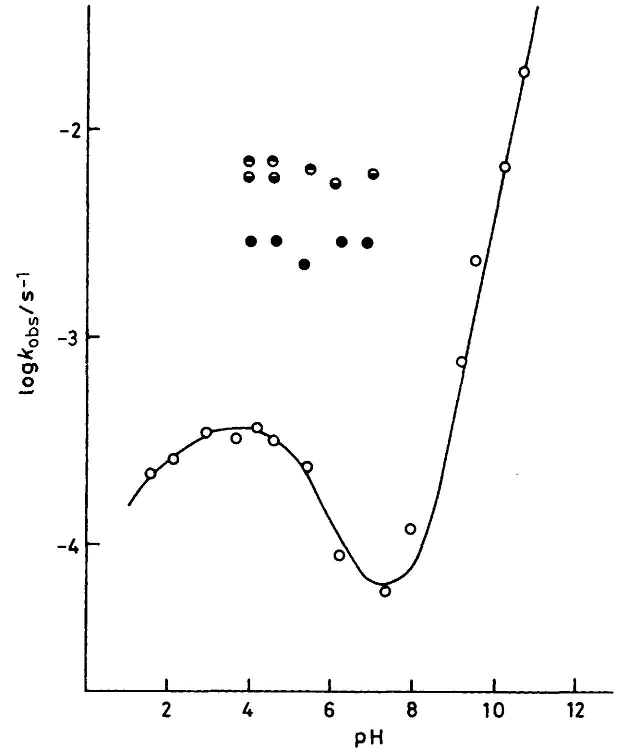


Figure 1. Plot of $\log k_{obs}$ *vs.* pH for the hydrolysis of 8-trifluoroacetamidoquinoline-2-carboxylic acid (**2**) in water at 70 °C (μ = 0.1 mol dm^{-3} with KCl) in the presence of 0.01 mol dm^{-3} Ni^{2+} (●), Co^{2+} (◒), Zn^{2+} (◓), and in the absence of metal ions (○). The slope is 1.0 at pH > 8.

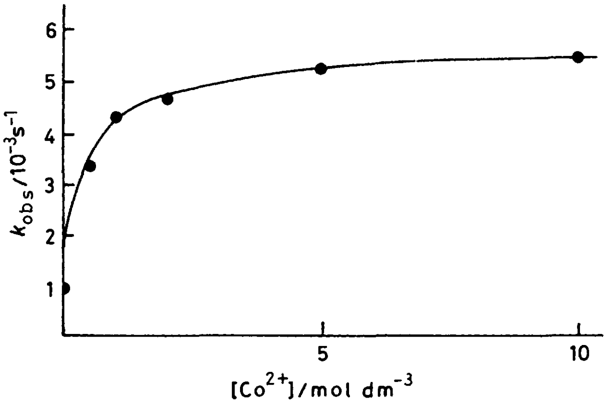


Figure 2. Plot of k_{obs} *vs.* the concentration of Co^{2+} for the hydrolysis of 8-trifluoroacetamidoquinoline-2-carboxylic acid (**2**) in water at 70 °C and pH 6.25.

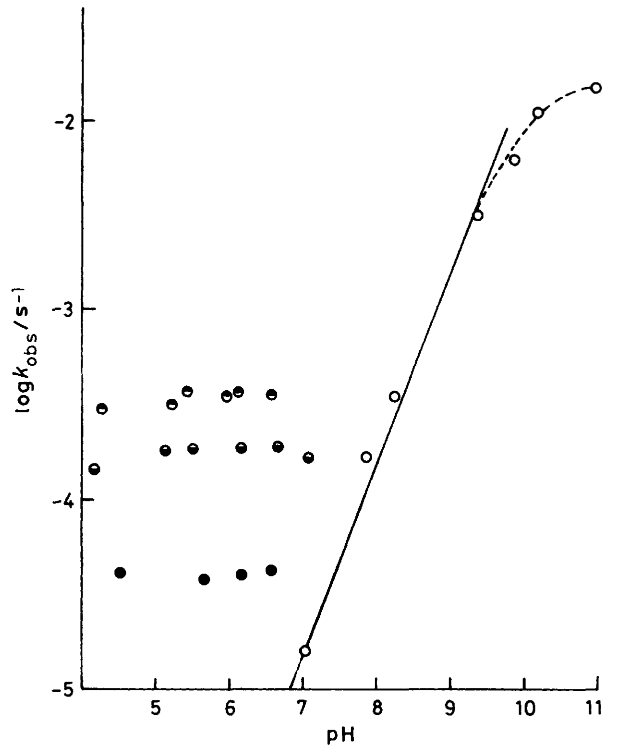


Figure 3. Plot of $\log k_{obs}$ *vs.* pH for the hydrolysis of *N*-(6-carboxypicolinyl)-2,4-dinitroaniline (**3**) in water at 70 °C (μ = 0.1 mol dm^{-3} with KCl) in the presence of 0.01 mol dm^{-3} Ni^{2+} (●), Co^{2+} (◒), Zn^{2+} (◓), and in the absence of metal ions (○). The slope is 1.0 at pH 7–9.

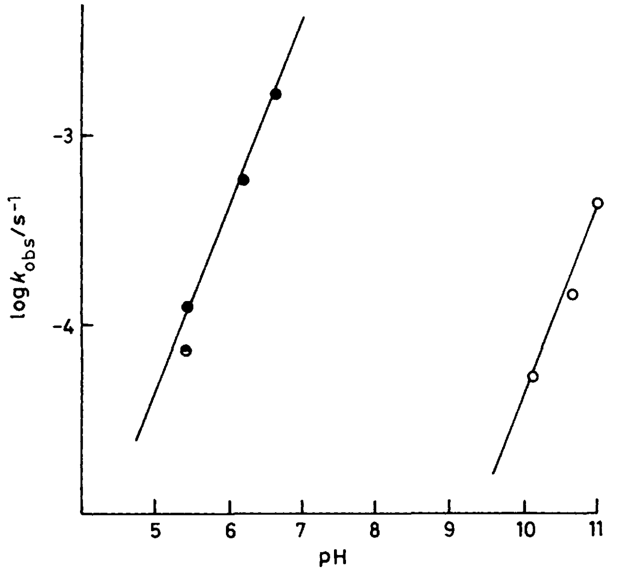


Figure 4. Plot of $\log k_{obs}$ *vs.* pH for the hydrolysis of *N*-(8-quinolyl)azetidin-2-one (**4**) in water at 70 °C (μ = 0.1 mol dm^{-3} with KCl) in the presence of 0.01 mol dm^{-3} Ni^{2+} (●), 0.02 mol dm^{-3} Zn^{2+} (◓), and in the absence of metal ions (○). The slopes are 1.0.

mol dm^{-3}. In the presence of a constant 0.01 mol dm^{-3} concentration of Ni^{2+} the hydrolysis of (**4**) is OH^- catalysed, as seen in Figure 4, with $k_{OH} = 2.1 \times 10^3$ dm^3 mol^{-1} s^{-1}, 10^5 times larger than in the absence of metal ion.

Discussion

The plot of $\log k_{obs}$ *vs.* pH for hydrolysis of the amide (**2**) in Figure 1 can be simply explained in terms of OH^- catalysed hydrolysis of the anionic species and water reactions of the anionic and zwitterionic species [equation (1)]. Breakdown of a tetrahedral intermediate is the rate-determining step in the OH^- catalysed hydrolysis of trifluoroacetanilide[19] and ring substituted *N*-trifluoromethyl acetanilides.[20] It is likely therefore that this is also the case in the hydrolysis of (**2**). The apparent water reactions of (**2**) are reactions of a type that are not commonly observed in amide hydrolysis, although a pH-independent reaction does occur in the hydrolysis of *N*-trifluoroacetyl-*p*-nitroaniline[21] and *N*-acylimidazoles.[16,22] The water reactions of (**2**) must be due to the electron withdrawal in the acyl group, which facilitates the addition of water to the

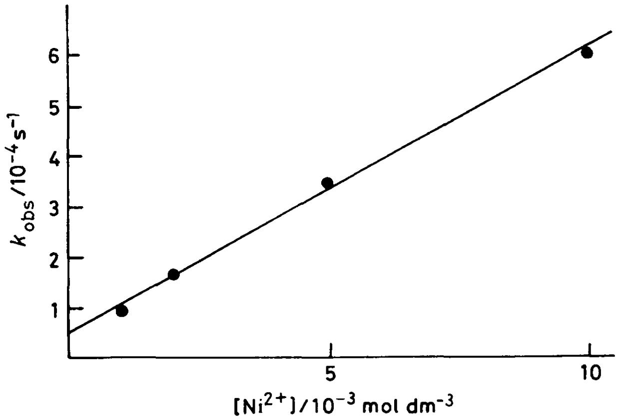


Figure 5. Plot of k_{obs} *vs.* the concentration of Ni^{2+} for the hydrolysis of *N*-(8-quinolyl)azetidin-2-one (**4**) in water at 70 °C and pH 6.18.

carbonyl group. Similar water reactions have been observed in the hydrolysis of acyl activated esters.[23–27]

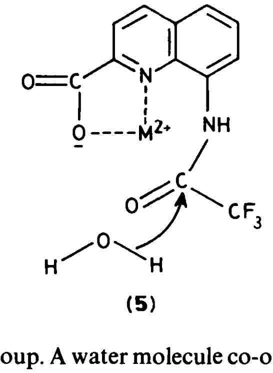
Kinetic equivalents exist, of course, in the reactions of (**2**), *e.g.*, the apparent water reaction of the anion (k_2) could proceed with attack of OH^- on the zwitterion [($k_{OH}'(OH^-)$ in the Scheme]. The bell-shaped profile at low pH is in accord with equation (1), but it is not evident that the protonated species of the reactant should be less reactive than the zwitterion. The rate retarding effect of increased hydronium ion concentration at pH < 4 may reflect protonation of a zwitterionic tetrahedral intermediate. The decline in k_{obs} with decreasing pH at pH < 4 could then reflect a change in rate-determining step in the water reaction since increasing acidity should favour expulsion of amine from a tetrahedral intermediate so that formation of the intermediate would be rate determining at low pH. Alternatively, all the reactions could be considered to proceed with hydroxide ion catalysis (see the Scheme); an OH^- catalysed reaction of the protonated species (governed by k_{OH}'') would produce a decline in k_{obs} with decreasing pH at pH values $< pK_1$. However, k_{OH}'' would then require a value of *ca.* 3×10^7 dm^3 mol^{-1} s^{-1} at 70 °C, which is quite large for that type of reaction.

The hydrolysis reactions of *N*-(6-carboxypicolinyl)-2,4-dinitroaniline (**3**) and the *N*-(8-quinolyl)azetidin-2-one (**4**) are also OH^- catalysed. The downward bend at pH 10 in the plot of log k_{obs} *vs.* pH in the hydrolysis of (**3**) (Figure 3) may reflect ionization of an OH group in a tetrahedral intermediate (ionization of a tetrahedral intermediate to a dianionic species occurs at higher OH^- concentration in the hydrolysis of *p*-nitroacetanilide.)[28] Ionization of the NH group of the amide reactant might also occur* (the analogous pK_a of *p*-nitroacetanilide is 13.8).[28] However, the ionized species of trifluoroacetanilide is not totally inert to OH^- catalysed hydrolysis.[19] The hydrolysis of *para*-substituted acetanilides at high pH involves breakdown of a tetrahedral intermediate.[29] Therefore, as with (**2**), the OH^- catalysed hydrolysis of (**3**) very likely proceeds with the significant breakdown of a tetrahedral intermediate to products. The appreciable OH^- catalysis of the reaction of (**4**) must be due to the β-lactam structure. Similar reactions have been observed previously in the hydrolysis of *N*-aryl substituted β-lactams.[30]

Metal-ion Catalysis.—There is significant catalysis of the hydrolysis reactions of (**2**)–(**4**) by the divalent metal ions Ni^{2+}, Co^{2+}, and Zn^{2+}. Binding of these metal ions to (**2**) and (**3**) is very strong because of the possibility of chelation by two functional groups as well as the amide carbonyl group. The principal binding sites are, of course, the 2-carboxy group and the quinoline nitrogen of (**2**), and the 6-carboxy group and the pyridine nitrogen of (**3**).† Note that association constants for metal-ion binding to (**2**) are similar to those for binding to quinoline-2-carboxylic acid.[31] As a consequence, saturation effects are observed at low metal-ion concentrations (< 0.01 mol dm^{-3}).

The rate of hydrolysis of (**2**) is increased 50–100 fold in the presence of 0.01 mol dm^{-3} Ni^{2+}, Co^{2+}, or Zn^{2+} at pH 7 (70 °C), and Cu^{2+} is a better catalyst than these metal ions at 30 °C by an additional factor of 10–20. Metal-ion chelation by the carbonyl oxygen is possible but would require a seven-membered ring and would therefore be sterically unfavourable. The marked catalytic effect by Cu^{II} indicates that the metal ion is not complexed to the carbonyl oxygen. Such complexation will reduce the pK_a of a neutral amide to 4–5 (8–10 in the presence of Co^{II} or Ni^{II}).[10,32,33] Ionization of the amide in the pH range 4–5 should greatly reduce its reactivity in the metal-ion promoted reactions.

At a constant saturating concentration of metal ion (0.01 mol dm^{-3}) the reactions of (**2**) are pH-independent. Thus, the metal-ion catalysed reactions could be water reactions in which a metal-ion-bound water or an external water molecule (**5**) attacks



(**5**)

at the carbonyl group. A water molecule co-ordinated to a metal ion would, of course, be weakly basic and therefore a poor nucleophile. Metal ion-promoted OH^- catalysed reactions are not observed in the hydrolysis of (**2**). Metal ion-promoted pH-independent water reactions also occur in the hydrolysis of anhydrides[34] and substituted phenolic esters of picolinic acid.[8] In the latter reactions the pK_a of the leaving group has little effect (β_{lg} *ca.* 0), which indicates that nucleophilic attack of water is rate determining. Water reactions, therefore, take place at pH < 7 when the leaving group is very good because attack of water can then be competitive with that of the low concentration of OH^- or metal-ion bound OH^-. In the case of the amide (**2**), however, the situation is quite different in that C–N bond breaking would be difficult, and breakdown of a tetrahedral intermediate to products should be rate determining. Proton transfer to the leaving group nitrogen must occur to facilitate C–N bond breaking.[23] Such proton transfer might take place from the attacking water molecule either in a stepwise or concerted reaction. Kinetically equivalent processes could involve attack of metal-ion bound or external OH^- on a protonated species or rate-determining breakdown of a zwitterionic tetrahedral intermediate, as depicted in (**6**).

Metal-ion binding to *N*-(6-carboxypicolinyl)-2,4-dinitroaniline (**3**) is also very strong. As in the reactions of (**2**), at constant saturating concentrations of the metal ions the k_{obs} values are independent of pH. Again there is no indication of

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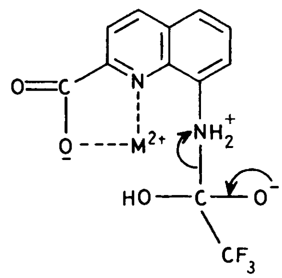
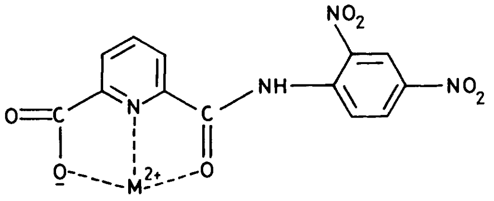
* The pK_a of 2,4-dinitroacetanilide is 10.4 at 50 °C.[29]

† Values of the logarithms of the stability constants at 25 °C for complexation of the metal ions with quinoline-2-carboxylic acid are: Cu^{II}, 5.91; Ni^{II}, 4.95; Co^{II}, 4.49; and Zn^{II}, 4.17. Values of the logarithms of the stability constants for complexation of the metal ions with picolinic acid at 25 °C are: Ni^{II}, 6.4; Co^{II}, 5.74; and Zn^{II}, 5.12.[31]



(6)

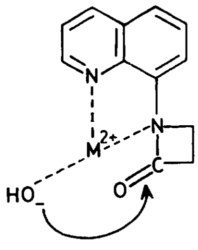
metal-ion enhancement of the OH^- catalysed reaction. Chelation by the amide carbonyl oxygen would in this case be sterically favourable in a 1 : 1 complex because a five-membered ring would be formed. At pH 7 the enhancements of the rate of hydrolysis of (**3**) due to Co^{II} and Zn^{II} are 13 and 23-fold. Since hydrolysis in the absence of the metal ions is OH^- catalysed these rate enhancements will increase by a factor of ten for each decrease in pH of one unit (assuming that spontaneous hydrolysis remains OH^- catalysed at pH < 7). The mechanism of the metal-ion promoted reaction must involve attack of a water molecule on the metal-ion complex (**7**) or a kinetic equivalent, although sterically favourable chelation by the amide carbonyl group is clearly not a requirement for a metal-ion-promoted water reaction in view of the similar reactions of (**2**). Again it would be expected that C–N bond breaking would be part of the rate-determining step, and that complete or partial proton transfer to nitrogen would be required for breakdown of the complex to products.[23] Note that the acyl group is the same as that of *N*-(6-carboxypicolinyl)benzimidazole with which the metal-ion-promoted OH^- catalysed reaction is very favourable (**1**).[15] Therefore, the differences in mechanism found for the benzimidazole and the anilide (**3**) are strictly due to the different leaving groups.



(7)

Electron withdrawal in the leaving group of the anilide derivatives has a large effect on the rate constants of both the OH^- catalysed reaction and the pH-independent metal-ion catalysed reactions. Substitution of chlorine for the nitro group in the 2-position reduces k_{OH} by a factor of 17 and reduces k_0 for the Zn^{II} catalysed reaction by a factor of five. Likewise, with hydrogen in the 2-position, k_{OH} is ten times less than that of the 2-chloro-4-nitro-derivative, and the rates of the metal-ion catalysed reaction, although detectable, are too slow to be accurately measured at 70 °C. Electron withdrawal in the leaving group will increase the ease of attack of the nucleophile at the carbonyl group and increase the ease of leaving group departure from the tetrahedral intermediate, but will decrease the ease of protonation of nitrogen. Thus, the rate enhancing effects of increased electron withdrawal in this system must reflect the relative importance of nucleophilic attack by OH^- or water and/or the increased ease of C–N bond breaking.

The identity of the rate-determining step in metal-ion catalysed reactions will be especially important if the carbonyl oxygen of the substrate is complexed by the metal ion. A metal ion will then be maximally efficient as a catalyst when nucleophilic attack is rate determining, because of the facilitating effect due to polarization of the carbonyl group. Indeed, it is difficult to envisage an exceptionally large catalytic role for the metal ion if breakdown of a tetrahedral intermediate is strictly rate determining. In that case complexation of the oxygen anion of the tetrahedral intermediate would stabilize the intermediate by reducing the necessary driving force for C–N bond breaking. An increase in the equilibrium concentration of the tetrahedral intermediate due to the presence of the metal ion should result in a moderate (or negligible) catalytic effect if breakdown of the intermediate to products is markedly inhibited. Stabilization of the leaving group in the transition state by direct co-ordination of the metal ion and nitrogen as the C–N bond breaks might produce a significant effect,[13,14] but such a mechanism would not explain why an apparent water reaction is observed with (**2**) and (**3**) at *ca.* pH 7 rather than OH^- catalysis. The water reaction implies the need for an additional proton. Also, the ability of the metal ions to bind to nitrogen would be decreased by increased electron withdrawal in the leaving group. If C–N bond breaking is sufficiently facile that nucleophilic attack at the carbonyl is rate determining, then OH^- catalysis would be expected at pH values near neutrality, as in the hydrolysis of *N*-acylimidazoles[15] and other acyl derivatives.[34] Thus, it is probable that C–N bond breaking is occurring in the rate-determining step, and as a consequence, the magnitudes of the rate enhancements in the pH-independent metal-ion-catalysed reactions are only moderately large. The reactions could reflect a transition state in which both bond making and C–N bond breaking have an important influence.



(8)

In contrast with the hydrolysis of (**2**) and (**3**), the metal-ion effects in the hydrolysis of the *N*-(8-quinolyl)azetidin-2-one (**4**) involve promotion of the apparent OH^- catalysed reaction. Thus, the reactions proceed with attack of metal-ion bound OH^- (**8**) or metal-ion enhancement of the attack of external OH^-. Attack of metal-ion bound OH^- will give plots of log k_{obs} *vs.* pH that are linear with slopes of 1.0 to the pK_a of the co-ordinated water molecule.* The rate enhancement produced by 0.01 mol dm^{-3} Ni^{2+}, a non-saturating concentration, is a factor of 10^5. Therefore, the metal-ion-promoted reaction is very favourable, even though the binding of the metal ion to (**4**) is weak and metal-ion complexation of the carbonyl oxygen would require a seven-membered ring. The similar large effects of divalent metal ions in the hydrolysis of *N*-acylimidazoles and *N*-acylbenzimidazoles[15] reflects the ease of nucleophilic attack at the carbonyl group and the ease of C–N bond breaking. The favourable OH^- catalysed hydrolysis of β-lactams has been attributed to release of steric strain in the four-membered lactam ring and to inhibition of resonance in the amide function, which will increase the ease of attack of the nucleophile at the carbonyl carbon.[30,36–38,]† Consequently, similar factors may

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* The pK_a values for the ionization of the aquo-complexes of Zn^{II}, Co^{II}, and Ni^{II} are 8.8, 8.9, and 10.6, respectively, at 25 °C.[35]

† M. I. Page has argued against such inhibition of resonance being an important factor in the hydrolysis of the β-lactam ring of penicillin derivatives.[36]

be influencing the metal-ion-catalysed reactions of *N*-acyl imidazoles and the β-lactam (**4**), although the rate constants for hydrolysis of the β-lactam are many orders of magnitude less than those in the reactions of the *N*-acylimidazoles. Gensmantel *et al.*[13,14] have suggested that divalent metal ions bind to the β-lactam nitrogen of benzylpenicillin and thereby enhance the ease of C–N bond breaking (but see ref. 33). In view of the large rate enhancement in the metal ion-promoted OH^- catalysed reaction of (**4**), it is likely that nucleophilic attack by OH^- is rate determining. Thus, either formation of a tetrahedral intermediate is the rate-determining step, or nucleophilic attack and C–N bond breaking occur simultaneously. The β_{lg} (slope of a plot of log k_{OH} *vs.* the pK_a of the leaving group) for hydrolysis of a series of *N*-substituted β-lactams is -0.44,[30,39] which supports rate-determining attack of OH^- in the alkaline hydrolysis of those compounds analogous to (**4**).

The striking contrast in the mechanisms of metal-ion catalysed hydrolysis of the amides (**2**)–(**4**) is very likely due to differences in the critical transition states brought about by the differences in the ease of C–N bond breaking. With (**2**) and (**3**), attack of the nucleophile at the carbonyl group will be enhanced by the strong electron withdrawal in either the acyl group (**2**) or the leaving group (**3**), but partial or complete protonation of nitrogen will be required to assist C–N bond breaking in both cases. The metal-ion catalysed reactions are then pH-independent, and only moderate rate enhancements are observed. In contrast, nucleophilic attack by OH^- is rate determining in the metal-ion-catalysed hydrolysis of *N*-acylimidazoles[15] and the β-lactam (**4**), and apparent OH^- catalysis is observed with exceedingly large rate enhancements. Metal-ion complexation of the amide should markedly enhance the attack step. If the reaction involves attack of metal-ion bound OH^- [mechanisms (**1**) and (**8**)], then the intramolecular nature of the reaction will lead to a further large rate enhancement. Consequently, the identity of the rate-determining step in amide hydrolysis determines which mechanism of metal-ion catalysis will occur.

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