Leaving Group Effect in the Cleavage of Picolinate Esters Catalyzed by Hydroxy-Functionalized Metallomicelles

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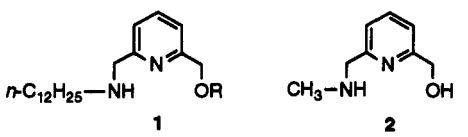
Micellar aggregates of complexes of transition metal ions with the hydroxy-functionalized surfactant **1a** are very effective catalysts of the cleavage of activated esters of α-amino acids. To ascertain their effectiveness toward unactivated esters, a systematic kinetic study was undertaken employing as substrates the picolinic acid esters **3a-l**, the pK_a of their alcoholic portion spanning more than 12 units from 3.6 to 16. The leaving group effect was investigated in water, pH = 6.3, in the absence and presence of Cu^{2+} ions, in the presence of the nonmicellar complex **2**·Cu^{2+}, and in the presence of micellar aggregates made of **1a**·Cu^{2+} or of its O-methylated analog **1b**·Cu^{2+}. In the presence of free metal ions the leaving group effect is negligible in the case of esters with good leaving groups (pK_a < *ca.* 12), and it becomes remarkably large in that of unactivated substrates. In the presence of Cu^{2+} complexes, either micellar **1a** or nonmicellar **2**, the leaving group effect is relatively small in the case of activated substrates (pK_a < 9, **1a**; pK_a < 11, **2**) and sharply increases in the case of unactivated substrates. A similar trend was observed in a less extensive kinetic investigation using Zn^{2+} ions at pH = 7.5. The largest rate enhancements were observed in the case of the most activated substrates in micellar solutions of the **1a**·Cu^{2+} complex (1.6×10^6 folds for **3b** over the rate in pure buffer), considerably larger than those in the presence of its nonmicellar analog (4.2×10^4 folds) or of the free metal ion (1.5×10^3 folds). However, in the case of unactivated esters, such kinetic benefits vanish out and the metal ion alone is even more effective (2×10^4 folds acceleration for **3l** in presence of Cu^{2+}) than its complexes, either in the monomeric (7.3×10^3 folds) or in the micellar form (4.6×10^3 folds). On the basis of the possible changes in the mechanistic pathway depending on the nature of the leaving group, a rationale is offered.

Interest in the role of metal-bound hydroxides as nucleophiles has been stimulated by several studies concerning organic systems that mimic or model metallohydrolases such as carboxypeptidase A.[1] Divalent transition metal ion catalysis in the hydrolysis of esters has been extensively studied.[2,3] In these reactions, the rate constants for hydroxide ion catalysis are markedly enhanced, and at least in the case of activated esters of amino acids, rate enhancements as large as 10^7 have been reported.[4]

More recently, ligand molecules containing a hydroxy function bound in the proximity to a metal ion chelating subunit have been synthesized and investigated. Early studies of simple systems, such as the one reported by Sigman and Jorgensen,[5] showed that the rate enhancements observed in the cleavage of the *p*-nitrophenyl ester of picolinic acid (PNPP) in neutral aqueous solutions is due to the activation of the neighboring hydroxyl that acts as the effective nucleophile. Remarkable results were reported for more complex metallocatalysts containing 2-(hydroxymethyl)imidazole or pyridine as chelating subunit.[6] We,[7] as well as other groups,[8] turned our attention to metalloaggregates (micelles or vesicles) made up of transition metal ion–ligand–surfactants. In aqueous solutions, such metalloaggregates would add the benefits of the hydrophobic interactions between the organic substrate and the activated micellar aggregate to those of the metal ion activation of the system. Our studies were mainly focused on amphiphilic molecules featuring a substituted pyridine as the basic chelating subunit and as a molecular junction for a hydroxy function, paraffinic chain(s), and, in some cases, other structural residues such as hydrophilic subunits and chiral centers. Among these, the lipophilic ligand **1a** (Chart 1) is a simple structure

Chart 1



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1a : R = H
1b : R = CH_3

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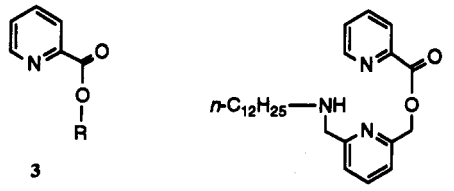
Chart 2



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R = **a**: 2,6-dichloro-4-nitrophenyl; **b**: 2,4-dinitrophenyl; **c**: pentachlorophenyl; **d**: 2-nitro-5-fluorophenyl; **e**: 4-nitrophenyl; **f**: 2-nitrophenyl; **g**: 3-nitrophenyl; **h**: 3-chlorophenyl; **i**: phenyl; **j**: trifluoroethyl; **k**: 2-methoxyethyl; **l**: ethyl

that has been recently investigated[7d] as a catalyst of ester hydrolysis in the presence of transition metal ions. The very large binding constant of **1a** for Cu^{2+} allowed us to explore several aspects of its metalloaggregates and simplify the kinetic analysis of the catalyzed reaction.

We report here a kinetic study of the hydrolysis of a number of esters of picolinic acid, **3a-l** (Chart 2), in the absence and presence of Cu^{2+} (and Zn^{2+}) alone or bound to metallomicelles of ligand **1a** aimed to define the effect of the leaving group and some mechanistic facets of the reaction. The kinetic study was complemented by exploring the effects of metallomicelles of **1b** (the OMe analog of **1a**) and those of the complexes of the hydrophilic ligand **2** (the analog of **1a** devoid of the paraffinic chain). Ester **4** was also synthesized and investigated for comparison purposes (see *infra*). This study of the leaving group effect was motivated by (a) Menger's *caveat*[9] concerning the use, justified only by convenience, of *p*-nitrophenyl esters and (b) Fife and Przystas' observation[10] that the Ni^{2+}- or Cu^{2+}-promoted alkaline hydrolysis of picolinate esters is virtually insensitive to changes in the leaving group as its pK_a changes from 4 to 12. The two above arguments led us to explore the possibility that the large rate accelerations observed in the case of PNPP, **3e**, using metallomicelles of **1a**·Cu^{2+}, could be observed also in the case of unactivated picolinates, such as simple alkyl esters (or amides) of picolinic acid, thus making the system a real biomimetic model as well as catalyst in its own right.

Results and Discussion

Kinetics in the Presence (and Absence) of Cu^{2+} and Zn^{2+}. The hydrolysis of picolinate esters in water in the absence and in the presence of divalent transition metal ions was thoroughly investigated by Fife and Przystas,[10] and the main results are summarized here. In the absence of metal ions, the reaction is catalyzed by hydronium ion, hydroxide ion, and water (or a kinetic equivalent in a pH independent region). In the presence of Ni^{2+} or Cu^{2+} large rate enhancements were observed, and the rate–pH profiles clearly indicate apparent hydroxide ion catalysis even at pH values below 4. The rate acceleration is due to chelation, since no significant metal ion catalysis is observed in the case of isonicotinate esters. The metal ion catalyzed reaction therefore implies a chelation preequilibrium, and the reaction may involve nucleophilic attack of a hydroxide ion bound to the metal ion or of an external ion on the activated carbonyl carbon of the complex.[11]

Table 1. Rate Constants (k_ψ, s^{-1}) for the Hydrolysis of Picolinate Esters 3 in the Presence of Cu^{2+} and Zn^{2+} Ions

compd	pK_a (leaving group)		Cu^{2+} [a,b]	Zn^{2+} [c]
		10^2k_o,[a] s^{-1}	10^2k_ψ, s^{-1}	10^4k_ψ, s^{-1}
3a	3.56[d]		4.6	
3b	4.1[e]	2.8×10^{-3}	4.3	
3c	5.26[f]		3.4	
3d	6.07[g]		9.5	
3e	7.10[e]	9×10^{-4}	5.6	7.62[h]
3f	7.22[h]		6.2	
3g	8.35[h]		6.6	
3h	9.12[i]		5.2	
3i	9.90[e]	4×10^{-5} [l]		6.8
3j	12.4[e]	3.2×10^{-5} [l]	1.9	2.95
3k	14.8[j]		0.083	1.25
3l	16.0[e]	1.5×10^{-6} [l]	0.03	0.42

[a] In MES buffer 0.05 M, pH 6.3, 25 °C. [b] $[Cu^{2+}] = 2.0 \times 10^{-4}$ M. [c] In HEPES buffer 0.05 M, pH 7.5, 25 °C, $[Zn^{2+}] = 6.0 \times 10^{-4}$ M. [d] Fisher, A.; Leary, G. J.; Topson, R. J.; Vaughan, J. *J. Chem. Soc.* **1967**, 686. [e] Reference 10. [f] Birchall, J. M.; Haszeldine, R. N. *J. Chem. Soc.* **1959**, 3653. [g] Hancock, C. K.; Clague, D. H. *J. Am. Chem. Soc.* **1964**, *86*, 4942. [h] Fernandez, L. P.; Hepler, L. G. *J. Am. Chem. Soc.* **1959**, *81*, 1783. [i] Bolton, P. D.; Hall, F. M.; Reece, I. H. *Spectrochim. Acta* **1966**, *22*, 1825. [j] Ballinger, P.; Long, F. A. *J. Am. Chem. Soc.* **1960**, *82*, 795. [k] $k_o = 2.8 \times 10^{-5}$ s^{-1}. [l] Extrapolated from the data measured in the range 35–50 °C.

The present results confirm the main evidence reported by the cited authors. We have focused our attention on the leaving group effects in the hydrolysis of picolinate esters **3a-l**, and the kinetic measurements were mainly made in 2-morpholinoethanesulfonic acid (MES) buffer at pH = 6.3 at 25 °C, unless otherwise indicated. In the absence of metal ions, most of the substrates react at a very slow rate, and some rate constants reported in Table 1 were estimated by extrapolation of rate data obtained at higher temperatures.

Addition of Cu^{2+} or Zn^{2+} remarkably enhances the rate of hydrolysis. Table 1 shows rate data for the hydrolysis of esters **3a-l** at a selected concentration of these metal ions. It is emphasized here that the rates were measured in a range of concentrations far below those approaching saturation conditions: as shown in Figure 1 in the case of picolinates **3a**, **3e**, **3j**, and **3k** the k_ψ values increase linearly (no evidence of downward curvature) with increasing metal ion concentration, and this indicates that the Cu^{2+} binding constant to picolinic acid esters is small.

Focusing attention on the leaving group effect, Figure 2 shows the Brönsted plots: log k_ψ *vs* the pK_a of the conjugate acid of the leaving group.[12] In the absence of metal ion, although taking into account the approximate values of most of the rate data for the slowest terms, the plot is apparently linear and the slope (β_{lg}) is –0.25. In the presence of 0.2 mM Cu^{2+} the plot shows a break at a pK_a value of *ca.* 11.5. There is virtually no effect for good leaving groups and a remarkable effect in the case of poorer leaving groups (the β_{lg} values change from *ca.* 0 to –0.5). Although the analysis was limited to a smaller number of substrates, the same trend is apparent in the case of Zn^{2+} ions, and this is in line with the findings reported by Fife and Przystas[10] using Ni^{2+} as a metal ion. It may be noticed that in the case of Cu^{2+} the effects for the worst leaving groups are apparently magnified. Interestingly, the approximate rate acceleration factors upon addition of Cu^{2+}

(9) Menger, F. M.; Ladika, M. *J. Am. Chem. Soc.* **1987**, *109*, 3145.
(10) Fife, T. H.; Przystas, T. J. *J. Am. Chem. Soc.* **1985**, *107*, 1041.
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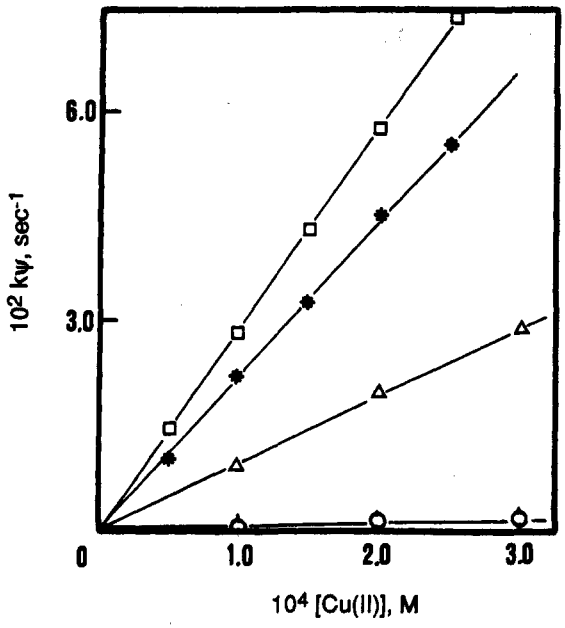


Figure 1. Rate *vs* Cu^{2+} concentration profiles for the hydrolysis of picolinate **3a** (□), **3e** (*), **3j** (△), and **3k** (○) in MES buffer, pH 6.3, and 25 °C.

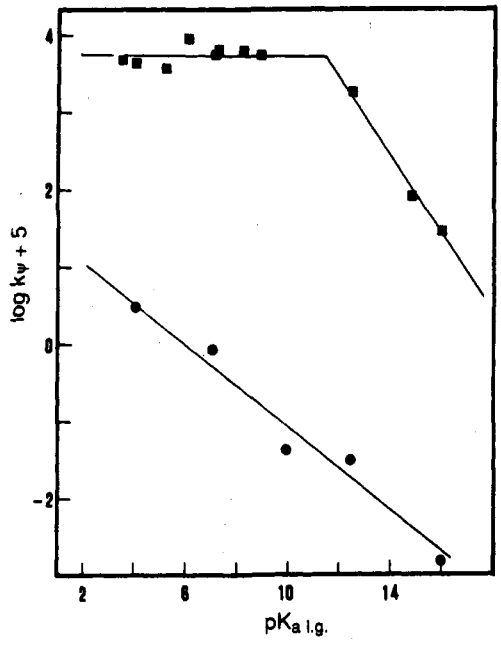
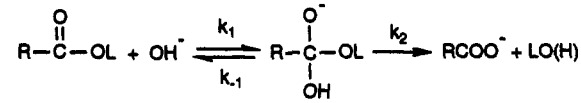


Figure 2. Log k_ψ *vs* pK_a of the conjugate acid of the leaving group for the hydrolysis of picolinates **3** in the absence (●) and in the presence (■) of Cu^{2+} (0.2 mM) in MES buffer, pH 6.3, and 25 °C.

are 2×10^3, 6×10^4, and 8×10^3 on going from the picolinate **3b** (2,4-dinitrophenyl) to **3j** (trifluoromethyl) and to **3l** (ethyl) esters, the largest rate benefits thus resulting for leaving groups with a pK_a value close to 11–12 as it appears from a scrutiny of Figure 2.

The metal ion catalyzed process involves a metal ion chelation preequilibrium, so that the observed rate constant is the product of the hydroxide ion reaction and the metal ion binding constant ($k_\psi = k_{OH}K_M$). The effect of the leaving group on K_M has been dismissed as negligible by Fife and Przystas[10] for Ni^{2+}. In the present case, although we cannot offer direct evidence, the observed effects (Figure 2) can hardly be ascribed to substantial changes in the preequilibrium metal ion binding: the flat

Scheme 1



portion for good leaving groups would imply that there is a very tight compensation of the k_{OH} and K_M values over a very wide spectrum of compounds and that such compensating effect would suddenly vanish out in the case of poor leaving groups.

The rationale for the data in Figure 2 may be based on the commonly accepted stepwise mechanism (Scheme 1) of hydroxide hydrolysis.[13] In the absence of metal ions, the nucleophilic species is mainly an OH^- from bulk water (pK_{nuc} larger than that of the leaving group of all esters), and following Scheme 1, the formation of the tetrahedral intermediate is, for each substrate, the rate-determining step.

In the presence of metal ions, the rate limiting step changes from the formation to the breakdown of the tetrahedral intermediate as the partitioning ratio (k_2/k_{-1}) changes from a value greater than 1 to one smaller than 1. The two straight lines of the plot intersect at p*K* 11.5: as a first approximation, at this value, $\Delta(pK) = (pK_{nuc} - pK_{lg}) = 0$. Hence, the nucleophilic species could be indicated as a hydroxide ion, quite likely resulting from a metal ion coordinated water molecule with an apparent $pK_a = 11$–12. When compared to other data[14] concerning the acidity of a Cu^{2+} bound water molecule, a $pK_a = 11$–12 is rather large; however, we wish to emphasize that this is an apparent value. Quite clearly, the reaction pathway shown in Scheme 1 is an oversimplified mechanism as seen by the fact that the role of the metal ion is not considered. Yet it is involved in both the tetrahedral intermediate and in the transition state(s) of the reaction. The metal ion stabilizes the intermediate, and depending on its lifetime, it may coordinate the oxygen atom of the leaving group, thus making its departure easier. If such is the case it becomes a better leaving group than expected from its thermodynamic pK_a value. This, eventually, may lead to an underestimation of the pK_{nuc} of the hydroxide ion from the break in the Brönsted plot.

The β_{lg} value close to zero for activated ester, in the presence of the metal ions, points to a transition state where bond breaking is virtually negligible at variance with that (0.25), in the absence of the metal ion, which indicates a transition state involving at least partial bond breaking. This confirms the idea that the tetrahedral intermediate is stabilized by the metal ion, possibly through coordination of the (former carbonyl) oxygen, in such a way that the negative charge is somehow delocalized without need of partial bond breaking. Thus, the coordinated metal ion may (a) provide the effective nucleophile, with the entropic benefits resulting from the chelation preequilibrium, and (b) favor the formation of the transient intermediate *via* Lewis acid catalysis (activation of the carbonyl ester) and, as a consequence, by stabilizing it.[11]

Kinetics in the Presence of Metal Ion Chelates of 1 and 2. Ligands **1a,b** are very lipophilic compounds, soluble in acidic water or in neutral solutions containing Cu^{2+} where they form micellar aggregates.[7d] They may

(13) Williams, A. *Acc. Chem. Res.* **1989**, *22*, 387.
(14) Basolo, F.; Pearson, G. P. *Mechanism of Inorganic Reactions*, Wiley: New York, 1967; p 32. Morrow, J. R.; Trogler, W. C. *Inorg. Chem.* **1989**, *28*, 2330.

Table 2. Rate Constants (k_ψ, s^{-1}) for the Hydrolysis of Picolinate Esters 3 in the Presence of 1a·Cu^{2+}, 1a·Zn^{2+}, 2·Cu^{2+}, and 1b·Cu^{2+}

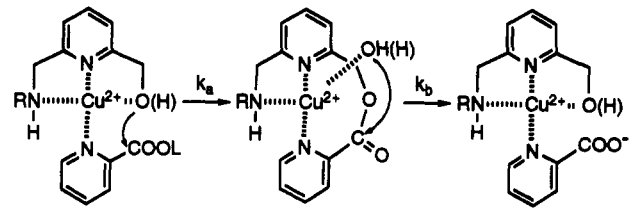
	catalyst			
compd	1a·Cu^{2+} [a] k_{fast}, s^{-1}	1a·Zn^{2+} [b] k_{fast}, s^{-1}	2·Cu^{2+} [a] k_{fast}, s^{-1}	1b·Cu^{2+} [a] k, s^{-1}
3b	45.1		1.17	
3d	11.2		0.27	
3e	7.0	0.16	0.15	0.056
3f	3.82		0.14	
3g	2.3		0.071	
3h	1.26			
3i		0.012		
3j	2.7×10^{-3}	3.0×10^{-4}	3.4×10^{-3}	9.0×10^{-3}
3k			1.3×10^{-4}	
3l	7×10^{-5}		1.1×10^{-4}	

[a] [Ligand] = [Cu^{2+}] = 0.2 mM, MES buffer 0.05 M, pH 6.3, 25 °C. [b] [1a] = 0.4 mM, [Zn^{2+}] = 0.6 mM, HEPES buffer 0.05 M, pH 7.5, 25 °C.

also be solubilized in an inert matrix of cetyltrimethylammonium bromide (CTABr); the present kinetic investigation was carried out in the presence of CTABr (in a constant 10-fold excess over the ligand) in order to explore a suitable concentration range of the chelates, well above the cmc of the comicelles: 1.7×10^{-5} M in the case of **1a**, and 1.6×10^{-5} in the case of 1a·Cu^{2+}. The hydrolytic cleavage of the picolinate esters in the presence of the metalloaggregates of **1a,b**, as well as in the presence of chelates of **2**, were followed spectroscopically by monitoring the changes of absorbance in the range 260–270 nm and also at higher wavelengths in the case of nitrophenolic derivatives (see Experimental Section). Particularly at high chelate concentrations, the measurements were complicated by a strong background absorption, and the absorbance changes observed during the reaction were generally small. For these reasons, reliable kinetic measurements could not be obtained in the case of picolinates **3c** (pentachlorophenyl), **3h** (*m*-chlorophenyl), and **3i** (phenyl) among those investigated.

Most notably, the changes in absorbance at 260–270 nm employing **1a** and **2** as ligands showed a biphasic behavior in the case of the most reactive substrates and a clean monophasic behavior for the less reactive terms. In the case of *p*-nitrophenyl picolinate, the rate constant of the first (faster) process (at 264 nm) was identical to that of the release of *p*-nitrophenol followed at 400 nm. This was confirmed in the kinetic experiments using all the other nitrophenolic derivatives and indicates that the fast process is the cleavage of the ester. On the other hand, the rate constant ($0.09 \pm 0.03\ s^{-1}$ under the conditions of Table 2) of the second, slower process was the same, within the experimental error, for all the substrates showing a biphasic behavior. This was diagnostic of a process involving a relatively stable species formed after the ester cleavage. Early investigations[7b] on the mode of action of chelates of 2-(hydroxymethyl)pyridino derivatives, the analogs of **1a**, schematically indicated in Scheme 2, prompted us to establish that the second slow step is the hydrolysis of the transacylation product **4**. In fact, this was isolated by quenching the chelate-catalyzed cleavage of **3e** and identified by comparison with an authentic sample of **4**. Moreover, during the hydrolysis of **4** in the presence of 1a·Cu^{2+} (0.2 mM), under the standard conditions used for the kinetic measurements of the hydrolytic cleavage of the picolinate esters, the same

Scheme 2



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spectral changes were recorded as in the slow step of the biphasic kinetics and the same rate constants were obtained.

That the hydrolytic cleavage of the picolinate esters with a good leaving group proceeds *via* a nucleophilic attack of the hydroxy function of **1a** to give **4** is further confirmed by kinetic experiments carried out using **1b**, the O-methylated ligand: under the same standard conditions, the rate of the hydrolytic cleavage of the esters with good leaving groups was much slower than that in the presence of comicelles of **1a** and very close to that of the hydrolysis in the absence of chelating micelles; in each case, no biphasic behavior was observed.

Table 2 shows the rate constants for the process (the fast one in the case of biphasic kinetics) obtained from measurements carried out under the standard conditions indicated.

As mentioned above, in the case of esters **3j-l** only monophasic kinetics were observed. In a set of experiments employing a constant [Cu^{2+}] = 0.2 mM, the rate of cleavage of these substrates was found to decrease with increasing concentrations of comicelles of **1a**.

The dicotomy in the set of esters explored is also evident from the Brönsted-type plot of Figure 3 which is defined by two straight lines with different slopes: −0.30 in the pK_a range 4–9 and −0.65 in the range 9–16. The leaving group effect obtained from rate data employing micellar solutions of 1a·Cu^{2+} is substantially confirmed by the plot of (the log) k_ψ values observed using 2·Cu^{2+}, a nonmicellar catalyst, as shown in Figure 3: the slopes are −0.30 and −0.50. The second value is lower than that with 1a·Cu^{2+} and very close to that obtained using Cu^{2+} alone. The pK_a values at which the two straight lines of the plot intersect are different: 9 and 11 (the latter one, again, very close to that obtained using Cu^{2+} alone). Unfortunately, the error affecting such values is larger than desirable, also due to the fact that the pivoting rate data concerning ester **3i** (pK_a = 9.9) in the presence of Cu^{2+} chelates could not be obtained (see Experimental Section).

The rate benefits observed in the reaction of the picolinate esters having good leaving groups in the presence of 1a·Cu^{2+} or 2·Cu^{2+} can be ascribed to the formation of a ternary complex (see Scheme 2) where the metal ion acts as a template and favors a pseudointramolecular nucleophilic attack of the hydroxy function of the ligand. As the leaving group of the esters is made increasingly poorer, the hydroxy function of the ligand becomes less and less effective up to the case of the trifluoroethyl picolinate, **3j**, that reacts virtually at the same rate in the presence of comicelles of 1a·Cu^{2+} or 1b·Cu^{2+}. Under the standard conditions employed, the cleavage of the esters having a poor leaving group is slowed down by addition of micellar (or nonmicellar) ligands to a Cu^{2+} solution (up to a 7-fold decrease in the k_ψ value). Yet, the rate of hydrolysis of these esters is much larger than that in pure buffer by at

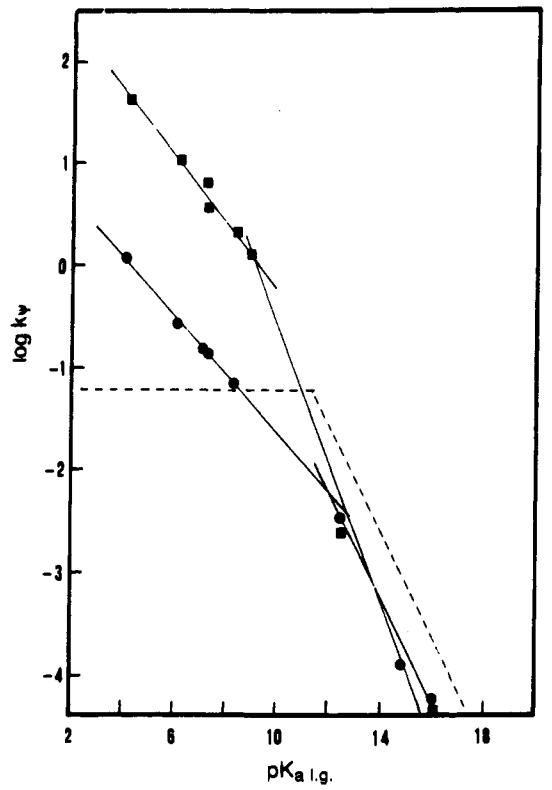
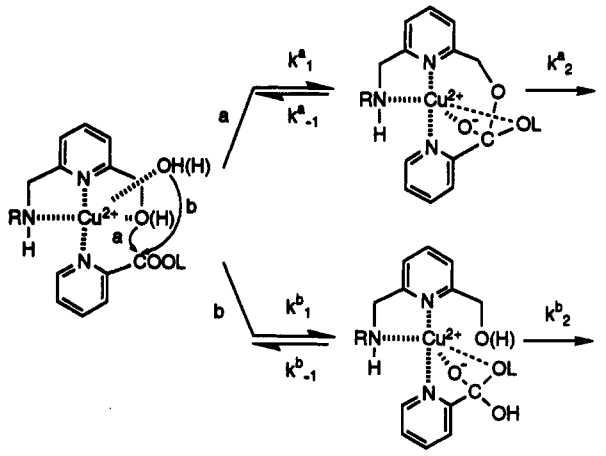


Figure 3. Log k_ψ vs pK_a of the conjugate acid of the leaving group for the hydrolysis of picolinates **3** in the presence of **1a**·Cu^{2+}/CTABr (■) (0.2 mM) and in the presence of **2**·Cu^{2+} (●) (0.2 mM), in MES buffer, pH 6.3, and 25 °C. The dotted lines indicate the plot obtained in presence of only Cu^{2+} (see Figure 2) for an easier comparison.

Scheme 3



least 4 orders of magnitude. Assuming that Cu^{2+} is virtually all bound to the ligand and the stability constant of the ternary complex does not remarkably change on changing the alcoholic portion of the picolinate esters, the reaction is likely to proceed via a nucleophilic attack of a water molecule bound to the metal ion in a ternary complex. Thus, within such complex, either a hydroxide ion or the ligand (dissociated) hydroxy group may compete for a nucleophilic attack to the carbonyl carbon of the ester as indicated in Scheme 3.

Apparently, the hydroxy function of the ligand is favored, possibly for entropic reasons, and is the effective nucleophile as long as the rate-determining step is the formation of the tetrahedral intermediate, *i.e.*, in the case of good leaving groups or, following Scheme 3 (route a), when $k^a_2 \gg k^a_{-1}$. On going to slower substrates and $k^a_2 < k^a_{-1}$, the effectiveness of the hydroxy function will vanish out and the hydroxide ion is the effective nucleophile (route b) *provided* that the partitioning factor k^b_2/k^b_{-1} is larger than k^a_2/k^a_{-1}. In other terms, within the ternary complex the hydroxide ion is less "available" for a nucleophilic attack to form the tetrahedral intermediate but, once it is formed, is slower to leave it than the competing ligand hydroxide function. Such a hypothesis is in agreement with the higher apparent acidity, by *ca.* 2 pK_a units, of 2-(hydroxymethyl)pyridine[15] than that of water.

The analysis of the data shown in Figure 3 when compared to those obtained in the presence of the metal alone (dashed line on Figure 3) points to (a) a strongest dependence of the log k_ψ on the pK_a of the leaving group (for a good leaving group) in the case of the ligands **1a** and **2** and (b) a break point *ca.* 2 pK_a units lower in the surfactant system. A rationale for the above observation suggests a later transition state within the ternary complex for the formation of the tetrahedral intermediate (in which bond breaking is more important) than in the case of the hydrolysis catalyzed by the metal ion alone. This may be the consequence of a lower electrophilicity of the metal ion when chelated to ligands; on one hand, this would diminish its ability to disperse the charge of the transition state, on the other hand, this decreases the efficiency in coordinating the oxygen of the leaving group. This latter point could be also associated to a diminished pK_a of the nucleophile (the ligand hydroxyl) due to micellization.

Conclusions

The present kinetic study has brought to light a rather interesting picture concerning the leaving group effects in the hydrolytic cleavage of a number of picolinate esters. Although less precise than desirable, the data of Figures 2 and 3 are descriptive of the impressive differences on moving from pure buffer to metal ion alone to hydroxy-functionalized chelates as monomers species or as micellar aggregates. In the case of *picolinates having poor leaving groups*, the metal ion alone accelerates the hydrolysis more effectively than when it is associated to the functionalized ligands; their (activated) hydroxy function is a less effective nucleophile than bound water and the overall result of the chelate formation is likely a kind of encumbrance leading to an apparent, albeit not dramatic, inhibition to the hydroxide ion effectiveness. In the case of *activated picolinates*, all the kinetic benefits of the metal ion are in full display: as the leaving group becomes better than trifluoroethanol the catalytic effects reach a sort of saturation level and any leaving group effect apparently fades out. When the metal ion is associated to the ligand molecules, it activates the chelate hydroxy function in such way that it becomes the effective nucleophile and the rate enhancements becomes more and more sizable as the leaving group pK_a decreases, and the catalytic effects are remarkably magnified in cationic micellar aggregates. In the case of the 2,4 dinitrophenyl picolinate **3b** the rate enhancements over that in pure buffer are 1.5×10^3 (Cu^{2+}, alone), 4.2×10^4 (**2**·Cu^{2+}), and 1.61×10^6 (**1a**·Cu^{2+}) under the standard (0.2 mM) quite low concentrations used.

To sum up, the present results indicate that catalysts such as the divalent transition metals chelates of **1a** or **2** are of much help for the less needy, more reactive

(15) Petitfaux, C. *Ann. Chim.* **1973**, *14*(8), 33.

substrates and fail to be effective in the case of more natural substrates. From the point of view of enzyme modeling, one should agree with Menger's[9] statement that the hydrolysis of "authentic" substrates presents a challenge that nature, but not organic chemistry, has already met. However, by modeling enzymes employing very simple molecular systems one cannot expect to match the results of the complex biological systems but rather to obtain indications that might be useful for more refined systems. From the investigation of the present metallocatalytic system the indications are that (a) the metal ion plays a crucial role in the activation of all the available nucleophilic functions and of the carbonyl carbon, (b) the ligand hydroxy function is very effective only in the case of most reactive substrates, and (c) a second function, slightly less acidic, an associated water molecule, then intervenes. Perhaps, an even less acidic functionality covalently bound to the ligand molecule could play a very effective role in the case of the "authentic" substrates and make a properly designed bifunctional ligand a more versatile "authentic" catalyst.

Experimental Section

General Methods. Melting points are uncorrected. ^{1}H-NMR spectra were recorded on a 200 MHz spectrometer, and chemical shifts in ppm are reported relative to internal Me_4Si. Surface tension measurements to evaluate the cmc of the aggregates were performed with a Kruss Type 8451 tensiometer. Microanalyses were performed by the Laboratorio di Microanalisi of our department.

Materials. $Cu(NO_3)_2$ and $Zn(NO_3)_2$ were analytical-grade products. Metal ion stock solutions were titrated against EDTA following standard procedures.[16] 2-Morpholinoethanesulfonic acid (MES) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), used as buffers, were a Fluka and a Sigma product, respectively, used as received. *n*-Hexadecyltrimethylammonium bromide (CTABr) was an analytical grade commercial product. 6-((*n*-Dodecylamino)methyl)-2-(hydroxymethyl)pyridine (**1a**) and 6-((*n*-dodecylamino)methyl)-2-((methyloxy)methyl)pyridine (**1b**) were prepared as reported.[7d] 2,4-Dinitrophenyl picolinate (**3b**), 4-nitrophenyl picolinate (**3e**), phenyl picolinate (**3i**), trifluoroethyl picolinate (**3j**), and ethyl picolinate (**3l**) were prepared by literature methods.[10]

6-((Methylamino)methyl)-2-(hydroxymethyl)pyridine (2). 6-(Bromomethyl)-2-(hydroxymethyl)pyridine[7b] (0.42 g, 2.1 mmol) was dissolved in 10 mL of a 33% solution of methylamine in EtOH (Fluka) cooled at 0 °C. After 6 h of stirring at room temperature, the solvent was evaporated, and the crude product obtained was dissolved in a few mL of MeOH and eluted with MeOH through a column loaded with a basic ion-exchange resin (IRA 401, Fluka). The free amine obtained was further purified by chromatography on a silica column (CH_2Cl_2/MeOH/NH_4OH (84/15/1)) yielding 0.26 g (82%) of pure **3** as an oil. ^{1}H-NMR δ (CD_3OD): 2.44 (s, 3H, NCH_3), 3.85 (s, 2H, CH_2NCH_3), 4.73 (s, 2H, CH_2OH), 7.32 (d, J = 7.32 Hz, 1H, H_5Py), 7.45 (d, J = 7.32 Hz, 1H, H_3Py), 7.83 (t, J = 7.32 Hz, 1H, H_4Py). Anal. Calcd for $C_8H_{12}N_2O$: C, 63.13; H, 7.95; N, 18.41. Found: C, 62.94; H, 8.01; N, 18.25.

(2-(6-((Dodecylamino)methyl)pyridyl))methyl Picolinate (4). To a solution of *N*-BOC-protected **1a**[7d] (0.40 g, 1.0 mmol) in 10 mL of pyridine were added picolinic acid (0.16 g, 1.3 mmol) and 1,3-dicyclohexylcarbodimide (0.27, g, 1.3 mmol). The reaction mixture, protected with a drying tube, was stirred at room temperature for 1 week. The slurry was then cooled in an ice bath, and a white precipitate was filtered off and washed with pyridine. The combined organic washings were evaporated at reduced pressure to leave a crude product that was first chromatographated on a silica column (toluene/ethyl acetate (7:3)) and then on a preparative plate (silica, same eluent) to give the *N*-BOC-protected ester. This derivative was dissolved in 4 mL of 33% HBr in acetic acid and stirred at room temperature for 15 min. Ten mL of Et_2O was then added, and the precipitate was collected and thoroughly washed with Et_2O. The solid was treated with a 10% solution of $NaHCO_3$, extracted with CH_2Cl_2, dissolved in acetone and precipitated again as the hydrochloride with a few drops of concd HCl. The hydrochloride was collected, treated with $NaHCO_3$, and extracted with CH_2Cl_2. The evaporation of the organic solvent afforded 0.2 g (49%) of **4**, mp 60–61 °C. NMR δ ($CDCl_3$): 0.81 (bt, 3H, $(CH_2)_nCH_3$), 1.21 (m, 18H, $CH_2(CH_2)_9CH_3$), 1.49 (m, 2H, $NCH_2CH_2(CH_2)_n$), 2.63 (t, J = 7.02 Hz, 2H, $NCH_2(CH_2)_n$), 2.73 (bs, 1H, NH), 3.90 (s, 2H, $PyCH_2N$), 5.49 (s, 2H, $PyCH_2O$), 7.20 and 7.30 (2d, J = 7.63 Hz, 2H, Py H_3 and H_5), 7.46 (m, 1H, Ac Pic H_5), 7.61 (t, J = 7.63, 1H, Py H_4), 7.80 (dt, J = 7.93 and 1.53 Hz, 1H, Ac Pic H_4), 8.14 (dt, J = 7.93 and 1.22 Hz, Ac Pic H_6), 8.80 (m, 1H, Ac Pic H_3). Anal. Calcd for $C_{25}H_{37}N_3O_2$: C, 72.95; H, 9.06; N, 10.2. Found: C, 72.42; H, 9.16; N, 9.95.

General Procedure for the Synthesis of Picolinate Esters (3). Method a. To a solution of picolinic acid (1.0 g, 8.1 mmol) in 70 mL of dry CH_2Cl_2 were added the proper phenol (8.1 mmol) and dicyclohexylcarbodiimide (1.7 g, 8.1 mmol). To the reaction mixture were added a few crystals of 4-(dimethylamino)pyridine. After being stirred for 48 h at room temperature, the slurry was cooled in an ice bath; a white precipitate was filtered off and washed with CH_2Cl_2. The evaporation of the organic solvent afforded a crude product that was purified as described below. **Method b.** The same amount of reactants as above was dissolved in 20 mL of dry pyridine. After being stirred for 48 h at room temperature the slurry was treated as above.

2,6-Dichloro-4-nitrophenyl Picolinate (3a) (Method a). Purified by crystallization from MeOH, yield 63%, mp 129–130 °C. ^{1}H-NMR δ ($CDCl_3$): 7.66 (m, 1H, H_5 Py), 7.99 (dt, J = 7.93 and 1.53 Hz, 1H, H_4 Py), 8.32 (s, 2H, H_3 and H_5 Ph), 8.36 (m, 1H, H_3 Py), 8.91 (m, 1H, H_6 Py). Anal. Calcd for $C_{12}H_6Cl_2N_2O_4$: C, 46.03; H, 1.93; N, 8.95. Found: C, 45.68; H, 1.86; N, 8.74.

Pentachlorophenyl Picolinate (3c) (Method a). The crude product was first chromatographated on a silica gel column (toluene/ethyl acetate (20:1)) and then crystallized twice from MeOH, yield 45%, mp 142–143 °C. ^{1}H-NMR δ ($CDCl_3$): 7.64 (m, 1H, H_5 Py), 7.97 (dt, J = 7.68 and 1.83 Hz, 1H, H_4 Py), 8.32 (dt, J = 7.68 and 1.10 Hz, 1H, H_3 Py), 8.90 (m, 1H, H_6 Py). Anal. Calcd for $C_{12}H_4Cl_5NO_2$: C, 38.80; H, 1.09; N, 3.77. Found: C, 38.55; H, 1.05; N, 3.84.

2-Nitro-5-fluorophenyl Picolinate (3d) (Method a). The crude product was first chromatographated on a silica gel column (toluene/ethyl acetate (9:1)) and then crystallized from MeOH, yield 46%, mp 97–98 °C. ^{1}H-NMR δ ($CDCl_3$): 7.18 (m, 2H, H_4 and H_6 Ph), 7.62 (m, 1H, H_5 Py), 7.97 (dt, J = 7.93 and 1.83 Hz, 1H, H_4 Py), 8.28 (m, 2H, H_3 Py and H_3 Ph), 8.88 (m, 1H, H_6 Py). Anal. Calcd for $C_{12}H_7FN_2O_4$: C, 54.97; H, 2.69; N, 10.68. Found: C, 54.50; H, 2.59; N, 10.46.

2-Nitrophenyl Picolinate (3f) (Method a). The crude product was first chromatographated on a silica gel column (toluene/ethyl acetate (9:1)) and then crystallized from CH_2Cl_2/hexane, yield 36%, mp 72–73 °C. ^{1}H-NMR δ ($CDCl_3$): 7.47 (m, 2H, H_4 and H_6 Ph), 7.60 (m, 1H, H_5 Py), 7.72 (dt, J = 7.66 and 1.80, 1H, H_5 Ph) 7.95 (dt, J = 7.70 and 1.53 Hz, 1H, H_4 Py), 8.20 (dd, J = 8.16 and 1.65, 1H, H_3 Ph), 8.29 (m, 1H, H_3 Py), 8.86 (m, 1H, H_6 Py). Anal. Calcd for $C_{12}H_8N_2O_4$: C, 59.02; H, 3.30; N, 11.47. Found: C, 58.90; H, 3.21; N, 11.42.

3-Nitrophenyl Picolinate (3g) (Method b). Purified by crystallization from MeOH, yield 82%, mp 128–129 °C. ^{1}H-NMR δ ($CDCl_3$): 7.61 (m, 1H, H_5 Py), 7.64 (m, 2H, H_5 and H_6 Ph), 7.97 (dt, J = 7.93 and 1.83 Hz, 1H, H_4 Py), 8.20 (m, 2H, H_2 and H_4 Ph), 8.31 (m, 1H, H_3 Py), 8.88 (m, 1H, H_6 Py). Anal. Calcd for $C_{12}H_8N_2O_4$: C, 59.02; H, 3.30; N, 11.47. Found: C, 58.97; H, 3.26; N, 11.36.

3-Chlorophenyl picolinate (3h) (Method b). Purified by crystallization from MeOH, yield 15%, mp 91–92 °C. ^{1}H-NMR δ ($CDCl_3$): 7.25 (m, 4H, H_2, H_4, H_5 and H_6 Ph), 7.58 (m, 1H, H_5 Py), 7.92 (dt, J = 7.8 and 1.53 Hz, 1H, H_4 Py), 8.26 (m, 1H, H_3 Py), 8.85 (m, 1H, H_6 Py). Anal. Calcd for $C_{12}H_8NO_2Cl$: C, 61.69; H, 3.45; N, 5.99. Found: C, 62.07; H, 3.66; N, 6.19.

(16) Holzbecher, Z. *Handbook of Organic Reagents in Inorganic Analysis*; Wiley: Chichester, 1976.

2-Methoxyethyl Picolinate (3k) (Method b). The crude product was purified by column chromatography on silica gel (toluene/ethyl acetate (4:1)) yielding 0.8 g of an oil (54%). ^{1}H-NMR δ ($CDCl_3$): 3.43 (s, 3H, CH_3), 3.77 (t, J = 4.76 Hz, $CH_2CH_2OCH_3$), 4.57 (t, J = 4.76 Hz, 2H, $CH_2CH_2OCH_3$), 7.47 (m, 1H, H_5 Py), 7.84 (dt, J = 7.7 and 1.73 Hz, 1H, H_4 Py), 8.15 (m, 1H, H_3 Py), 8.77 (m, 1H, H_6 Py). Anal. Calcd for $C_9H_{11}NO_3$: C, 59.66; H, 6.12; N, 7.73. Found: C, 59.45; H, 6.02; N, 7.63.

Kinetic Studies. Solutions were prepared in MES (0.05 M, pH 6.3) buffer or in HEPES (0.05 M, pH 7.5) buffer. No changes in pH were observed during the kinetic runs. Slower reactions were followed on a spectrophotometer equipped with a thermostated cell holder and faster reactions on an Applied Photophysics SF.17MV stopped-flow spectrometer. Reaction temperature was maintained at 25 ± 0.1 °C. The substrate concentration was (2–4) × 10^{-5} M, and the kinetics follow in each case a first-order law up to 90% of the reaction. The release of the differents alcohols was monitored at the following wavelengths: **3a**, **3b**, **3d**, and **3e** at 400 and 264 nm, **3c** at 264 and 315 nm, **3f** at 350 and 264 nm, **3g** at 335 and 264 nm, and **3h**, **3i**, **3j**, **3k**, and **3l** at 264 nm. The rate constants were obtained by nonlinear regression analysis of the absorbance *vs* time data (using the software package Enzfitter: Leatherbarrow, R. J. *Enzfitter*; Elsevier: Amsterdam, 1987). The fit error on the rate constants was always less than 1% except in the case of **3c**, **3h**, and **3i** in the presence of ligands **1** and **2**. With these three substrates the small absorbance changes observed during the reaction and the strong absorbance background due to the ligand **1** or **2** hampered any reliable kinetic measurements.

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