# Metallomicelles as Catalysts of the Hydrolysis of Carboxylic and Phosphoric Acid Esters

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*Received February 5, 1990*

Lipophilic ligands **2–4** have been synthesized. In the presence of Cu(II) ions, they form metallomicelles that are catalytically active in the cleavage of the *p*-nitrophenyl esters of acetic, hexanoic, and dodecanoic acids. Catalysis was also observed in the cleavage of *p*-nitrophenyl diphenyl phosphate. The apparent $pK_a$ of the Cu(II)-coordinated hydroxyl of ligand **2a**, in the micellar aggregate, is 7.7, as estimated from the rate vs pH profiles for both classes of esters: this suggests that the hydroxyl is involved as a nucleophile in the hydrolytic cleavage, which proved to be a really catalytic process. The ligand bearing the free hydroxyl (**2a**) is more effective in the cleavage of carboxylate esters than ligands with the methylated alcoholic group (**2b**) or devoid of it (**4**); the opposite behavior is observed in the cleavage of the phosphate triester. A rationale is offered that calls for the difference in the coordination ability to the metal center of phosphate and carboxylate esters.

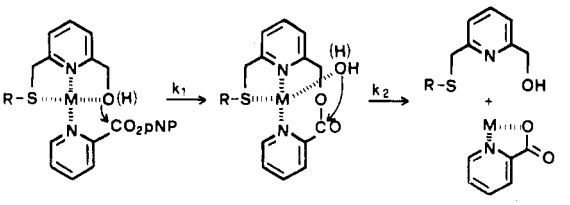
The hydrolysis of carboxylic and phosphoric acid esters is of paramount importance in biological[1,2] and industrial processes. Furthermore, interest in the cleavage of phosphate esters is prompted by the importance of decontaminating areas exposed to some pesticides or chemical weapons.

Functional micelles[3] and, more recently, vesicles[4] are investigated as effective catalytic systems as they provide the hydrophobic environment for binding the substrate and the function that may perform the catalytic process. Many hydrolytic processes in enzymes involve metal cations that are assumed to activate a water molecule or other nucleophilic groups as well as the electrophilic center of the substrate. Moving along these lines, our as well as other research groups, were stimulated to construct and investigate the properties of metal chelating surfactants[5] or lipophilic ligands[6] enbedded or not in a micellar matrix, as catalysts of the cleavage of carboxylic or phosphoric acid esters.

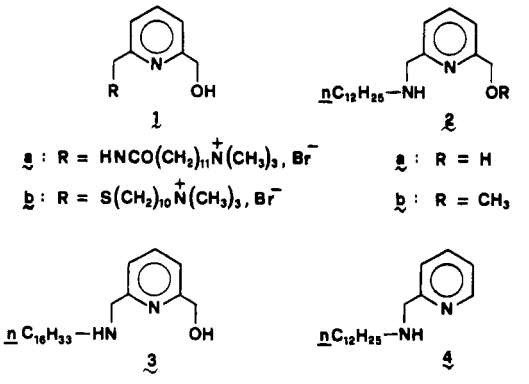
We have recently reported[5a] that 2-(hydroxymethyl)-pyridine-functionalized surfactants **1** are powerful catalysts of the cleavage of *p*-nitrophenyl picolinate (PNPP) in the presence of Cu(II) or Zn(II) ions. The critical feature of the catalytic process was the formation of a ternary complex involving ligand, metal ion, and substrate in which the metal cation activated the hydroxyl nucleophile in a pseudo-intramolecular process (see Scheme I).

Rather disappointingly these systems did not accelerate the cleavage of substrate other than PNPP or $\alpha$-amino acid esters, i.e., esters that are also strong ligands. On the other hand, a Cu(II) surfactant containing a diaminoethane

**Scheme I**




subunit has been recently reported by Menger[6d] to be a good catalyst of the cleavage of a phosphate triester but not of carboxylic acid esters.[7] In order to shed light on these results we synthesized ligands **2–4** and tested their $Cu^{2+}$ complexes as catalysts of the cleavage of carboxylic acid esters of various hydrophobicity, namely, the *p*-nitrophenyl esters of acetic, *n*-hexanoic, *n*-dodecanoic acids (PNPA, PNPH, and PNPD, respectively), and *p*-nitrophenyl diphenyl phosphate (PNPDPP) as well. The results of the kinetic study are here reported together with a possible rationale of the effects observed.




## Results

**Ligands.** Ligands **2** and **3** have been synthesized by reaction of 2-(carboxymethyl)-6-formylpyridine[8] with the proper alkylamine followed by reduction with $NaBH_4$. Ligand **4** has been obtained by reduction of the imine formed by reacting 2-formylpyridine with *n*-dodecylamine. None of them is dispersible in neutral aqueous solutions; nevertheless, in the presence of at least 1 equiv of $Cu^{2+}$ ions ligands **2** and **3** form clear dispersions at pH = 6.3 (MES buffer) made of micellar aggregates of the ligand·$Cu^{2+}$ complex. In the case of **3** solubilization requires sonication

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**Table I. Observed Rate Constants, $k_\psi$, for the Cleavage of PNPH and PNPDPP by Different Catalysts[a]**

| | | | PNPH | | PNPDPP | |
|---|---|---|---|---|---|---|
| entry | catalyst[b] ($10^3$ conc, M) | additive[c] | $10^4k_\psi$, $s^{-1}$ | $k_\psi/k_{CTABr}$ | $10^4k_\psi$, $s^{-1}$ | $k_\psi/k_{CTABr}$ |
| 1 | none[d] | CTABr[d] | 0.037 | 1 | 1.1 | 1 |
| 2 | **2a**[e] (1) | CTABr | 0.34 | 9 | 1.6 | 1.5 |
| 3 | **2a**·$Cu^{2+}$ (1) | none | 36.4 | 984 | 31.9 | 29 |
| 4 | **2a**·$Cu^{2+}$ (1) | CTABr | 4.5 | 122 | 4.1 | 4 |
| 5 | **2a**·$Cu^{2+}$ (2) | none | 51.8 | 1400 | 38.5 | 35 |
| 6 | **2b**·$Cu^{2+}$ (1) | none | 1.6 | 43 | 31.9 | 29 |
| 7 | **2b**·$Cu^{2+}$ (1) | CTABr | 0.65 | 17 | 14.2 | 13 |
| 8 | **2b**·$Cu^{2+}$ (2) | none | 2.5 | 68 | 79.5 | 72 |
| 9 | **3**·$Cu^{2+}$[f] (0.4) | CTABr | 5.12 | 138 | | |
| 10 | **4**·$Cu^{2+}$ (2) | CTABr | 0.43 | 12 | 15 | 14 |

[a] 0.05 M MES buffer, pH = 6.25, 35 °C. [b] In all cases [ligand] = [$Cu^{2+}$]. [c] [Additive] = 10[catalyst]. [d] Conditions: [$Cu^{2+}$] = $1 \times 10^{-3}$ M; [CTABr] = $4 \times 10^{-3}$ M. [e] Without metal ion. [f] This was the highest allowed concentration of catalyst for stable solutions (see text).

of a suspension of the ligand in the buffer solution containing the metal cations. The dynamic light scattering analysis of the aggregates formed after sonication suggests the coexistence of multimodal structures, probably micellar assemblies and other aggregates. They are rather unstable and turbidity appears after ca. 1.5–2 h at room temperature after sonication. Solutions of aggregates obtained with **2**·$Cu^{2+}$ complexes are, on the contrary, stable for months. The $Cu^{2+}$ complex of ligand **4** is not appreciably dispersible in water and could be studied only co-micellized in a cetyltrimethylammonium bromide (CTABr) matrix. The critical micelle concentration (cmc), determined tensiometrically, was $1.3 \times 10^{-4}$ M in 0.05 M MES buffer at pH = 6.3 for ligand **2a**·$Cu^{2+}$ complex; the cmc for **3**·$Cu^{2+}$, being lower than $5 \times 10^{-6}$ M, could not be precisely determined. The binding constants of these ligands with $Cu^{2+}$ have not been directly determined; they have been estimated in the range $10^9$–$10^{10}$ $M^{-1}$ reasonably close to that reported[9] for the formation constant ($K_b = 10^{9.5}$ $M^{-1}$) of the 1:1 complex of 2-(aminomethyl)pyridine with $Cu^{2+}$. However, the complex formation constant may be lower (by 1 or 2 orders of magnitude) when the ligand aggregates in cationic micelles,[10] particularly in co-micelles with CTABr, due to electrostatic repulsion of the metal ion.

**Kinetic Studies.** Kinetics were performed under pseudo-first-order conditions, monitoring the appearance of *p*-nitrophenol at 317 nm and at 35 °C in 0.05 M MES buffer (pH = 6.3). The temperature was chosen in order to explore a sufficiently high concentration range. The detailed analysis of the system has been done for ligand **2a** as aggregates made of **2a**·$Cu^{2+}$ are much more stable than those made of **3**·$Cu^{2+}$.

The data are reported in Tables I and II. Those in Table I allow a comparison between the different catalysts in the cleavage of esters PNPH and PNPDPP and indicate the kinetic benefits relative to the reaction measured in CTABr and $Cu^{2+}$ only, without the ligand ($k_{CTABr}$). The hydrolysis rate in pure buffer solution ($k_0$) at pH = 6.3 was not accessible for the most hydrophobic substrates (PNPH, PNPD, and PNPDPP). Table II reports the kinetic and thermodynamic parameters obtained from the nonlinear regression analysis of the kinetic data obtained for the four esters investigated. The value $k_{lim}$ represents the extrapolated rate constant expected for the substrate totally bound to the micelle as obtained by using the binding constant reported in the same table. The last column of Table II also reports very approximate values of the overall accelerations, as the ratio $k_{lim}/k_0$, the $k_0$ term having been estimated from extrapolations of literature data (see

**Table II. Kinetic and Thermodynamic Parameters for the Cleavage of the Investigated Substrates by Catalyst 2a•$Cu^{2+}$[a]**

| substr | $10^3k_{lim}$[b] $s^{-1}$ | $K_b$[b], $M^{-1}$ | $k_{lim}$[b,c]/$k_{CTABr}$ | $k_{lim}/k_0$ |
|---|---|---|---|---|
| PNPA | 21.3 | 94 | 5070 | 6090 |
| PNPH | 9.6 | 628 | 2590 | (4500)[d] |
| PNPD | 9.1 | 2600 | 2680 | (17500)[d] |
| PNPDPP | 4.6 | 2500 | 42[e] | (26500)[f] |

[a] In MES buffer, pH = 6.25, and at 35 °C. [b] From the nonlinear regression analysis of the kinetic data. [c] Owing to the poor solubility of substrates PNPH, PNPD, and PNPDPP in water solution and the difficulty to get acceptable kinetics at this pH in the absence of catalyst, we compared the $k_{lim}$ with those obtained in the presence of CTABr and $Cu^{2+}$ which are reliable. [d] The $k_0$ values for the evaluation of these ratios were extrapolated from literature data taking into account differences in pH and temperature (Bonora, G. M.; Fornasier, R.; Scrimin, P.; Tonellato, U. *J. Chem. Soc., Perkin Trans. 2* **1985**, 367. Guthrie, J. P. *J. Chem. Soc., Chem. Commun.* **1972**, 897; *Can. J. Chem.* **1973**, *51*, 3494. Tee, O. S.; Enos, J. A. *Can. J. Chem.* **1988**, *66*, 3027). [e] The reasons for this low acceleration are discussed in the text. [f] Extrapolated from: Bunton, C. A.; Farber, S. J.; Fendler, E. J. *J. Org. Chem.* **1968**, *33*, 29.

footnotes of the table). Accordingly these ratios must be taken with caution. It is relevant to notice that the acceleration effect exerted by CTABr on the rate of hydrolysis of the carboxylic acid ester is quite modest (less than 1 order of magnitude) while it is rather remarkable for PNPDPP[11] (ca. 600 times). The relative rates reported in Table I should be read keeping in mind this point. The reported rate constants of Table I for homomicelles (no CTABr added) and those of Table II underestimate the highest rate constants attainable with ligand **2a** since they have been determined for 1:1 complexes while the most active species (see below) are the 2:1 (ligand:metal) complexes that were not soluble in the absence of additive.

Figures 1 and 2 show the rate vs catalyst concentration profiles determined in the presence of catalysts **2a**·$Cu^{2+}$ and **2b**·$Cu^{2+}$ for substrates PNPH and PNPDPP. Methylation of the hydroxyl causes a relevant decrease of the catalytic effect in the cleavage of PNPH, the observed rate constant being 21 times slower (at[catalyst] = [$Cu^{2+}$] = $2 \times 10^{-3}$, see entries 5 and 8 of Table I). In the case of PNPDPP, the opposite trend is observed: the observed rate constant, $k_\psi$, is almost 2 times faster (same conditions and entries as above) in the case of **2b** than in that of **2a**. Catalyst **4**·$Cu^{2+}$ behaves exactly as **2b**·$Cu^{2+}$ (compare entries 7 and 10 in Table I), thus indicating that the $OCH_3$ group is not involved in the process.

As mentioned above the most efficient stoichiometry with ligand **2a**, either in the cleavage of PNPH or PNPDPP, is 2(ligand):1($Cu^{2+}$). This is clearly shown by the

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(10) Scrimin, P.; Tecilla, P.; Tonellato, U.; Vendrame, T. *J. Org. Chem.* **1989**, *54*, 5988.
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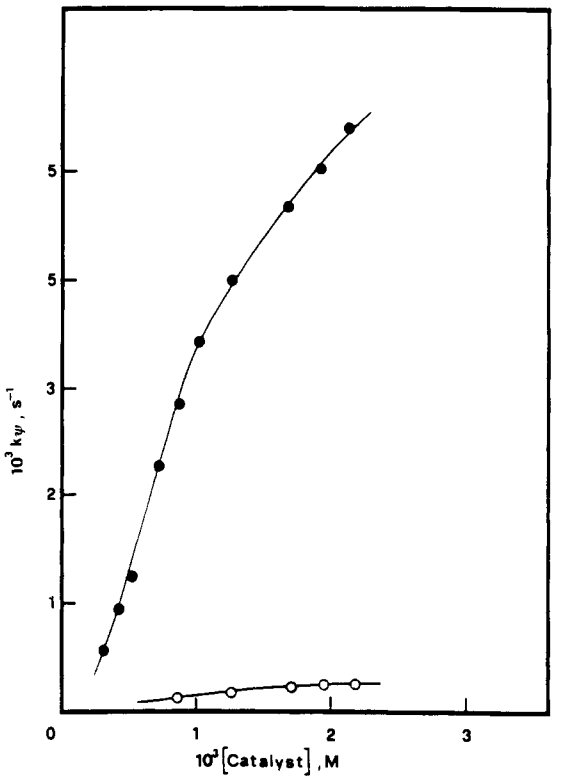



**Figure 1.** Observed rate constant, $k_\psi$, vs concentration profiles for the cleavage of PNPH: (●) catalyst = **2a**·$Cu^{2+}$; (○) catalyst = **2b**·$Cu^{2+}$ (MES buffer, pH = 6.3, 35 °C.

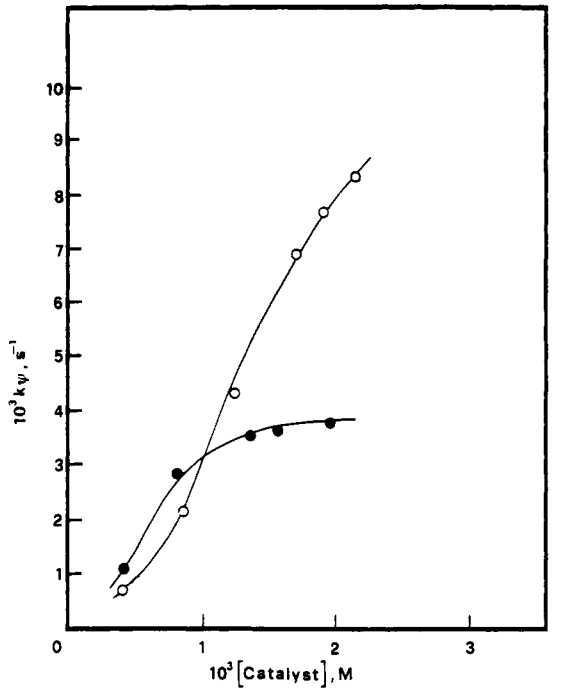



**Figure 2.** Observed rate constant, $k_\psi$, vs concentration profiles for the cleavage of PNPDPP: (●) catalyst = **2a**·$Cu^{2+}$; (○) catalyst = **2b**·$Cu^{2+}$ (MES buffer, pH = 6.3, 35 °C).

plots reported in Figure 3. The kinetic runs have been performed at different $Cu^{2+}$ concentration at a fixed [ligand] co-micellized with CTABr([CTABr] = 10[ligand]). The use of co-micelles was necessary since when $[Cu^{2+}] <$ [ligand] no stable solutions could be obtained. With both substrates the curves show a maximum at $[Cu^{2+}]/$[catalyst] = 0.5. Surprisingly, in the case of the methylated ligand (which shows an enhanced reactivity with PNPDPP), the optimum stoichiometry switches to 1:1 (see Figure 3 inset).

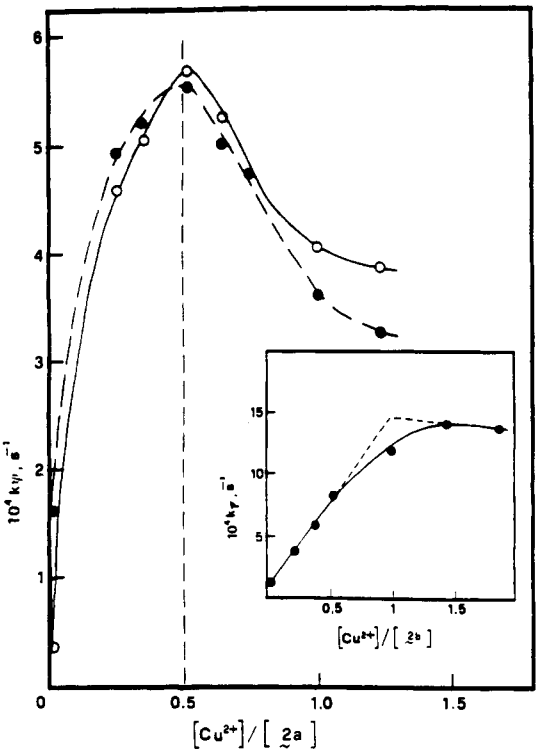



**Figure 3.** Changes of the observed rate constant, $k_\psi$, as a function of the $[Cu^{2+}]/$[**2a**] ratio for substrates PNPH (○) an PNPDPP (●). Inset: Same profile observed for ligand **2b** and substrate PNPDPP. Conditions in all cases: [CTABr] = $9.1 \times 10^{-3}$ M; [ligand] = $9.1 \times 10^{-4}$ M; MES buffer, pH = 6.3, 35 °C.

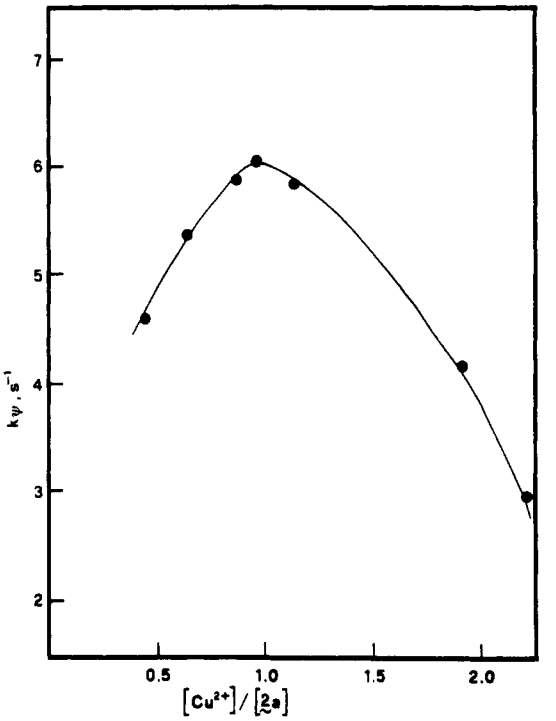



**Figure 4.** Changes of the observed rate constant, $k_\psi$, as a function of the $[Cu^{2+}]/$[**2a**] ratio for substrate PNPP ($[Cu^{2+}] = 1.8 \times 10^{-4}$ M; **2a** was used as a 1:10 blend with CTABr; MES buffer, pH = 6.3, 35 °C).

Interestingly, the same 1:1 stoichiometry leads to the most efficient system in the case of the cleavage of PNPP with **2a** (see Figure 4). Owing to the strong dependence of the rate of the hydrolysis of PNPP[12] on $[Cu^{2+}]$, the plot of

(12) Fife, T. H.; Przystas, T. J. *J. Am. Chem. Soc.* **1985**, *107*, 1041.

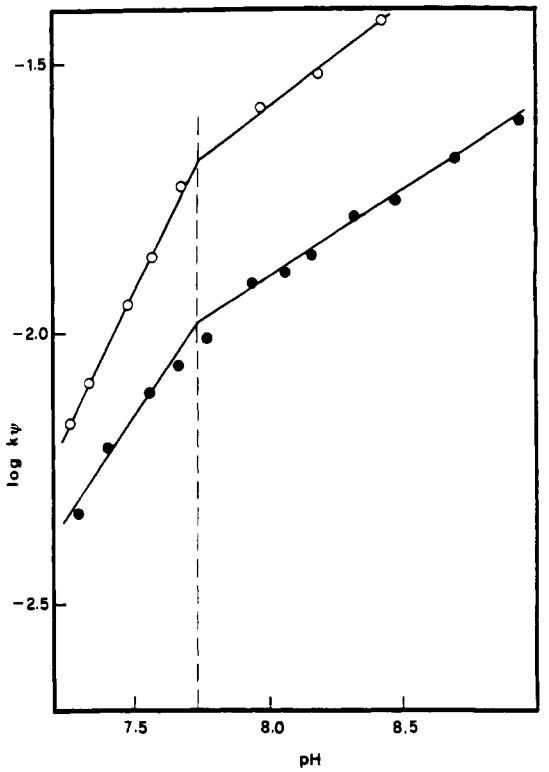


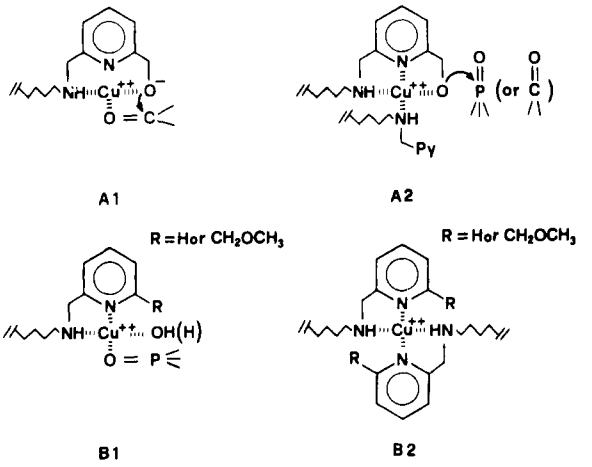

**Figure 5.** Observed rate constant, $k_\psi$, vs pH profiles for the cleavage of PNPH (○) and PNPDPP (●) by catalyst **2a** $Cu^{2+}$ ([**2a**] = [$Cu^{2+}$] = $1 \times 10^{-3}$ M, 35 °C).

Figure 4 has been obtained by keeping constant [$Cu^{2+}$] and changing the concentration of the ligand.

**p$K_a$ Determination.** A pH–rate constant profile was determined for reactions of both substrates PNPH and PNPDPP with catalyst **2a**·$Cu^{2+}$. Buffers used were MES (pH = 6–6.8), HEPES (pH = 6.8–7.8), EPPS (pH = 7.7–8.7), and CHES (pH = 8.7–9.7). The concentration of these buffers was 0.01 M at variance with all other experiments where buffer concentration was 0.05 M. At this higher buffer concentration, we observed a dependence of the slope on the nature of the buffer used. The pH value was checked before and after any kinetic run and proved to be constant within ±0.03 pH unit. A plot of log $k_\psi$ vs pH (Figure 5) gave, in the case of both substrates, a sharp break at pH 7.7, which we take as the systematic p$K_a$ of the hydroxyl bound to the $Cu^{2+}$ ion under our micellar reaction conditions. A p$K_a$ = 7.8 has been recently reported for a $Cu^{2+}$ co-ordinated hydroxyl by Tagaki[5b] in homomicellar anionic aggregates. It would be very interesting to know the p$K_a$ of the $Cu^{2+}$-bound water molecule, which should be responsible of the cleavage of PNPDPP with ligand **2b**. Regrettably solutions of the complex **2b**·$Cu^{2+}$ were unstable at pH > 7.0. Up to that pH we did not observe any break in the log $k_\psi$ vs pH profile.[13]

**Turnover Experiments.** Experiments in the presence of excess substrate were performed either with PNPH or PNPDPP in order to test the real catalytic behavior of the **2a**·$Cu^{2+}$ system. At pH = 6.3 and 35 °C, using a co-micellar blend 1:20 with CTABr (being [**2a**·$Cu^{2+}$] = $2 \times 10^{-5}$ M), we observed a nearly quantitative release of *p*-nitrophenol, with no evidence of "burst" kinetics, using up to a 10-fold PNPH and 5-fold PNPDPP excess over catalyst. This is clear evidence of turnover at least in the case of this catalyst and both substrates. Control experiments indicated that the *p*-nitrophenol released in the cleavage process does not affect the rate of the cleavage process, at variance with the observation reported by Trogler and Morrow.[14]

**Chart I**




## Discussion

The metal–ion complexes of surfactants **2–4** here investigated are good catalysts of the hydrolysis of both carboxylic and phosphoric acid esters at variance with the metallomicelles of **1** and $Cu^{2+}$ or $Zn^{2+}$, which are rather efficient in the cleavage of the ligand substrate PNPP but virtually inert toward PNPA and PNPH.[5a] Control experiments[15] indicate that this difference is due to the failure of the aggregates of **1** to provide a micellar pseudophase hydrophobic enough to host these substrates. In the case of the more classical aggregates studied here, the pronounced hydrophobic environment is such as to ensure an effective catalyst–substrate interaction.

A rationale for the catalytic process should account for the following points: (i) The hydroxy group is a requisite for the cleavage of the carboxylic acid esters but not for that of the phosphate triester. (ii) The complex with 1:2 (metal ion:ligand)stoichiometry appears, in the case of ligand **2a**, the most active with both substrates PNPH and PNPDPP while the methylated sibling **2b**, particularly active with PNPDPP, operates with a preferential 1:1 stoichiometry.

Four ternary complexes, schematically represented in the Chart I, may be envisaged as effective species in the routes to the cleavage of the substrates investigated. A1 and A2 are the 1:1 and 1:2 complexes involving the hydroxyl-bearing ligands; B1 and B2 are the stoichiometric analogues for the O-methylated ligand. In the case of A1 and B1 the substrate is part of the complex, whereas in the case of A2 and B2 the substrate is not included in the complex, although it may be kept in close proximity to it by the hydrophobic forces at play within the micellar aggregate. B2 appears as the most unlikely situation for a productive mode of action as neither co-ordination of the substrate or activation of a nucleophilic species ($H_2O$ or hydroxyl) occurs.

As discussed elsewhere,[5a] complex A1 is the relevant species involved in the cleavage of PNPP and other α-amino acid esters, using hydroxyl bearing ligands.

With ligand **2a**, complex A2 appears as the most effective system leading to the cleavage of carboxylate (PNPH)

(13) A p$K_a$ lower than 6 is suggested by Menger in his system (see ref 6d).

(14) Morrow, J. R.; Trogler, W. C. *Inorg. Chem.* **1989**, *28*, 2830.

(15) Spectroscopic measurements give no evidence of increased solubility of these substrates in the presence of the bolaform aggregates.

and phosphate (PNPDPP) esters on the basis of the following evidence: the 1:2 stoichiometry (see Figure 3), the same apparent $pK_a$ for both types of sustrates (see Figure 5), indicating the $Cu^{2+}$ activated hydroxyl as the nucleophilic function, the absence of inhibition due to the liberated *p*-nitrophenol in the turn over experiments, and, hence, the lack of competition for the complexation to the metal ion.

In the case of ligand **2b** (with the methylated hydroxyl), a mechanistic route via complex B1 is here suggested as the effective mode of cleavage in the case of PNPDPP. Recent work from the laboratories of Menger[6d,16] and Trogler[14] reached similar conclusions with ligands devoid of an hydroxy group. The major difference between the two kind of esters lays, according to the proposed mechanisms, in their ability to be involved in the $Cu^{2+}$ coordination: such a possibility is open only for the phosphate triester or the ligand ester PNPP but not for the carboxylic acid esters. This suggestion is supported by recent data by Chin and Jubian,[17] who evaluated the binding constant of methyl acetate to the $Cu^{2+}$·2,2′-dipyridylamine complex to be as low as $2.6 \times 10^{-3}$ $M^{-1}$. Accordingly, under our conditions ([**2a**·$Cu^{2+}$] = $1 \times 10^{-3}$ M, [substrate] = $1 \times 10^{-5}$ M) the concentration of the ternary complex (ligand–metal ion–substrate) would be lower than $10^{-10}$ M. On the contrary the affinity of the P=O group for $Cu^{2+}$ is much greater[18] and mode B1 becomes more likely.

Still it is not clear why, in the case of ligand **2a**, a route via complex A1 is less efficient in the cleavage of PNPDPP than that via A2 in spite of the fact that the analogous complex B1 is, by all kinetic evidence, effective in the case of ligand **2b**. A possible explanation calls for the strain in the transition state, which should involve a complex structure where the oxygen is part of both a four-membered chelate and a five-membered chelate. Such a situation is not present in a mechanism involving B1 where the nucleophilic is a (deprotonated) coordinated water molecule. Sargeson[19] has recently shown how the formation of a four-membered chelate phosphate ester is strongly dependent on the size of the metal ion as a result of the strain present in such a structure.

## Conclusion

The present results, in line with previous reports,[5,6] point to the effectiveness of $Cu^{2+}$ metallomicelles as catalysts of the cleavage of phosphate or carboxylate esters. In the light of kinetic evidence, it appears that the two kind of esters may be cleaved following different routes. Accordingly, the implementation of an effective metallocatalyst must take into account the different structural features they require for optimum catalysis.

Furthermore, the present mechanistic interpretation may provide interesting suggestions for better understanding the mechanism of the cleavage of peptides or phosphate esters in metalloenzymes. Thus, two different hypotheses have been recently formulated on the mode of action of zinc-containing proteases like carboxypeptidase-A; one[20] favors the prominent role of the metal ion as Lewis acid catalyst, the other[21] prefers a mechanism involving a metal hydroxide as the nucleophile. It appears from these results that (at least in the case of carboxylate esters) C=O coordination to the metal ion with consequent Lewis acid catalysis, if operative in the enzyme, must be the result of a specific coordination mode of the substrate. When the complexation geometry is rather flexible as in micelles, this kind of catalysis does not occur.

We are currently involved in the synthesis of a metallocatalyst with well-defined geometry specifically designed for ester cleavage.

## Experimental Section

**General Methods.** Melting points are uncorrected. NMR spectra were measured with a Bruker WP 200 SY spectrometer operating at 200 MHz, and chemical shifts are reported relative to internal $Me_4Si$. Surface tension measurements were performed with a Kruss type 8451 tensiometer. Kinetic traces were recorded on a Perkin-Elmer Lambda 5 spectrophotometer equipped with a thermostatted cell holder. Dynamic light scattering measurements were made on a Nicomp 370 particle size autocorrelator equipped with a Spectra-Physics 2016 argon laser. Microanalyses were performed by the Laboratorio di Microanalisi of our department.

**Materials.** $Cu(NO_3)_2$ was analytical grade Carlo Erba product. Metal ion stock solutions were titrated against EDTA following standard procedure.[22] Buffers were made up from glass-bidistilled water. The buffer components[23] were used as supplied by the manufacturers: MES (Fluka), HEPES (Sigma), EPPS, CHES (Aldrich). Abbreviations for buffers used are as follows: MES, 4-morpholinoethanesulfonic acid, HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, EPPS, 1-(2-hydroxyethyl)-piperazine-4-(3-propanesulfonic acid), CHES, 2-(cyclohexylamino)ethanesulfonic acid. PNPA, PNPH, and PNPD were Sigma products used as received. PNPP[24] and PNPDPP[25] were prepared and purified by literature methods.

**6-((*n*-Dodecylamino)methyl)-2-(hydroxymethyl)pyridine (2a).** 6-Formyl-2-(carboxymethyl)pyridine[8] (800 mg, 4.85 mmol) was dissolved in a THF solution (20 mL) containing *n*-dodecylamine (900 mg, 4.85 mmol) and freshly activated 4-Å molecular sieves and kept for 12 h under stirring, at room temperature in a nitrogen atmosphere. The slurry was subsequently filtered off, and the THF was evaporated under reduced pressure to leave the imine derivative as an oily material. The $^1H$ NMR ($CDCl_3$) spectrum shows the disappearance of the aldehyde CH signal ($\delta$ = 10.21) substituted by the imine CH ($\delta$ = 8.51). Next, 500 mg (1.5 mmol) of this crude material was dissolved in dry ethanol and 235 mg (6.2 mmol) of $NaBH_4$ (*CAUTION:hydrogen evolution!*) was added to the solution. After gas evolution ceased, 690 mg (6.2 mmol) of finely powdered $CaCl_2$ was added and the slurry kept under magnetic stirring for 1 h at room temperature. Excess hydride was cautiously destroyed with water and the ethanol rotary-evaporated. The remaining milky water solution was extracted with chloroform (3 × 100 mL), and the organic layer was dried ($Na_2SO_4$) and evaporated to leave the amino alcohol as a white solid. This was recrystallized from acetone: 438 mg (95% yield), mp 52.5–53 °C.

NMR $\delta_{CDCl_3}$: 0.88 (br t, 3 H, $CH_3$), 1.28 (m, 18 H, $CH_2(CH_2)_9$), 1.55 (m, 2 H, $NCH_2CH_2$), 2.10 (br s, 2 H, NH and OH), 2.68 (t, $J$ = 7.02 Hz, 2 H, $NCH_2(CH_2)_n$), 3.94 (s, 2 H, $NCH_2Py$); 4.74 (s, 2 H, $OCH_2Py$), 7.11 and 7.21 (2d, $J$ = 7.63 Hz, 2 H, Py H3 and H5), 7.64 (t, $J$ = 7.63 Hz, 1 H, Py H4).

Anal. Calcd for $C_{19}H_{34}N_2O$: C, 74.46; H, 11.18; N, 9.14. Found: C, 74.42; H, 11.42; N, 9.17.

**6-((*n*-Hexadecylamino)methyl)-2-(hydroxymethyl)-pyridine (3).** This compound was synthesized by following the same procedure as followed in the synthesis of **2a**, using *n*-hexadecylamine. The product has mp 68–69 °C (from pentane).

NMR $\delta_{CDCl_3}$: 0.88 (br t, 3 H, $CH_3$), 1.29 (m, 26 H, $(CH_2)_{13}$), 1.52 (m, 2 H, $NCH_2CH_2$), 2.00 (br s, 2 H, NH and OH), 2.66 (t, $J$ = 7.32 Hz, 2 H, $NCH_2(CH_2)_n$), 3.92 (s, 2 H, $NCH_2Py$), 4.74 (s, 2 H, $OCH_2Py$), 7.10 and 7.21 (2d, $J$ = 7.63 Hz, 2 H, Py H3 and H5),

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7.64 (t, $J$ = 7.63 Hz, 1 H, Py H4).

Anal. Calcd for $C_{23}H_{42}N_2O$: C, 76.19; H, 11.68; N, 7.72. Found: C, 75.88; H, 12.04; N, 7.63.

**6-((*n*-Dodecylamino)methyl)-2-((methyloxy)methyl)-pyridine (2b).** The amino alcohol **2a** (320 mg, 1.04 mmol) was dissolved in dry dioxane (10 mL) containing triethylamine (106 mg, 1.1 mmol). Di-*tert*-butyl dicarbonate (260 mg, 1.1 mmol) was subsequently added and the solution stirred for 3 h at room temperature. The dioxane was rotary-evaporated and the crude material obtained was taken up with slightly basic water (50 mL) and extracted with chloroform (3 × 80 mL). Evaporation of the dried ($Na_2SO_4$) organic solution gave quantitatively the protected derivative, which was used without any further purification. This was slowly added to a suspension of NaH (1.5 mmol) in dry THF to which $CH_3I$ (1.5 mmol) was subsequently added. The reaction mixture was kept in a nitrogen atmosphere at 40 °C for 1 h and then stirred overnight at room temperature. The slurry was next quenched (cautiously!) with water and extracted with $CHCl_3$. Evaporation of the dried chloroform yielded the methylated alcohol, which was purified by column chromatography ($SiO_2$, $CHCl_3/CH_3OH$ 20:1). After purification 300 mg of material was collected. Deprotection was achieved by following standard treatment with $HBr/CH_3COOH$ (15 min, room temperature), yielding, after workup, 210 mg of pure **1b** as an oil.

NMR $\delta_{CDCl_3}$: 0.87 (br t, 3 H, $(CH_2)CH_3$), 1.25 (m, 18 H, $(CH_2)_9$), 1.52 (m, 2 H, $NCH_2CH_2$), 1.98 (br s, 1 H, NH), 2.64 (t, $J$ = 7.32 Hz, 2 H, $NCH_2(CH_2)_n$), 3.47 (s, 3 H, $OCH_3$), 3.88 (s, 2 H, $NCH_2Py$), 4.57 (s, 2 H, $OCH_2Py$), 7.20 and 7.28 (2d, $J$ = 7.63 Hz, 2 H, Py H3 and H5), 7.65 (t, $J$ = 7.63 Hz, 1 H, Py H4).

Anal. Calcd for $C_{20}H_{36}N_2O$: C, 74.95; H, 11.32; N, 8.74. Found: C, 74.81; H, 11.40; N, 8.60.

**2-((*n*-Dodecylamino)methyl)pyridine (4).** 2-Formylpyridine (563 mg, 5.2 mmol) and *n*-dodecylamine (952 mg, 5.2 mmol) were dissolved in benzene (50 mL) and refluxed under Dean-Stark conditions for 2 h. The solvent was then rotary-evaporated and the crude imine dissolved in ethanol (20 mL). $NaBH_4$ (400 mg, 10.5 mmol) was added in portions and the solution stirred at room temperature for 4 h. Excess hydride was destroyed by addition (caution!) of water. Ethanol was evaporated and the milky water solution extracted with chloroform (3 × 50 mL). Evaporation of the dried ($Na_2SO_4$) organic layer gave the crude amine as a greasy material (1.2 g, 87% yield). Pure **4** was obtained after column chromatography ($SiO_2$, $CHCl_3/CH_3OH$, 20:1).

NMR $\delta_{CDCl_3}$: 0.88 (br t, 3 H, $CH_3$), 1.27 (m, 18 H, $(CH_2)_9$), 1.54 (m, 2 H, $NCH_2CH_2$), 1.95 (br s, 1 H, NH), 2.65 (t, $J$ = 7.32 Hz, 2 H, $NCH_2CH_2$), 3.91 (s, 1 H, $NCH_2Py$), 7.16 (ddd, $J$ = 7.63, 4.88, 1.83 Hz, 1 H, Py H5), 7.31 (ddd, $J$ = 7.63, 1.83, 1.22 1 H, Py H3), 7.64 (td, $J$ = 7.63, 1.83 Hz, 1 H, Py H4), 8.56 (ddd, $J$ = 4.88, 1.83, 1.22 Hz, 1 H, Py H6).

Anal. Calcd for $C_{18}H_{32}N_2$: C, 78.28; H, 11.67; N, 10.13. Found: C, 78.02, H, 11.52; N, 10.24.

**Kinetic Studies.** Solutions were prepared in the proper buffer (0.05 M) at 35 °C. Reaction temperature was maintained at 35 ± 1 °C. Release of *p*-nitrophenol[26] was followed at 317 nm. Each kinetic run was initiated by injecting a 20-40-μL portion of substrate ($1 \times 10^{-3}$ M in $CH_3CN$) into the cuvette containing 2 mL of the buffer solution. Rate constants were obtained either by linear plots of log $(A_\infty - A_t)$ vs time or nonlinear regression analysis[27] of the absorbance data vs time. Calculations in aggregate solutions were made by assuming, in each case, that the reaction medium is a homogeneous solution.

**Acknowledgment.** This research was sponsored by the Italian National Reserch Council (CNR, Rome) in the frame of the special National Project on "Chimica Fine e Secondaria". P.S. thanks Professor Fred Menger, Emory University, for discussion. We are indebted to Mr. Enzo Castiglione for his skillful technical assistance.

**Registry No.** **2a**, 130496-97-8; **2a** *O*-*t*-Bu derivative, 130496-98-9; **2b**, 130496-99-0; **3**, 130497-00-6; **4**, 130497-01-7; PNPA, 830-03-5; PNPH, 956-75-2; PNPD, 1956-11-2; PNPDPP, 10359-36-1; 6-formyl-2-(carboxymethyl)pyridine, 130497-02-8; *n*-dodecylamine, 124-22-1; *n*-hexadecylamine, 143-27-1; 2-formylpyridine, 1121-60-4.

(26) Breslow (see ref 6c) has shown that the cleavage of PNPDPP yields *p*-nitrophenol as the principal product though some alternative hydrolysis with loss of phenol may also occur. This is probably true in our case too, though we did not investigate the products composition. The rate constants evaluated from the spectrophotometric appearance of *p*-nitrophenol are, however, not affected by the possibly competing hydrolysis to liberate phenol (see ref 14).

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# Reactions of Thermally Generated *tert*-Butyl and Di(*tert*-alkyl)ketyl Radicals in Toluene: Cage Effects and Hydrogen Transfer

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*Received April 17, 1990*

Thermolysis of di(1-adamantyl)-*tert*-butylmethanol (**2a**) in toluene at 145-185 °C gives mainly bibenzyl, di(1-adamantyl) ketone, di(1-adamantyl)methanol, and the cross-product, 1,1-di(1-adamantyl)-2-phenylethanol. In the presence of benzophenone (BP) or benzenethiols as hydrogen-accepting and hydrogen-donating radical scavengers, respectively, the di(1-adamantyl)methanol/di(1-adamantyl) ketone ratio tends to steady values as the scavenger/**2a** ratio is increased, while the cross-product disappears. At 165 °C the secondary alcohol minimum is 8% (BP) and the ketone minimum 11% (thiol). These represent the contributions of geminate hydrogen atom transfer reactions to the overall yields, i.e., the cage effects. With BP the major cross-product is 1,1,2-triphenylethanol. Products from the self- and cross-reactions of benzyl and thiyl radicals are found when thiol is present, the diaryl disulfide predominating at high thiol concentration. In both cases, cross-products resulting from reaction of the *tert*-butyl radical with the scavenger-derived radical are detected in small amounts, being of greater importance in deuteriated toluene. The *tert*-butyl radical is considered, therefore, to be less reactive in hydrogen atom abstraction than the 1-adamantyl radical. Cage effects for other di(*tert*-alkyl)-*tert*-butylmethanols that thermolyze with exclusive *t*-Bu-C bond fission have also been measured and the product composition of the scavenger-free reaction interpreted by kinetic simulation based on the steady state approximation. Rate constants for hydrogen abstraction by the *tert*-butyl radical from toluene are not accurately determined by this procedure but seem, nevertheless, to indicate that the literature value (14.4 $M^{-1}$ $s^{-1}$ at 48 °C) is an overestimate. Solvent hydrogen abstraction by the ketyl radical shows a small but well-defined steric effect.

In the thermolysis of tri(*tert*-alkyl)methanols in toluene, one of the key products is the secondary alcohol, resulting from hydrogen atom transfer to the ketyl radical intermediate. If all the *tert*-alkyl groups are bridgehead, then

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