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A Macrocyclic Enzyme Model System. An Electrostatic-Hydrophobic Double-Field Catalysis by a [20]Paracyclophane in the Deacylation of *p*-Nitrophenyl Hexadecanoate†

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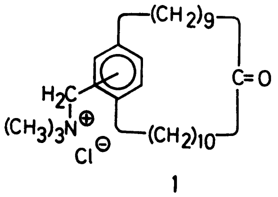
The catalytic functions of 10-oxo[20]paracyclophanyl-22(23)-methyltrimethylammonium chloride (**1**) in the hydrolysis and aminolysis reactions of *p*-nitrophenyl hexadecanoate (PNPP) were investigated in 1% (v/v) methanol–1% (v/v) dioxane-water over the pH 9—10 region at 40.0 °C and $\mu=0.2$ (NaCl) with the initial PNPP concentration of 1.00×10^{-5} M. The paracyclophane (0.88×10^{-5} M) accelerated the hydrolysis of PNPP by one order of magnitude at pH 9.7. The presence of either ethanolamine or glycine in the **1**-catalyzed deacylation of PNPP resulted in further rate enhancement. The rate enhancement was brought about by the aminolysis reaction of PNPP with the amines as confirmed by the product analysis. The greater reactivity of glycine relative to that of ethanolamine was attributed to the electrostatic interaction between the anionic carboxylate group of glycine and the cationic ammonium group of **1**. The novel electrostatic-hydrophobic double-field catalysis was discussed for the glycinolysis reaction.

[20]Paracyclophanes have been shown to function as host molecules in aqueous media for the incorporation of *p*-nitrophenyl carboxylates bearing a sufficiently long alkyl chain *via* hydrophobic interaction.[1–6] An efficient acyl transfer from the bound substrate to either oxime[1–5] or amino group[6] placed on the paracyclophane skeleton has been observed. In the absence of an effective nucleophile on the macrocycle, the bound substrate would be susceptible to an attack by any nucleophile present in the bulk phase. In such occasions, the reactivity of the bimolecular deacylation reaction would be controlled by the geometry and spatial orientation of a bound substrate against an attacking nucleophile as well as by the approaching mechanism played by the latter species. This state of affairs should be even more pronounced when the macrocycle carries a functional group which may interact with a nucleophile. In this study, we have investigated the hydrolysis and aminolysis of *p*-nitrophenyl hexadecanoate as catalyzed by a [20]-paracyclophan-10-one carrying a quaternary ammonium group on its benzene ring (**1**). The specific catalytic function of the ammonium group is to be characterized from the kinetic viewpoints.



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Experimental

Materials. 10-Oxo[20]paracyclophanyl-22(23)-methyltrimethylammonium chloride (**1**) was prepared as described previously.[4] The crude product was purified by repeated high speed liquid chromatography using a Hitachi 635 liquid chromatograph equipped with a column of Hitachi gel 3019, methanol being used as an eluant. *p*-Nitrophenyl hexadecanoate was the product of E. Merck, Darmstadt, West Germany and used after inspection of its purity by elemental analysis. Glycine obtained from commercial source was recrystallized from water. Ethanolamine was distilled *in vacuo* immediately before use.

Kinetic Measurements. Rates of *p*-nitrophenoxide liberation were measured at 400 nm. Each run was initiated by adding 30 µl of 1.0×10^{-3} M *p*-nitrophenyl hexadecanoate in dioxane to 2.97 ml of a reaction medium which was pre-equilibrated at 40.0 ± 0.1 °C in a thermostatted cell set in a Hitachi 124 recording spectrophotometer. The reaction medium was prepared by mixing 30 µl of a stock solution of **1** in methanol with 2.94 ml of an aqueous buffer solution of $Na_2B_4O_7$–Na_2CO_3, or otherwise either of glycine–sodium glycinate or ethanolamine–ethanolammonium chloride. The ionic strength was maintained constant at 0.2 with sodium chloride.

pH *and* pK_a *Measurements.* pH-Values of the buffer solutions were measured at 40.0 ± 0.1 °C with a Beckman expandomatic SS-2 pH meter equipped with a Metrohm EA-125 combined electrode after calibration with a combination of appropriate standard buffers. The potentiometric titrations were carried out at 40.0 ± 0.1 °C and $\mu=0.20$ (NaCl) with sodium hydroxide to determine pK_a values of glycine and ethanolamine.

Analysis of Glycinolysis Product. A solution of *p*-nitrophenyl decanoate (100 mg) and **1** (1.53×10^{-3} mmol) in a mixture of glycine buffer (pH 9.7, 1000 ml containing 0.07 mol of glycine, 0.03 mol of sodium hydroxide, and 0.07 mol of sodium chloride) and dioxane (70 ml) was stirred at room temperature for 65 h. The reaction mixture was acidified (pH 2) with hydrochloric acid and then extracted with ether (100 ml × 2) and chloroform (100 ml × 3). The combined extracts were extracted with 5% aqueous sodium hydroxide. The aqueous extract was acidified and extracted with ether (100 ml × 3). After drying over sodium sulfate, the ether extract was evaporated to give an oil (30 mg) which was subsequently chromatographed on a column (2.4 × 10 cm) of silica gel (Wako gel C-100) after esterification with excess diazomethane. *p*-Nitrophenol (15 mg) and methyl decanoate (small amount) showing a C=O stretching band (neat) at 1730 cm^{-1} were eluted with benzene and benzene-chloroform (1 : 1), respectively. Further elution with chloroform afforded glycinolysis product **2** (15 mg). IR (neat): 3320

$CH_3(CH_2)_8CONHCH_2CO_2CH_3$

2

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TABLE 1. FIRST-ORDER RATE CONSTANTS FOR THE p-NITROPHENOL RELEASE FROM PNPP IN THE PRESENCE AND ABSENCE OF **1** AT $\mu=0.2$ (NaCl), 40.0±0.1 °C[a)]

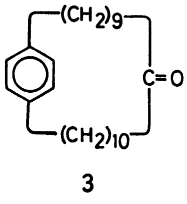
$[\mathbf{1}]\times10^5$ (M)	pH	Buffer system[b)]	$k_{obs}\times10^4$ (s^{-1})
None	9.70	$Na_2B_4O_7$-Na_2CO_3	0.14
None	9.70	$H_2NCH_2CO_2^-$-$H_3N^+CH_2CO_2^-$	0.15
None	9.70	$H_2N(CH_2)_2OH$-$H_3N^+(CH_2)_2OH$	0.23
0.88	9.32	$Na_2B_4O_7$-Na_2CO_3	0.78
0.88	9.73		1.3
0.88	10.03		2.2
0.88	10.16		2.8
0.88	10.33		3.3
0.88	8.80	$H_2NCH_2CO_2^-$-$H_3N^+CH_2CO_2^-$	1.3
0.88	9.10		1.8
0.88	9.40		3.3
0.88	9.75		5.3
0.88	10.07		5.9
0.88	10.25		6.4
0.88	8.96	$H_2N(CH_2)_2OH$-$H_3N^+(CH_2)_2OH$	0.58
0.88	9.33		1.4
0.88	9.79		2.4
0.88	10.10		3.1

a) $[PNPP]_0=1.00\times10^{-5}$ M in 1%(v/v)methanol-1%(v/v)dioxane-water. b) The total amine concentration was kept constant at 0.05 M in the case of amine buffers. The total buffer concentration was kept constant at 0.0098 M in the case of borate-carbonate buffers.

(NH str.), 1730 (ester C=O str.), 1640 (amide C=O str., amide I band), and 1545 cm^{-1} (NH bend + CN str., amide II band). NMR ($CDCl_3$, TMS) : δ 6.02 (1H, broad s, NH), 4.09 (2H, d, NH$\underline{CH_2}$), 3.78 (3H, s, OCH_3), 2.29 (2H, t, CH_2-CO), 1.9—1.1 (14H, m, methylene), and 0.89 (3H, distorted t, $\underline{CH_3}CH_2$).

Results and Discussion

The hydrolysis of p-nitrophenyl hexadecanoate (PNPP) was accelerated by the ammonium salt (**1**) in reference to the spontaneous hydrolysis under the same conditions: initial PNPP concentration of 1.00×10^{-5} M, $\mu=0.2$ with NaCl, in 1%(v/v)methanol–1%(v/v)dioxane–water, at 40.0±0.1 °C. The observed rates are 1.3×10^{-4} and 0.14×10^{-4} s^{-1} for catalyzed and spontaneous reactions at pH 9.7, respectively. The rate enhancement is significant for the presence of a small amount of **1** (0.88×10^{-5} M). The catalytic effect of **1** must be developed by the quaternary ammonium group since [20]paracyclophan-10-one (**3**) which lacks in a positive charge showed no acceleration effect in the PNPP hydrolysis. Two kinetically indistinguishable mechanisms can be cited for the catalytic function of **1**. The ammonium group concentrates anionic hydroxide ions around it as in cationic micelles.[7)] Alternatively, the ammonium group may serve as a super acid which neutralizes a negative charge developing at the carbonyl oxygen of bound PNPP in the transition state in a manner as predicted for the deacylation of PNPP catalyzed by 10-hydroxyimino [20] paracyclophanyl - 22 (23) - methyltrimethyl - ammonium chloride (**4**).[4)] There is a significant difference between these two mechanisms. The former involves the direct electrostatic interaction between the positive ammonium group of the catalyst and the hydroxide ion of nucleophilic nature prior to the rate-determining step while the latter does not. In order to examine this criterion, the kinetic behavior of glycine in the deacylation of PNPP was investigated in reference to that of ethanolamine which has a similar pK_a (amine) as glycine. Glycine is a nucleophile of significant choice since it contains a neutral amino nucleophile as well as an anionic carboxylate having a potentiality to interact electrostatically with the ammonium group of **1**.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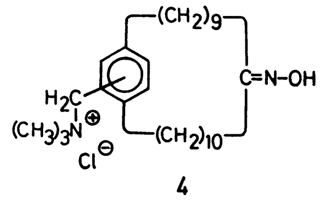
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A large excess of ethanolamine (0.05 M) in the absence of **1** gave out some 1.5-fold increase in the deacylation rate of PNPP at pH 9.70, whereas glycine hardly affected the reaction rate in the same concentration range. The **1**-catalyzed deacylation of PNPP, on the other hand, was enhanced with glycine (0.05 M) by a factor of 4 relative to the **1**-catalyzed alkaline hydrolysis without added glycine. Ethanolamine also showed a similar enhancement effect but to a lesser extent. The effect of pH variation in the pH range 8.8—10.4 at a constant amine concentration (0.05 M) and the effect of an amine concentration in the 0.01—0.1 M range at constant pH (9.70) on the **1**-catalyzed deacylation were investigated to go through a more critical assessment of the effects of amine bases. The observed first-order rate constants (k_{obsd}) for the deacylation of PNPP are summarized in Tables 1 and 2. The pH-rate and amine concentration-rate correla-

Table 2. Effects of concentration changes of glycine and ethanolamine on the p-nitrophenol release from PNPP as catalyzed by **1** at pH 9.70±0.02, μ=0.2 (NaCl), and 40.0±0.1 °C[a)]

[Glycine] (M)	$k_{\text{obs}}\times10^4$ (s^{-1})	[Ethanolamine] (M)	$k_{\text{obs}}\times10^4$ (s^{-1})
0.01	3.5	0.013	2.5
0.03	5.7	0.033	3.0
0.05	7.1	0.05	3.4
0.075	9.2	0.07	4.3
0.088	10.8	0.1	4.3
0.1	12.3		

a) $[\text{PNPP}]_0=1.00\times10^{-5}$ M, $[\mathbf{1}]=1.53\times10^{-5}$ M in 1% (v/v)methanol–1%(v/v)dioxane-water.

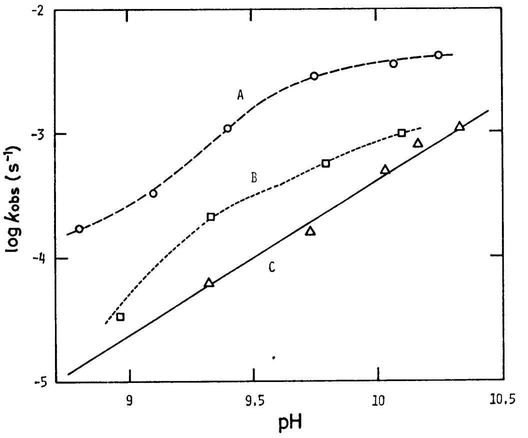

Fig. 1. pH-Rate correlation for the p-nitrophenol release from PNPP as catalyzed by **1** (0.88×10^{-5} M) at μ=0.2 (NaCl), and 40.0±0.1 °C: A, glycine buffer (0.05 M); B, ethanolamine buffer (0.05 M); C, borate-carbonate buffer (0.01 M).

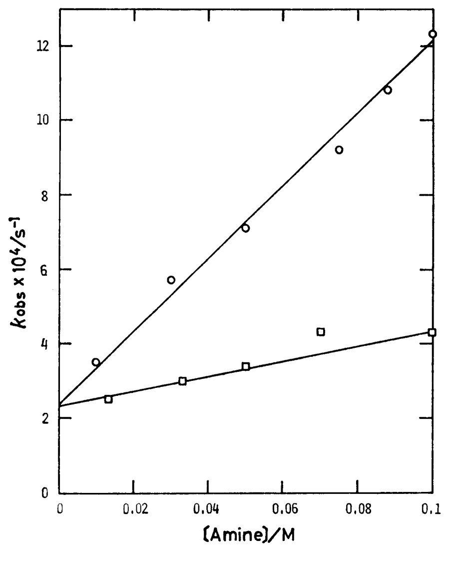

Fig. 2. Correlations between first-order rate constant for the p-nitrophenol release from PNPP as catalyzed by **1** (1.53×10^{-5} M) and concentrations of glycine (○) and ethanolamine (□) at pH 9.70±0.02, μ=0.2 (NaCl), and 40.0±0.1 °C.

tions are shown in Figs. 1 and 2, respectively. Aminolysis by the added amine may certainly be responsible for the rate enhancement. That the aminolysis was actually taking place was confirmed by the product analysis using p-nitrophenyl decanoate instead of hexadecanoate as a substrate; the decanoate ester in a glycine buffer (pH 9.7) containing a catalytic amount of **1** afforded the glycinolysis product. Judging from the nucleophilicities of glycine and hydroxide toward p-nitrophenyl acetate, second-order rate constants being respectively 155 and 890 M^{-1} min^{-1} at 25 °C,[8)] the kinetic effect of glycine observed for the present system is surprisingly small. This is most likely ascribed to a steric factor pertaining to such a bulky substrate as PNPP and its complex with **1**.

At constant pH (9.70), the **1**-catalyzed deacylation rate (k_{obsd}) can be correlated linearly with the amine concentration (Fig. 2); $k_{\text{obsd}}=\kappa(\text{OH})+k_{\text{amine}}[\text{Amine}]$ where $\kappa(\text{OH})$[9)] represents the hydrolysis rate factor as a function of pH and k_{amine} the catalytic rate constant effected by the presence of glycine or ethanolamine. It is evident that the k_{amine} value for glycine is 5 times as large as that for ethanolamine. Thus, a novel reaction mechanism must take place in the degradation of PNPP effected by glycine in consistent with the following facts. Firstly, the basicities of glycine and ethanolamine are mutually comparable; the pK_a (amine) values determined potentiometrically under the kinetic condition are 9.4 and 9.3 for glycine and ethanolamine, respectively. The difference in nucleophilic reactivity between the two bases toward a p-nitrophenyl ester would be in a neighborhood of 1.2-fold judging from the rate-basicity correlation established for amine bases in the deacylation of p-nitrophenyl acetate.[8)] Secondly, there is a good reason to expect that a hydrophobic environment provided by the inclusion complex formed between **1** and PNPP would favor the attack of neutral ethanolamine rather than that of charged glycine. In fact the deacylation of PNPP in the absence of **1** was moderately accelerated by ethanolamine but hardly affected by glycine (*vide supra*). Furthermore, the uncharged paracyclophane (**3**) was confirmed qualitatively to show a rate enhancement effect in the aminolysis but not in the hydrolysis of PNPP; ethanolamine was found to show greater enhancement effect than glycine in the aminolysis reaction. Thirdly, steric interaction between an amine nucleophile and the inclusion complex formed between **1** and PNPP may possibly be an important factor governing the reactivity of the amine. However, it is unreasonable that ethanolamine is more bulky than glycine.[10)]

On the basis of these considerations, the enhanced reactivity of glycine should be ascribed to the favorable electrostatic interaction between the negative carboxylate group of glycine and the positive ammonium group of **1** into which PNPP is incorporated (Fig. 3). If there is no special interaction between an amine and **1**, an amine nucleophile attacks unimolecularly on the substrate and the deacylation rate (k_{obsd}) is given by $k_{\text{obsd}}=\kappa(\text{OH})+k_{\text{amine}}[\text{Amine}]$, where $\kappa(\text{OH})$ represents the hydrolysis rate factor[9)] and is evaluated from line C in Fig. 1. Thus, ($k_{\text{obsd}}-\kappa$-

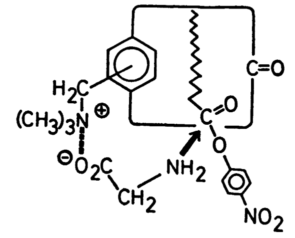

Fig. 3. A plausible reactiom mechanism for the glycinolysis of PNPP: the anionic carboxylate group of glycine is anchored on the ammonium group and the amine nucleophile attacks on PNPP which is incorporated into the hydrophobic cavity of **1**.

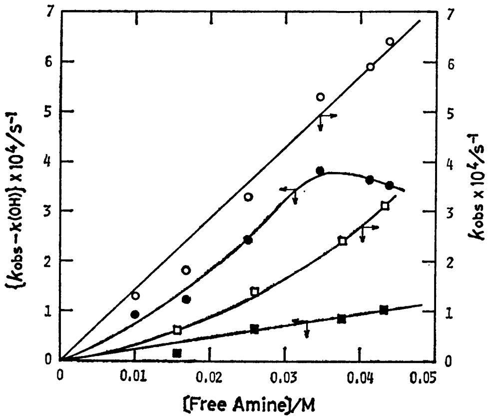

Fig. 4. Correlations between PNPP-deacylation rate and free amine concentration at constant total concentration of amine species (0.05 M) based on the data shown in Fig. 1:

(○, ●), glycine; (□, ■), ethanolamine.

(OH)) should give a linear correlation with the concentration of free amine evaluated from its pK_a value while the total concentration of amine is maintained constant. This is what is observed for the ethanolamine system (Fig. 4). Such a correlation, however, fails to be applied to the glycine system. Free glycine concentrations correlate more satisfactorily in a linear manner with k_{obsd} rather than ($k_{obsd}-\kappa$(OH)) (Fig. 4), and the ($k_{obsd}-\kappa$(OH)) curve is bent down beyond pH 9.7. These results are indicative of an important difference in kinetic behaviors of the two amines. The glycinate ion in a large excess amount seems to exclude the hydroxide ion from the vicinity of the ammonium group of **1** and the glycinolysis becomes a major reaction for the glycine system in a whole pH region studied.

The present work has made a step toward the development of an effective organic double-field catalyst (C) with which the two reacting molecules (A and B) are complexed through two different kinds of interaction forces effected by functions (C_A and C_B) placed in C (Fig. 5). The reactivity and selectivity of the bimolecular reaction of A and B would be much affected by such complexation. The present work demonstrates that the ammonium salt (**1**) should be regarded as a hydrophobic-electrostatic double-field catalyst. The hydrophobic field provided by the polymethylene chain and benzene ring incorporates the substrate (PNPP), while the electrostatic field provided by the ammonium group exhibits a kinetically significant interaction with the anionic carboxylate of the other reactant, glycine (Fig. 3). Another important role played by the hydrophobic cavity of [20]paracyclophanes is the deaggregation of carboxylic esters bearing a long alkyl chain. Such carboxylic esters are known to exist in a self-aggregated form in bulk phase.[11] The incorporation into the hydrophobic cavity tends to set the substrate more or less in an elongated form so that the site of ester bond becomes open and susceptible to the attack of nucleophile.

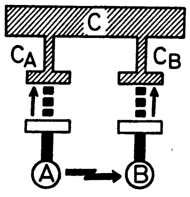

Fig. 5. Schematic representation of double-field catalysis:
A and B, reacting molecules; C, catalyst; C_A and C_B, catalytic functions of different nature provided by the catalyst.

References

1) Y. Murakami, J. Sunamoto, and K. Kano, *Bull. Chem. Soc. Jpn.*, **47**, 1238 (1974).

2) Y. Murakami, J. Sunamoto, H. Okamoto, and K. Kawanami, *Bull. Chem. Soc. Jpn.*, **48**, 1537 (1975).

3) J. Sunamoto, H. Okamoto, H. Kondo, and Y. Murakami, *Tetrahedron Lett.*, 2761 (1975).

4) Y. Murakami, Y. Aoyama, and K. Dobashi, *J. Chem. Soc., Perkin II*, in the press.

5) Y. Murakami, Y. Aoyama, and K. Dobashi, *J. Chem. Soc., Perkin II*, in the press.

6) Y. Murakami, J. Sunamoto, H. Kondo, and H. Okamoto, *Bull. Chem. Soc., Jpn.*, submitted for publication.

7) J. H. Fendler and E. J. Fendler, "Catalysis in Micellar and Macromolecular Systems," Academic Press, New York, N. Y. (1975).

8) W. P. Jencks and J. Carriuolo, *J. Am. Chem. Soc.*, **82**, 1778 (1960).

9) κ(OH) can not be simply correlated with spontaneous (k_0) and hydrolysis (k_{OH}) rate constants by $\kappa(OH)=k_0+k_{OH}[OH^-]$ under the present experimental conditions (refer to Fig. 1). This may be attributed to the direct electrostatic interaction of hydroxide ions with the ammonium group of **1** and to the hydrophobic effect exerted by the inclusion complex formed between **1** and PNPP.

10) E. L. Eliel, N. L. Allinger, S. L. Angyal, and G. A. Morrison, "Conformational Analysis," John Wiley & Sons, Inc., New York, N. Y. (1965), Chap. 7.

11) F. M. Menger and C. E. Portnoy, *J. Am. Chem. Soc.*, **90**, 1875 (1968).