A Macrocyclic Enzyme Model System. Catalytic Properties of 10-Amino-[20]paracyclophane in the Deacylation of *p*-Nitrophenyl Carboxylates

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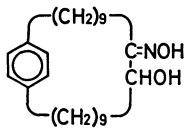
(Received April 21, 1977)

The catalytic efficiency of 10-amino[20]paracyclophane in the deacylation of *p*-nitrophenyl carboxylates was investigated in 10.9 or 20.8% (v/v) aqueous ethanol at $\mu=0.10$ (KCl). The present catalyst exhibited marked catalytic effects not only in the free amine but in the ammonium form. The observed saturation-type kinetics is consistent with a reaction mechanism which involves pre-equilibrium complexation between the aminoparacyclophane and the substrate at a 1 : 1 molar ratio, followed by pseudo-intramolecular catalysis effected by the amino group of the macrocycle. Studies on the inhibition effect by 1-dodecanol and the modification of the catalyst by 2,4-dinitrofluorobenzene confirmed the effective binding ability of the present paracyclophane toward hydrophobic substrates. The free amine form of the catalyst acted as an effective nucleophile to give the acylated aminoparacyclophane as confirmed by the product analysis. On the other hand, the protonated amine form also enhanced the ester degradation, retaining a turnover behavior. On the basis of the kinetic solvent isotope effect and the exceedingly minor kinetic effect of [10-oxo[20]paracyclophan-22(23)-ylmethyl]trimethylammonium chloride in the ester degradation in the neutral pH region, a plausible reaction mechanism has been discussed.

[20]Paracyclophanes are synthetic macrocycles designed to have a hydrophobic cavity of sizable diameter into which an appropriate hydrophobic substrate can be incorporated. Upon formation of the inclusion complex, the cleavage of an ester bond of the incorporated substrate is subjected to the catalysis by a functional group or groups placed on the edge of a macrocyclic skeleton. The previously developed catalysts in this series, *i.e.*, 10-hydroxy-11-hydroxyimino[20]paracyclophane (**1**)[1,2] and substituted 10-hydroxyimino[20]paracyclophanes,[3] have shown characteristic enzyme-like behavior in the deacylation of *p*-nitrophenyl carboxylates bearing a long aklyl chain. In those systems, the reaction had to be carried out at relatively high pH's to observe moderate catalysis because of relatively high pK_a values of the hydroxyimino group. Another macrocycle, 10-amino[20]paracyclophane (**2**), was prepared previously by Murakami *et al.*[4] and investigated as for its catalytic efficiency in this work. Since a free amino group is expected to act as either a nucleophile or a general base and a protonated amino group as either a general acid or an electrostatic catalyst in a moderate pH region, some novel catalysis different from those demonstrated by the hydroxyimino group would be expected in this study.

Amino acid residues as components constituting many enzymes often bear primary amine substituents. These amino groups play indispensable roles in the enzymatic reactions in one way or another. While the aminolysis of carboxylic esters is one of the most thoroughly investigated reactions,[5] the catalytic roles of amino groups seem less understood in the enzymatic reactions in spite of their importance. In the present investigation, the catalytic functions of an amino group placed in the hydrophobic field are to be clarified to obtain a clue to development of more elaborated enzyme model systems.



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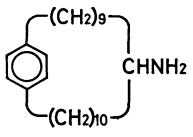

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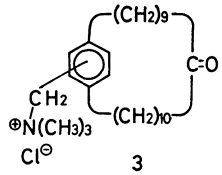

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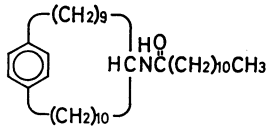

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Experimental

Materials. 10-Amino[20]paracyclophane (**2**) was prepared by a three-step procedure from 11-hydroxy[20]paracyclophan-10-one and isolated as the hydrochloride form.[4] Preparation of [10-oxo[20]paracyclophan-22(23)-ylmethyl]-trimethylammonium chloride (**3**) was described previously.[3] *p*-Nitrophenyl dodecanoate (PNPL) and hexadecanoate (PNPP) were the same as those described previously.[1,2] 2,4-Dinitrofluorobenzene was obtained as a guaranteed reagent from Nakarai Chemicals, Ltd. 1-Dodecanol was also obtained as a guaranteed grade from Nakarai Chemicals, Ltd. and distilled *in vacuo* before use. Deuterium oxide (99.75%) and ethanol-d_1 (99%) were the products of E. Merck AG and Commissariat al' Energie Atomique de France, respectively, and used without further purification.

Kinetic Measurements.[6] The deacylation rates of *p*-nitrophenyl carboxylates were determined by measuring the absorption at 400 nm either on a Hitachi 124 recording spectrophotometer or on a Shimadzu-Bausch & Lomb Spectronic 88 equipped with a Riken Denshi SP-G3 recorder. The procedure for the kinetic measurements was essentially the same as those described previously.[1,2] Each run was initiated by adding an appropriate amount of a substrate dissolved in ethanol to a reaction medium which was pre-equilibrated at an appropriate temperature in a thermostatted cell set in the spectrometer. The reaction medium was prepared by mixing appropriate amounts of 10-amino[20]paracyclophane, ethanol, potassium chloride, and buffer salts. The initial substrate concentration was always maintained at 1.00×10^{-5} M in order to make critical comparisons of kinetic data possible, since the hydrolytic rates of *p*-nitrophenyl carboxylates bearing a long alkyl chain

were reported to vary by the change in their initial concentrations.[7,8]

pH Measurements. The pH and pD values of reaction mixtures were measured with a TOA HM-5A pH meter equipped with a TOA GS-135C combined electrode. The pH meter was calibrated by using a combination of appropriate aqueous buffer solutions. The pH values thus determined were converted into stoichiometric hydrogen ion concentrations ($-\log[H^+]$) by titrating perchloric acid with standard sodium hydroxide in the same solvent as used for the kinetic runs (10.9 or 20.8% (v/v) aqueous ethanol). The difference between pH in 20.8% (v/v) aqueous ethanol and pD in 20.8% (v/v) ethanol-d_1–79.2% (v/v) deuterium oxide was directly estimated from the pH-meter readings measured in phosphate buffer for both solvent systems.

Product Analysis.[9] A solution of **2** (14 mg) and PNPL (50 mg) dissolved in a mixture of aqueous borate-carbonate buffer (pH 9.4, μ=0.1 with KCl, 800 ml) and ethanol (200 ml) was stirred at 40 °C for 58 h, and the mixture was extracted with ether (150 ml×6). The ether extracts were washed with water (200 ml×2), dried over sodium sulfate, and evaporated to give an oil. The oily material was chromatographed on a column (2.4×15 cm) of silica gel (Wakogel C-100) with dichloromethane–benzene (1 : 1) as an eluant to afford *ca.* 4 mg of the acylated amine (**4**). The authentic sample was prepared independently as follows for the identification of the product. A solution of **2** (20 mg) and dodecanoyl chloride (100 mg) in dry ether (25 ml) was stirred under reflux for 8 h. The cooled solution was washed with 5% aqueous sodium carbonate (20 ml×3), 5% aqueous sodium hydroxide (20 ml×5), and then water (30 ml×2) in this sequence. The usual work-up gave an oily material (*ca.* 40 mg) which was applied on a chromatographic column in a manner as described above. The amide fraction eluted with dichloromethane-benzene (1 : 1) was further purified by a Hitachi 635 liquid chromatograph equipped with a column of Hitachi gel 3019. Methanol was used as eluant and components eluted were detected by UV absorption at 254 nm. Spectral data identified the acylated amine (**4**) with the isolated authentic sample (*ca.* 5 mg). IR (neat): 3280 (NH str.), 1635 (amide C=O str.), and 1552 cm^{-1} (amide NH bend. and CN str.). NMR ($CDCl_3$, TMS): δ 7.11 (s, aromatic), 5.13 (broad d, NH), 3.51 (m, *H*CNHCO), 2.64 (t, benzyl methylene), 2.16 (t, CH_2CO), and 1.9—0.8 (m, methylene).

Results

The deacylation reaction of *p*-nitrophenyl carboxylates was investigated in aqueous ethanol both in the absence and in the presence of **2** as listed in Table 1. The initial concentration of a substrate was adjusted at 1.00×10^{-5} M and total amount of **2** was in the same concentration range. The pseudo-first-order rate constants were obtained from the early stage, since good first-order correlations were observed for that range under the present conditions. While **2** showed little catalytic effect in the degradation of substrates bearing a comparatively short alkyl chain in a manner as observed with **1**,[1,2] it showed marked rate acceleration in the reaction of carboxylic esters bearing a longer alkyl chain, such as PNPL and PNPP. The extents of rate acceleration in the ester decomposition are 1380- and 4750-fold for PNPL and PNPP, respectively, relative to their spontaneous hydrolyses as shown in Table 2.

pH-Rate profiles for the reaction between **2** and PNPL in 20.8% (v/v) aqueous ethanol and in 20.8% (v/v) ethanol-d_1–79.2% (v/v) deuterium oxide are a characteristic sigmoid type for which the acid dissociation process is responsible (Fig. 1). This feature is consistent with the presence of two forms for the present catalyst, *i.e.*, protonated (**2a**) and free amine species (**2b**), the latter being far more effective (Eq. 1). Figure 2 shows the solvent isotope effect on the specific base catalyzed hydrolysis of PNPL, plotted as log k_{hyd} *vs.* $-\log[H^+]$ or $-\log[D^+]$.

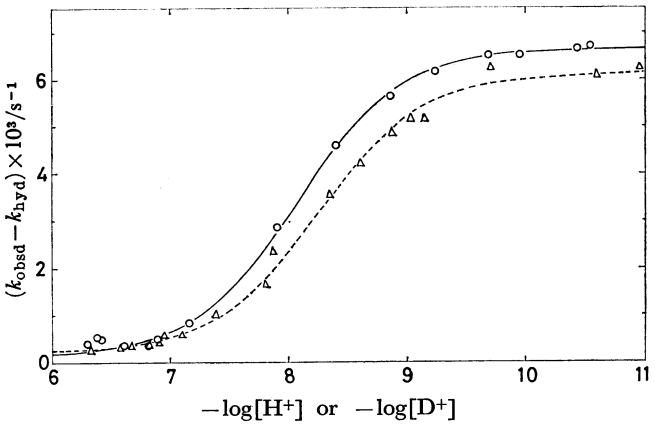


Fig. 1. pH-Rate profiles for the deacylation of PNPL as catalyzed by **2** in 20.8%(v/v) aqueous ethanol (○) and in 20.8%(v/v) ethanol-d_1–79.2%(v/v) deuterium oxide (△) at 40.0±0.1 °C and μ=0.10 (KCl) with the initial concentrations: PNPL, 1.00×10^{-5} M; **2**, 0.998×10^{-5} M. Solid lines are theoretical curves calculated by using parameters given in Table 3.

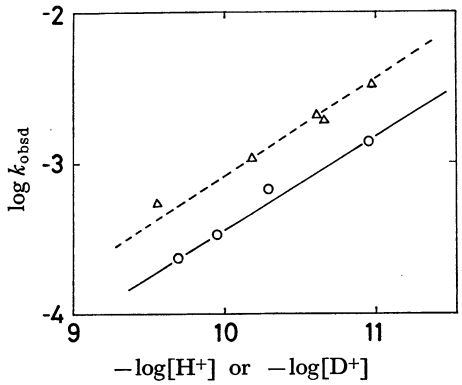
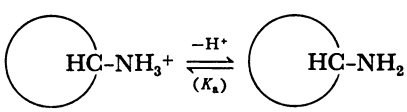


Fig. 2. pH-Rate profiles for the hydrolysis of PNPL as catalyzed by the specific base (hydroxide ion) in 20.8 %(v/v) aqueous ethanol (○) and in 20.8%(v/v) ethanol-d_1–79.2%(v/v) deuterium oxide (△) at 40.0± 0.1 °C and μ=0.10 (KCl) with the initial PNPL concentration of 1.00×10^{-5} M. The kinetic solvent isotope effect ($k^{(H)}/k^{(D)}$) is 0.47.



(1)

The kinetic pK_a values for the aminoparacyclophane in the presence of PNPL and PNPP are listed in Table 3 along with their deacylation rates as catalyzed by the two functional species, **2a** and **2b**. It must be noted

Table 1. Apparent first-order rate constants for the *p*-nitrophenol release from *p*-nitrophenyl carboxylates in the presence of **2** in aqueous ethanol at 40.0±0.1 °C and μ=0.10 (KCl)

$-\log[H^+]$	$[\mathbf{2}]\times10^5$/M	$[S]^{a)}\times10^5$/M	$k_{obsd}\times10^3/s^{-1}$	$-\log[H^+]$	$[\mathbf{2}]\times10^5$/M	$[S]^{a)}\times10^5$/M	$k_{obsd}\times10^3/s^{-1}$
Substrate:	PNPL[b]			6.83[d]	0.998	1.00	0.363
6.30	0.998	1.00	0.387, 0.403	6.90[d]	0.998	1.00	0.423
6.39	0.998	1.00	0.403	6.95[d]	0.998	1.00	0.583
6.42	0.998	1.00	0.300	7.10[d]	0.998	1.00	0.587, 0.590
6.61	0.998	1.00	0.290, 0.333	7.38[d]	0.998	1.00	1.01
6.82	0.998	1.00	0.370, 0.330	7.81[d]	0.998	1.00	1.65
6.89	0.998	1.00	0.473	7.87[d]	0.998	1.00	2.35
7.00	0.300	1.00	0.148	8.35[d]	0.998	1.00	3.54
7.00	0.400	1.00	0.190	8.61[d]	0.998	1.00	4.21
7.00	0.500	1.00	0.271	8.87[d]	0.998	1.00	4.83
7.00	0.700	1.00	0.348	9.03[d]	0.998	1.00	5.13
7.00	0.800	1.00	0.398	9.15[d]	0.998	1.00	5.15
7.00	0.900	1.00	0.490	9.55[d]	0	1.00	0.555
7.00	0.998	1.00	0.571	9.71[d]	0.998	1.00	6.25
7.00	1.00	1.00	0.571	10.18[d]	0	1.00	1.08, 1.07
7.00	1.10	1.00	0.692	10.61[d]	0.998	1.00	8.17
7.00	1.20	1.00	0.833	10.65[d]	0	1.00	2.10
7.00	1.40	1.00	1.07	10.66[d]	0	1.00	1.95
7.00	1.50	1.00	1.05	10.97[d]	0	1.00	3.38
7.00	1.60	1.00	1.20	10.97[d]	0.998	1.00	9.62
7.00	2.00	1.00	1.41	Substrate:	PNPP[e]		
7.16	0.998	1.00	0.825	6.43	0.995	1.00	0.625
7.90	0.998	1.00	2.89	6.97	0.301	0.996	0.072
8.40	0.998	1.00	4.66	6.97	0.502	0.996	0.162
8.40	0	1.00	0.023	6.97	0.703	0.996	0.213
8.86	0.998	1.00	5.79	6.97	0.904	0.996	0.353
8.86	0	1.00	0.059	6.97	1.00	0.996	0.410
9.24	0.998	1.00	6.29	6.97	1.21	0.996	0.532
9.24	0	1.00	0.073	6.97	1.41	0.996	0.517
9.69	0.998	1.00	6.77	6.97	1.51	0.996	0.748
9.69	0	1.00	0.233	6.97	1.61	0.996	0.895
9.95	0	1.00	0.328	6.97	1.71	0.996	0.998
9.95	0.300	1.00	1.90	6.97	1.81	0.996	1.15
9.95	0.400	1.00	2.77	6.97	1.91	0.996	1.23
9.95	0.500	1.00	3.46	6.97	2.01	0.996	1.23
9.95	0.700	1.00	5.15	7.44	0.955	1.00	0.583
9.95	0.800	1.00	5.85	7.74	0.955	1.00	1.67
9.95	0.900	1.00	6.54	8.24	0.955	1.00	3.02
9.95	0.998	1.00	6.84	8.61	0.955	1.00	4.53
9.95	1.00	1.00	7.29	8.99	0.955	1.00	5.41
9.95	1.10	1.00	8.25	9.61	0.955	1.00	5.53
9.95	1.20	1.00	8.80	9.94	0.955	1.00	5.90
9.95	1.30	1.00	9.00	10.27	0.955	1.00	6.20
9.95	1.40	1.00	9.75	10.29	0	0.996	0.067
9.95	2.00	1.00	10.8	10.29	0.301	0.996	1.14
10.29	0	1.00	0.658	10.29	0.401	0.996	1.68
10.44	0.998	1.00	7.33	10.29	0.502	0.996	2.35
10.54	0.998	1.00	7.50	10.29	0.703	0.996	3.50
10.77	0	1.00	0.962	10.29	0.803	0.996	4.19
10.96	0	1.00	1.39	10.29	0.904	0.996	4.89
Substrate:	PNPL[c]			10.29	1.00	0.996	5.77
6.33[d]	0.998	1.00	0.250	10.29	1.11	0.996	5.54
6.58[d]	0.998	1.00	0.303	10.29	1.21	0.996	6.38
6.67[d]	0.998	1.00	0.326	10.29	1.31	0.996	6.64
6.82[d]	0.998	1.00	0.347	10.29	1.41	0.996	6.92

Table 1. continued

$-\log[H^+]$	$[2]\times10^5$/M	$[S]^{a)}\times10^5$/M	$k_{obsd}\times10^3/s^{-1}$	$-\log[H^+]$	$[2]\times10^5$/M	$[S]^{a)}\times10^5$/M	$k_{obsd}\times10^3/s^{-1}$
10.29	1.51	0.996	7.00	Substrate:	PNPP[f)]		
10.29	2.01	0.996	7.72	6.38	0.955	1.00	1.66
10.67	0.955	1.00	6.23	6.81	0.955	1.00	2.20
10.79	0.955	1.00	6.16	7.38	0.955	1.00	3.59
				7.86	0.955	1.00	4.16

a) Initial concentration of a *p*-nitrophenyl carboxylate. b) Buffer systems: KH_2PO_4-$Na_2B_4O_7$ for $-\log[H^+]$ values of 6.0—9.0; Na_2CO_3-$Na_2B_4O_7$ for $-\log[H^+]>9.0$. Solvent: 20.8%(v/v) aqueous ethanol. c) Buffer systems: KH_2PO_4-Na_2HPO_4 for $-\log[D^+]$ values of 6.33—7.87; KH_2PO_4-$Na_2B_4O_7$ for $-\log[D^+]$ values of 8.35—9.15; Na_2CO_3 for $-\log[D^+]>9.55$. Solvent: 20.8%(v/v) ethanol-d_1-79.2%(v/v) deuterium oxide. d) $-\log[D^+]$. e) Buffer systems: KH_2PO_4-$Na_2B_4O_7$ for $-\log[H^+]$ values of 6.0—9.0; Na_2CO_3-$Na_2B_4O_7$ for $-\log[H^+]>9.0$ but Na_2CO_3 for $-\log[H^+]=10.29$. Solvent: 20.8%(v/v) aqueous ethanol. f) Buffer system: KH_2PO_4-$Na_2B_4O_7$. Solvent: 10.9%(v/v) aqueous ethanol.

Table 2. Rate enhancement effected by 10-amino-[20]paracyclophane (**2**) in the deacylation of PNPL and PNPP[a)]

Substrate	$[2]\times10^5$/M	k_{OH}[b)] $M^{-1}s^{-1}$	k_c[c)] $M^{-1}s^{-1}$	k_c/k_{OH}
PNPL	None	0.49±0.00[d)]		1380
	0.998		683[e)]	
PNPP[f)]	None	0.12		4750
	1.00		570	

a) Solvent, 20.8%(v/v) aqueous ethanol; $\mu=0.10$(KCl); 40.0±0.10 °C. b) $k_{OH}=k_{hyd}/[OH^-]$. c) $k_c=(k_{obsd}-k_{hyd})/[2]$. d) $[PNPL]_0=1.00\times10^{-5}$ M; $-\log[H^+]=$ 9.95—10.97 e) $[PNPL]_0=1.00\times10^{-5}$ M; $-\log[H^+]=$ 9.95. f) $[PNPP]_0=0.996\times10^{-5}$ M; $-\log[H^+]=10.29$.

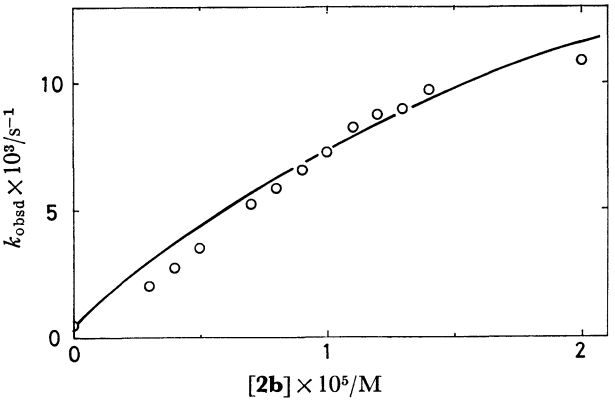


Fig. 3. Saturation-type kinetics for the reaction of PNPL with **2b** in 20.8% (v/v) aqueous ethanol at 40.0±0.1 °C, $\mu=0.10$ (KCl), and $-\log[H^+]=9.95$ with the initial substrate concentration of 1.00×10^{-5} M. Solid line is the theoretical curve calculated by using parameters listed in Table 5.

that the kinetic pK_a value decreases with the decrease in an ethanol content, even though it is little effected by a small change in the alkyl-chain length of the substrates. The equilibrium solvent isotope effect ($pK_a^{(D)}-pK_a^{(H)}$) is 0.20. The kinetic solvent isotope effects for reactions of PNPL with **2a** and **2b**, $k^{(H)}/k^{(D)}$, are 0.67 and 1.09, respectively.

The saturation-type kinetics was observed as exemplified by the correlation between concentration of aminocyclophane **2b** and rate constant for the deacylation of PNPL (Fig. 3). These results are consistent with a reaction mechanism which involves pre-equilibrium complexation of the aminoparacyclophane and the substrate at a 1 : 1 molar ratio, followed by the pseudo-intramolecular catalysis effected by the amino group of the paracyclophane.

The addition of 1-dodecanol resulted in the depression of the catalytic efficiency of **2** in the degradation of PNPL and PNPP, while the alcohol alone (10^{-4} M, 10-fold amount of the substrates) did not show any effect on the hydrolysis of both substrates.

The extent of inhibition by 1-dodecanol increased with the increase in its concentration, and both PNPL- and PNPP-systems show a similar behavior (Fig. 4). On the other hand, 2,4-dinitrofluorobenzene (DNFB) in-

Table 3. Acid dissociation constants of the protonated amino group of **2** and rate constants for the degradation of PNPL and PNPP as catalyzed by conjugated functional species **2a** and **2b**[a)]

Substrate	$[2]\times10^5$/M	Ethanol content %(v/v)	k_{OH}[b)]/$M^{-1}s^{-1}$	$k_{(2a)}\times10^4/s^{-1}$	$k_{(2b)}\times10^3/s^{-1}$	pK_a
PNPL	0.998	20.8	0.49±0.00	1.4±0.5	6.65±0.10	8.07
PNPL[c)]	0.998	20.8	1.06±0.00	2.1±0.3	6.11±0.31	8.25
PNPP	0.995	20.8	0.12	2.4±0.2	6.14±0.43	8.26
PNPP	0.995	10.9	—	1.0±0.1	4.74±0.14	7.08

a) $[Substrate]_0=1.00\times10^{-5}$ M; 40.0±0.1 °C; $\mu=0.10$ (KCl). pK_a-Values were evaluated graphically for the first approximation from the corresponding pH-rate profiles (see Fig. 1), and $k_{(2a)}$ and $k_{(2b)}$ values as well as pK_a were determined from computations which provide best fits to the corresponding kinetic (pH-rate) data. b) The second-order rate constant for the specific base catalyzed hydrolysis in the absence of **2**; $k_{OH}=k_{hyd}/[OH^-]$ or $k_{hyd}/[OD^-]$. c) In 20.8% (v/v) ethanol-d_1-79.2% (v/v) deuteruim oxide.

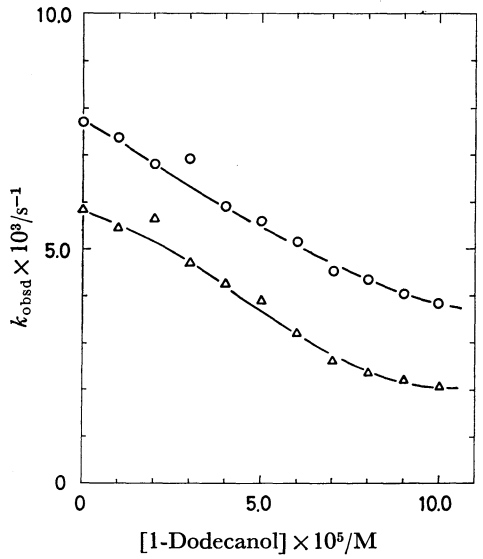


Fig. 4. Inhibition of the **2**-catalyzed degradation of PNPL (○) or PNPP (△) by 1-dodecanol in 20.8% (v/v) aqueous ethanol at 40.0±0.1 °C, μ=0.10 (KCl), and $-\log[H^+]=10.29$ with the initial concentrations: PNPL, 0.997×10^{-5} M; PNPP, 0.996×10^{-5} M; **2**, 1.00×10^{-5} M.

Table 4. The effect of chemical modification of **2** with 2,4-dinitrofluorobenzene in the catalytic degradation of PNPL[a)]

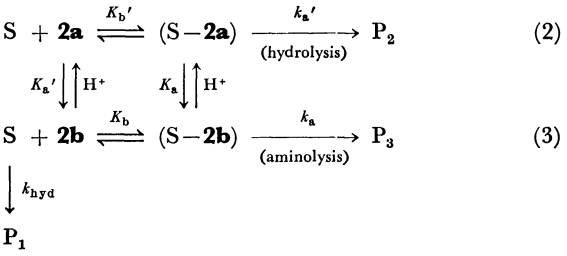
$[2]\times10^5$ M	$[DNFB]\times10^5$ M	Incubation time h	$k_{obsd}\times10^4$ s^{-1}
None	None	—	1.53
1.00	None	—	62.7
1.00	0.515	24	16.7
1.00	0.515	60	16.3
1.00	1.03	24	1.03
1.00	1.03	60	1.11
1.00	5.15	24	1.13
1.00	5.15	60	0.92

a) $[PNPL]_0=1.00\times10^{-5}$ M; solvent, 20.8% (v/v) aqueous ethanol; μ=0.10(KCl); $-\log[H^+]=9.26$; 40.0±0.1 °C.

hibited the aminoparacyclophane-catalyzed reaction in a manner different from 1-dodecanol. The pre-incubation of **2** with DNFB resulted in an appreciable reduction of the catalytic efficiency as listed in Table 4. Examination of the effects of incubation time and DNFB concentration on the reaction rate confirms that the amino group of **2** reacted quantitatively with DNFB at a 1 : 1 molar ratio in 24 h. It must be noted that the dinitrophenylated aminoparacyclophane reduced the rate of PNPL decomposition even below the catalyst absence level.

Discussion

10-Amino[20]paracyclophane is characterized by two functions: a hydrophobic binding site formed by polymethylene chains and a benzene ring, and a catalytic center provided by an amino group. In fact, dinitrophenylation of **2** with DNFB, which is the potent re-



P_1: Carboxylate and *p*-nitrophenolate ions,
P_2: carboxylate, *p*-nitrophenolate, and regenerated **2a**,
P_3: *p*-nitrophenolate ion and acylated **2**.

Scheme 1.

agent to modify an essential amino group of enzymes,[10] resulted in the complete disappearance of catalytic effect. Accordingly, it is evident that the amino group placed on the paracyclophane skeleton plays an indispensable role in the catalytic degradation of the carboxylic esters. Formation of an inclusion complex between **2** and the substrate was confirmed by the inhibition experiment with 1-dodecanol. The catalytic efficiency exhibited by **2** gradually decreases with the increase in inhibitor concentration as shown in Fig. 4. Since the inhibitor and the substrate (PNPL or PNPP) bear a hydrophobic alkyl chain of similar length, they can compete with each other in occupying the hydrophobic cavity of the catalyst.

In consistent with the pH-rate correlation (Fig. 1) and the saturation-type kinetics (Fig. 3), the reaction scheme is given by Scheme 1 in a manner similar to those applied to the hydroxyiminoparacyclophane catalysis described previously.[2] This reaction scheme means that the pre-equilibrium complexation of the aminoparacyclophane with the substrate at a 1 : 1 molar ratio is followed by the pseudo-intramolecular catalysis to decompose the substrate. Reactions 2 and 3 in Scheme 1 can be treated separately by analyzing the kinetic data obtained in sufficiently lower and higher pH regions, respectively, relative to the pK_a value of the catalyst. The binding constant K_b and the rate constant for substrate decomposition k_a were computed by the nonlinear least-squares method to obtain best fit to the kinetic data (see Appendix). The rate constants of simple hydroxide-catalyzed hydrolysis (k_{hyd}) were obtained independently from kinetic runs in the absence of the catalyst (Table 5).

Nucleophilic Catalysis in the Higher pH Region.[11] In general, the free amine takes part in the decomposition of carboxylic esters in two different ways;[5] the general base catalysis to give the same product as observed in the corresponding spontaneous hydrolysis, and the direct nucleophilic attack of the amino group on the ester carbonyl to give the acylated amine. When both substrate and amine bear long hydrocarbon chains, the overall rate of ester degradation was accelerated drastically due to the mutual hydrophobic interaction which facilitate to place the amino group in a close vicinity of the ester carbonyl.[7,12] At the same time, the aminolysis becomes the major reaction among competing ones. Similar proximity effect is expected for the present paracyclophane system and the amino group attacks on the ester carbonyl as confirmed by the prod-

Table 5. Kinetic parameters for the deacylation reactions of PNPL and PNPP as catalyzed by **2b**[a)]

Substrate	$-\log[H^+]$	$k_{hyd}\times10^4/s^{-1}$	$k_a\times10^2/s^{-1}$	$K_b\times10^{-3}/M^{-1}$	U[b)]
PNPL	9.95	3.28	2.40	59	4.32×10^{-6}
PNPP	10.29	*ca.* 0.1—0.5	1.38	100	2.76×10^{-6}

a) $[PNPL]_0=1.00\times10^{-5}$ M and $[PNPP]_0=0.996\times10^{-5}$ M; solvent, 20.8% (v/v) aqueous ethanol; 40.0±0.1 °C; $\mu=0.10$ (KCl). b) A residual sum of squares for the computational procedure, see Appendix.

uct analysis. The observed small isotope effect on the specific rate constant, $k_{(2b)}^{(H)}/k_{(2b)}^{(D)}=1.09$ for PNPL as shown in Table 3, is consistent with the direct nucleophilic attack by the amino group. For the nucleophilic displacement, the kinetic solvent isotope effect is usually very close to unity: phenyl acetate with imidazole, 1.07;[13)] *p*-nitrophenyl acetate with imidazole, 1.0;[13)] and *p*-nitrophenyl acetate with trimethylamine, 0.9.[14)]

The ester degradation was even slower than the corresponding spontaneous hydrolysis in the presence of the dinitrophenylated aminoparacyclophane. This means that the modified paracyclophane can still incorporate the substrate and the incorporated substrate is less susceptible to the nucleophilic attack by hydroxide ion due to the steric hindrance effect exerted by the bulkyl substituent. A similar state of affairs has been noted by Bender *et al.* for the reaction between phenyl acetates and dodecamethylcyclohexaamylose.[15)] Since the present and related paracyclophanes may be regarded to exist largely in a monomeric form under the kinetic conditions,[3)] the inclusion complex is most plausibly formed at a 1 : 1 molar ratio as shown in Scheme 1. The cyclic skeleton of **2b** shows a profound tendency to bind the substrates in a manner similar to those observed for other [20]paracyclophanes.[1-3)] Thus, there is no doubt that [20]paracyclophane skeleton provides an effective hydrophobic binding site for carboxylic esters bearing a long alkyl chain.

Another prominent feature found in the present study is that the acyl transfer rates as catalyzed by the amino group (**2b**) are comparable to those by the hydroxyimino group (**1**); $k_a=1.82\times10^{-2}$ s^{-1} at 43.1 °C and pH 10.3 for PNPL, and 4.45×10^{-2} s^{-1} at 39.9 °C and pH 10.7 for PNPP with the hydroxyimino group in 10.9% (v/v) aqueous acetone.[1)] This result seems to be rather surprising since the basicity of the hydroxyimino group of **1** is greater than that of the amino group of **2b** by an order of approximately 10^4. The large basicity as well as the additional α-effect should in general enhance the nucleophilic reactivity of the hydroxyimino group relative to the amine. The complexation of **2b** with the substrate bearing a long alkyl chain may provide a profound hydrophobic reaction field for the amino group of **2b**. This field effect (microsolvent effect) as well as the proximity effect seems to enhance the nucleophilicity of the amino group in the present system.

Rate Acceleration in the Neutral pH Region. The present paracyclophane gave out an appreciable catalysis in the degradation of PNPL and PNPP in a pH region where the amino group is protonated predominantly and the spontaneous hydrolysis of the substrate esters proceeds to a negligible extent. In an alkaline region where **2b** is the dominant species, the rate plots always deviated downwards, relative to the linear first-order correlation line, because the catalytic amino group was consumed along with the progress of reaction. On the other hand, the rate plots deviated upwards in the neutral pH region in a manner as observed in the spontaneous hydrolysis of PNPP over the whole pH range. In the cases of the hydrophobic substrates, such as PNPL or PNPP, the substrate becomes progressively free from the intermolecular aggregation along with the progress of reaction because of the decrease of substrate concentration.[8)] The kinetic behavior mentioned above suggests that the deaggregated substrate is more favorably incorporated into the paracyclophane. In the neutral pH region, in addition, it is clear that the catalytic group is not consumed during the course of reaction. The protonated amino group most plausibly plays a catalytic function by either of the following two mechanisms in the hydrolysis of carboxylic esters. The first one is the electrostatic catalysis effected by the positively charged ammonium group which stabilizes the anionic tetrahedral intermediate. The alkaline hydrolysis of acetylcholine proceeds 32 times as fast as that of 2-(dimethylamino)ethyl acetate.[16)] This acceleration effect was attributed to the electrostatic stabilization of the anionic tetrahedral intermediate by the positively charged ammonium group of the former species. An appropriate spatial orientation of an ammonium group relative to an ester carbonyl was suggested to be responsible for the development of such electrostatic catalysis.[17)] In a previous work,[3)] [10-hydroxyimino[20]paracyclophan-22(23)-ylmethyl]trimethylammonium chloride was found to enhance the deacylation of PNPL and PNPP in an alkaline solution due to an electrostatic effect provided by the positively charged ammonium group. In order to examine the possibility of electrostatic catalysis by the protonated amino group of **2a**, the kinetic effect of [10-oxo[20]paracyclophan-22(23)-ylmethyl]trimethylammonium chloride (**3**) in the degradation of PNPL was investigated under the following conditions: pH 7.28; solvent, 20.8% (v/v) aqueous ethanol; temp, 40.0 °C; ionic strength, 0.10 with KCl; initial concentration of PNPL, 1.00×10^{-5} M.[9)] The observed first-order rate constants (2.33×10^{-5} s^{-1} with 0.88×10^{-5} M of **3**, and 2.92×10^{-5} s^{-1} with 1.53×10^{-5} M of **3**) are much smaller than those observed in the presence of **2a**, even though they are larger than that of the spontaneous hydrolysis (*ca.* 1.3×10^{-5} s^{-1}) under the same conditions. Thus, it is concluded that contribution of electrostatic effect may be ruled out under the present experimental conditions, even if the structural difference between **2a** and **3** needs to be taken into consideration. In addition, the observed solvent isotope effect (0.67) seems

to support this view, since no isotope effect would be expected for the electrostatic catalysis which involves no proton transfer process. An alternative mechanism is the general acid catalysis[18] in which the ammonium group of **2a** attacks on the ester carbonyl as a general acid and the nucleophilic attack of a water molecule is facilitated accordingly. Even though the solvent deuterium isotope effect is usually greater than unity for this mechanism, there are other examples in which the isotope effect is smaller than unity: the dehydration step of oxime formation, 0.30;[19] the acid-catalyzed addition of thiol to aldehyde, 0.59.[20] Thus, the protonated amino group most plausibly attacks on the ester carbonyl as a general acid catalyst. This work presents undoubtedly a novel type of catalysis in any sense played by the protonated amino group placed on the hydrophobic cyclic skeleton in the hydrolysis of carboxylic esters.

In brief summary, the present aminoparacyclophane demonstrated marked catalytic effects on the deacylation of *p*-nitrophenyl carboxylates bearing a long alkyl chain not only in the free base but also in the protonated form. The nucleophilic attack of the free amino group of **2** on the ester carbonyl takes place pseudo-intramolecularly upon complexation with the substrate, which afforded the acylated aminocyclophane. On the other hand, the general acid catalysis is exercised by the protonated amino group of **2** in the deacylation of the same substrates. Even though its absolute catalytic efficiency is not so large as that of the free base group, a characteristic turnover behavior was observed.

Appendix

Determination of Kinetic Parameters for Saturation-type Kinetics. When the reaction is carried out in a sufficiently high pH region relative to the pK_a value of the catalyst, the predominant catalyst species is **2b** and Scheme 1 can be simplified to Scheme 2.

$$\mathrm{S} + \mathrm{C} \underset{}{\overset{K_b}{\rightleftharpoons}} (\mathrm{S-C}) \overset{k_a}{\rightleftharpoons} \mathrm{P}$$
$$\Big\downarrow k_{hyd}$$
$$\mathrm{P'}$$

Scheme 2.

The kinetic parameters given in Scheme 2 are evaluated by the following computations. Abbreviations are made here for convenience.

S: a substrate species
C: a catalyst species (**2b**)
S–C: a substrate-catalyst inclusion complex
P and P′: products
K_b: a binding constant
k_a: a catalytic rate constant for the aminolysis step
k_{hyd}: a rate constant for spontaneous or simple alkaline hydrolysis
k_ϕ: an overall rate constant

Mass balances in consistent with Scheme 2 are established as follows.

$$[\mathrm{S}]_T = [\mathrm{S}] + [\mathrm{S-C}] \tag{4}$$

$$[\mathrm{C}]_T = [\mathrm{C}] + [\mathrm{S-C}] \tag{5}$$

The binding constant is given by

$$K_b = \frac{[\mathrm{S-C}]}{[\mathrm{S}][\mathrm{C}]} \tag{6}$$

Combination of Eqs. 4, 5, and 6 gives

$$[\mathrm{S-C}] = \frac{1}{2}\left[[\mathrm{S}]_T + [\mathrm{C}]_T + \frac{1}{K_b} - \left\{\left([\mathrm{S}]_T + [\mathrm{C}]_T + \frac{1}{K_b}\right)^2 - 4[\mathrm{S}]_T[\mathrm{C}]_T\right\}^{1/2}\right] \tag{7}$$

The overall rate can be expressed as follows:

$$k_\phi[\mathrm{S}]_T = k_{hyd}[\mathrm{S}] + k_a[\mathrm{S-C}] \tag{8}$$

$$k_\phi = \left(1 - \frac{[\mathrm{S-C}]}{[\mathrm{S}]_T}\right)k_{hyd} + \frac{[\mathrm{S-C}]}{[\mathrm{S}]_T}k_a \tag{9}$$

The equation derived by combination of Eqs. 7 and 9 is nonlinear with respect to parameters K_b and k_a. An iterative calculation was performed to minimize the residual sum of squares (U):

$$U = \sum_{i=1}^{n}\{k_\phi(\mathrm{obsd})_i - k_\phi(\mathrm{calcd})_i\}^2,$$

where n is the number of observations at different concentrations of the catalyst and i is the observation-number index.

All calculation processes were programmed by means of the Fortran language for use with a FACOM 270-20/30 electronic computer of the Computation Center of Nagasaki University.

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