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A Macrocyclic Enzyme Model System. Deacylation of *p*-Nitrophenyl Carboxylates as Effected by a [20]Paracyclophane Bearing an Imidazole Group[†]

Yukito Murakami,* Yasuhiro Aoyama, Masaaki Kida, and Akio Nakano

Department of Organic Synthesis, Faculty of Engineering, Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812

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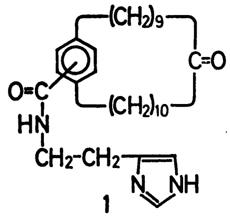
The kinetic effect of a [20]paracyclophane bearing an imidazole moiety (**1**) on the deacylation of various *p*-nitrophenyl carboxylates was investigated in 10.9%(v/v) ethanol–1.0%(v/v) dioxane–water of μ 0.10 (KCl) at 40.0±0.1 °C. The facile reaction of **1** with a hydrophobic ester resulted in the accumulation of the N^{Im}-acyl derivative of **1** along the progress of reaction, and the rate of regeneration of **1** (turnover) was negligibly small under present experimental conditions compared to the corresponding acylation rate. The overall feature for deacylation reactions of *p*-nitrophenyl carboxylates bearing a hydrophobic acyl moiety in the presence of **1** is consistent with the reaction scheme which involves the reactions of the monomeric and aggregated forms of **1** with substrate esters. The second-order rate constants for reactions of the monomeric species of **1** with relatively hydrophobic esters exceed considerably the corresponding second-order rate constants for imidazole-catalyzed reactions, suggesting the significant hydrophobic interaction between **1** and each hydrophobic ester. The binding constants (K) for complex formation of a micelle (aggregation number, N) with significantly hydrophobic substrates are greater than 10^5 M^{-1}. The second-order rate constants (k_mK/N) for reactions of the imidazole group of **1** with the long-chain carboxylates in micellar phase are the largest ever achieved for reactions of synthetic 4-substituted imidazoles with *p*-nitrophenyl esters.

Our recent studies have characterized the catalytic functions of [20]paracyclophanes bearing an oxime nucleophile in ester hydrolyses.[1–5] The novel and highly efficient catalysis played by those macrocycles have been attributed to their marked hydrophobic effect, which gives out the proximity effect through formation of a substrate-paracyclophane complex, and nucleophilic-electrostatic bifunctional character acted on the incorporated substrate. In this study, we have prepared an imidazole derivative of [20]paracyclophane (**1**) and investigated its esterase-like activity. Since the present paracyclophane is catalytically active in its neutral form, the aggregation behavior in a mixed aqueous-organic solvent system and its kinetic consequences in deacylation of various *p*-nitrophenyl carboxylates have been clarified.



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Experimental

Vibrational spectra were measured with a JASCO DS-403G grating spectrophotometer. ^{1}H NMR spectra were obtained with either a Varian A-60 or a Bruker WH-90 FT spectrometer and tetramethylsilane (TMS) was used as an internal reference. Fluorescence spectra were recorded on a Shimadzu spectrofluorophotometer RF-500. High speed liquid chromatography for preparative purpose was performed on a Hitachi 635 liquid chromatograph with Hitachi gel 3019. Gel filtration was carried out on a column packed with Sephadex LH-20. Methanol was used as eluant and components eluted were detected by UV absorption at either 254 or 265 nm for both chromatographic techniques.

N-*(Imidazol-4-ylethyl)-10(11)-oxo[20]paracyclophane-22-carboxamide (1).* A mixture of 10(11)-oxo[20]paracyclophane-22-carboxylic acid[6] (70 mg) and thionyl chloride (3 ml) was stirred at room temperature for 2 h. Excess thionyl chloride was removed *in vacuo* from the reaction mixture, into which a small amount of dry tetrahydrofuran was added subsequently with stirring. The mixture was evaporated *in vacuo* to give the acid chloride. A tetrahydrofuran solution (15 ml) of the acid chloride was added at room temperature in a period of 1 h to a mixture of histamine dihydrochloride (300 mg) and sodium hydroxide (500 mg) in water (20 ml) with vigorous stirring, and stirring was continued for another 6.5 h. Then, the mixture was poured into water (50 ml) and extracted with ether (50 ml×6). The ether extract was washed with 50 ml of water, dried over sodium sulfate and evaporated. The crude product was extracted with methanol, and then purified by means of repeated preparative liquid chromatography and gel filtration chromatography to afford **1** as an oil; yield 25%. IR (neat): 1718 (ketone C=O str.), 1640 (amide C=O str.), and 1530 cm^{-1} (NH bend. and C–N str.). NMR (CD$_3$OD, TMS): δ 7.71 and 6.98 (both s, H's on imidazole ring), 7.18 (s, meta and para H's relative to the amide substituent on benzene ring), 7.13 (s, H ortho to the amide on benzene ring), 3.62 (t J=7 Hz, $C\underline{H}_2CH_2NHCO$ or $CH_2C\underline{H}_2NHCO$), 3.1—2.1 (complex m, CH$_2$CO, benzyl protons, and $CH_2C\underline{H}_2NHCO$ or $C\underline{H}_2CH_2NHCO$), and 1.8—1.2 (m, methylene protons). Found: C, 75.02; H, 9.64; N, 7.87%. Calcd for $C_{32}H_{48}N_3O_2$: C, 75.69; H, 9.73; N, 8.27%.

p-*Nitrophenyl Carboxylates.* *p*-Nitrophenyl acetate (**2**), hexanoate (**3**), decanoate (**4**), dodecanoate (**5**), hexadecanoate (**6**), phenylacetate (**12**), and α-naphthoate (**13**) were prepared by condensation of commercially available carboxylic acids or acid chlorides with *p*-nitrophenol. The esters were identified by elemental analyses and spectral measurements before use.

(a) p-*Nitrophenyl Cyclohexanecarboxylate (7):* A mixture of cyclohexanecarbonyl chloride (6.5 g) and *p*-nitrophenol (5.0 g), magnesium ribbon (45 mg), and a small amount of iodine in dry benzene (20 ml) was refluxed for 4 h. After cooling down to room temperature, the organic layer was

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[†] Contribution No. 440 from this Department.

* To whom correspondence should be addressed.

separated, washed successively with cold water, saturated aqueous sodium bicarbonate, and cold water, and dried over sodium sulfate. The organic layer was then evaporated *in vacuo* and the residue was distilled; bp 174 °C/0.7 mmHg, yield 6.8 g (62%). NMR ($CDCl_3$, TMS): δ 8.21 and 7.21 (ABq J=9 Hz, aromatic H's), and 2.85—1.00 (m, H's on cyclohexane ring). Found: C, 62.75; H, 6.16; N, 5.40%. Calcd for $C_{13}H_{15}NO_4$: C, 62.64; H, 6.06; N, 5.61%.

(b) p-Nitrophenyl Cyclohexylacetate (8): The ester was prepared in a similar manner from cyclohexylacetyl chloride; yield 57%. NMR ($CDCl_3$, TMS): δ 8.23 and 7.23 (ABq J=9 Hz, aromatic H's), 2.46 (distorted d J=7 Hz, CH_2CO), and 1.70, 1.29, and 1.15 (m, H's on cyclohexane ring). Found: C, 63.77; H, 6.51; N, 5.34%. Calcd for $C_{14}H_{17}NO_4$: C, 63.86; H, 6.50; N, 5.32%.

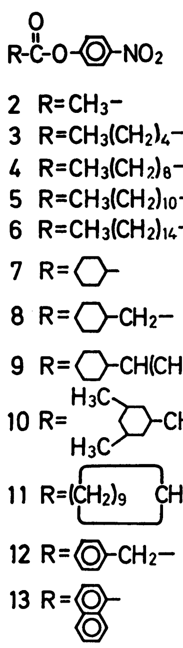
(c) p-Nitrophenyl α-Cyclohexylpropionate (9): The Reformatsky reaction of cyclohexanone (4.0 g) with methyl α-bromopropionate (6.0 g), which has been carried out under standard conditions,[7] gave *1-(1-methoxycarbonylethyl)cyclohexan-1-ol* (**14**; $R^1=CH_3$, R^2=H, n=6); bp 78—81 °C/2 mmHg, yield 52%. NMR ($CDCl_3$, TMS): δ 3.68 (s, OCH_3), 2.50 (q J=7 Hz, methine proton), 2.17—1.24 (broad s centered at 1.47, H's on cyclohexane ring and OH), and 1.17 (d J=7 Hz, $CHC\underline{H}_3$). The hydroxy ester was then dehydrated to give the unsaturated ester. The hydroxy ester (20 g) was added to a cold solution of thionyl chloride (30 ml) in benzene (50 ml) containing pyridine (15 ml) at 0 °C. The mixture was allowed to stand at room temperature for a half hour and poured into ice water (200 ml). The organic layer was separated, washed with water, dried over sodium sulfate, and evaporated *in vacuo*, and then the residue was distilled to afford *methyl α-(1-cyclohexenyl) propionate* (**15**; $R^1=CH_3$, R^2=H, n=6); bp 56.5 °C/1 mmHg, yield 80%. NMR ($CDCl_3$, TMS): δ 5.52 (broad s, CH=C), 3.62 (s, OCH_3), 3.01 (distorted q J=7 Hz, methine proton), 1.95 and 1.56 (m, H's on cyclohexane ring), and 1.22 (d J=7 Hz, $CHC\underline{H}_3$). Methyl α-(1-cyclohexenyl)propionate (14.6 g) in ethyl acetate (100 ml) was hydrogenated in an autoclave at 28 °C with 10% palladium carbon (2.0 g) as catalyst, initial hydrogen pressure being adjusted at 50 kg/cm^2. The catalyst was removed by filtration through celite 545 (Wako Pure Chemicals, Ltd.). The solvent was removed *in vacuo* and the residue was distilled to afford *methyl α-cyclohexylpropionate* (**17**; $R^1=CH_3$, R^2=H, n=6); bp 93—96 °C/15 mmHg, yield 69%. NMR ($CDCl_3$, TMS): δ 3.65 (s, OCH_3), 2.22 (distorted q J=7 Hz, methine proton), 1.65 and 1.11 (m, H's on cyclohexane ring), and 1.12 (d J=7 Hz, $CHC\underline{H}_3$). The methyl ester (10.0 g) was saponified with sodium hydroxide (15.0 g) in 50%(v/v) aqueous methanol (40 ml) to give *α-cyclohexylpropionic acid*, and recrystallized from ethyl acetate, mp 60.5—62.5 °C, yield 95%. Found: C, 69.14; H, 10.32%. Calcd for $C_9H_{16}O_2$: C, 69.19; H, 10.29%. The acid was treated with thionyl chloride to give the acid chloride (bp 84—86 °C/9 mmHg, yield 70%), which was converted to the corresponding *p*-nitrophenyl ester (**9**) in a manner as described above; bp 130 °C/0.05 mmHg, yield 57%. NMR ($CDCl_3$, TMS): δ 8.25 and 7.25 (ABq J=9 Hz, aromatic H's), 2.48 (distorted q J=7 Hz, $C\underline{H}CH_3$), 1.70 (m, H's on cyclohexane ring), and 1.26 (d J=7 Hz, $CHC\underline{H}_3$). Found: C, 65.13; H, 6.96; N, 4.96%. Calcd for $C_{15}H_{19}NO_4$: C, 64.96; H, 6.90; N, 5.05%.

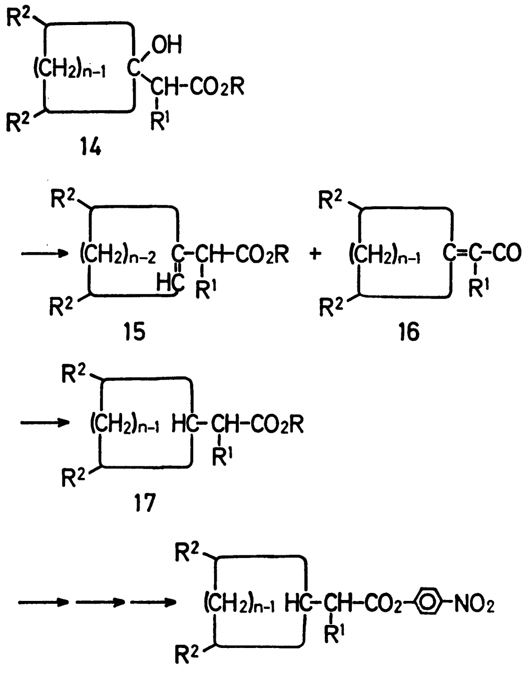
(d) p-Nitrophenyl 3,5-Dimethylcyclohexylacetate (10): The ester was obtained by a preparative procedure similar to that described for **9**. The Reformatsky reaction of ethyl bromoacetate with 3,5-dimethylcyclohexanone obtained by oxidation of 3,5-dimethylcyclohexanol with sodium dichromate afforded the corresponding hydroxy ester (**14**; R^1=H, $R^2=CH_3$, n=6); bp 92—95 °C/0.5 mmHg yield 32%. NMR (CCl_4, TMS): δ 4.12 (q J=7 Hz, $C\underline{H}_2CH_3$), 3.29 (s, OH), 2.45 and 2.33 (both s, CH_2CO), 1.76 and 1.59 (m, H's on cyclohexane ring), 1.27 (t J=7 Hz, $CH_2C\underline{H}_3$), and 0.86 (distorted d J=6.5 Hz, CH_3's on cyclohexane ring). Found: C, 67.22; H, 10.27%. Calcd for $C_{12}H_{20}O_3$: C, 67.24; H, 10.37%. The hydroxy ester was dehydrated to give *3,5-dimethyl-1-ethoxycarbonylmethyl-1-cyclohexene* (**15**; R^1=H, $R^2=CH_3$, n=6) and *ethyl 3,5-dimethylcyclohexylideneacetate* (**16**; R^1=H, $R^2=CH_3$, n=6) in a ratio of 1.6: 1 as judged by the NMR spectrum; bp 92—94 °C/4.5 mmHg, total yield 87%. NMR (CCl_4, TMS): δ 5.47 (s, C=CH–CO for **16**), 5.27 (broad s, CH=C for **15**), 4.06 (q J=7 Hz, $C\underline{H}_2CH)_3$, 2.80 (s, CH_2CO for **15**), 2.12 and 1.72 (m, H's on cyclohexane ring), 1.24 (t J=7 Hz, $CH_2C\underline{H}_3$), and 0.96 (distorted d J=6 Hz, CH_3's on cyclohexane ring). The mixture of unsaturated esters was hydrogenated in ethanol over either 10% palladium carbon or 5% rhodium carbon for 10 h at 80—100 °C and at the initial hydrogen pressure of 100 kg/cm^2 to give *ethyl 3,5-dimethylcyclohexylacetate* (**17**; R^1=H, $R^2=CH_3$, n=6), yield 85%. NMR (CCl_4, TMS): δ 4.07 (q J=7 Hz, $C\underline{H}_2CH_3$), 2.30 and 2.05 (both s, CH_2CO), 1.77 and 1.58 (m, H's on cyclohexane ring), 1.25 (t J=7 Hz, $CH_2C\underline{H}_3$), and 0.89 (double d J=2 and 7 Hz, CH_3's on cyclohexane ring). The ester was saponified and treated with thionyl chloride. The resulting acid chloride underwent reaction with *p*-nitrophenol to afford the *p*-nitrophenyl ester (**10**); bp 140 °C/0.2 mmHg, yield (from **17**) 42%. NMR (CCl_4, TMS): δ 8.21 and 7.21 (ABq J=9 Hz, aromatic H's), 2.34 with additional peaks at 2.43 and 2.55 (each s, CH_2CO), 1.97 and 1.63 (m, H's on cyclohexane ring), and 0.90 (d J=6 Hz, CH_3's on cyclohexane ring). Found: C, 65.89; H, 7.31; N, 4.75%. Calcd for $C_{16}H_{21}NO_4$: C, 65.95; H, 7.28; N, 4.80%.

(e) p-Nitrophenyl Cyclodecylacetate (11): The ester was prepared from cyclodecanone[8] in a manner similar to those, described for **8**, **9**, and **10**. NMR (CCl_4, TMS): δ 8.20 and 7.20 (ABq J=9 Hz, aromatic H's), 2.38 (distorted s, CH_2CO), and 1.54 (s, H's on cyclodecane ring). Found: C, 67.43; H, 7.85; N, 4.42%. Calcd for $C_{18}H_{25}NO_4$: C, 67.67; H, 7.90; N, 4.38%. The intermediates obtained in this synthesis were identified as follows. *1-Ethoxycarbonylmethylcyclodecan-1-ol* (**14**; $R^1=R^2$=H, n=10); bp 125 °C/0.35 mmHg, yield 38%. NMR (CCl_4, TMS): δ 4.18 (q J=7 Hz, $C\underline{H}_2CH_3$), 3.15 (broad s, OH), 2.37 (s, CH_2CO), 1.58 (m, H's on cyclodecane ring), and 1.33 (t J=7 Hz, $CH_2C\underline{H}_5$). *1-Ethoxycarbonylmethyl-1-cyclodecene* (**15**; R^1= R^2=H, n=10) and *ethyl cyclodecylideneacetate* (**16**; R^1= R^2=H, n=10) in a ratio of 3: 1; bp 102—110 °C/7.5 mmHg, total yield 87%. NMR (CCl_4, TMS): δ 5.60 (broad s, C=CH–CO for **16**), 5.26 (t J=8 Hz, CH=C for **15**), 4.06 (q J=7 Hz, $C\underline{H}_2CH_3$), 2.87 (s, CH_2CO for **15**), 2.5—2.0 (m, H_2C–C=C), 1.40 (m, H's on cyclodecane ring), and 1.23 (t J=7 Hz, $CH_2C\underline{H}_3$). Found: C, 73.48; H, 11.54%. Calcd for $C_{14}H_{24}O_2$: C, 74.27; H, 11.60%. *Ethyl cyclodecylacetate* (**17**; R^1= R^2=H, n=10). NMR (CCl_4, TMS): δ 4.07 (q J=7 Hz, $C\underline{H}_2CH_3$), 2.12 (broad s, CH_2CO), 1.51 (s, H's on cyclodecane ring), and 1.23 (t J=7 Hz, $CH_2C\underline{H}_3$).

Kinetic Measurements. Rates of *p*-nitrophenol liberation from *p*-nitrophenyl esters were measured at 400 nm with a Union Giken high sensitive spectrophotometer SM-401 under instrumental conditions; response 2 s and sensitivity 0.02 OD/full scale. Each run was initiated by adding a dioxane solution (30 μl) of *p*-nitrophenyl ester (7.0×10^{-5} M) to a reaction medium (3.0 ml) which was pre-equilibrated at 40.0±0.1 °C in a thermostatted cell set in the spectrophotometer. The reaction medium was prepared by mixing a buffer solution (2.7 ml, buffer concentration 0.01 M) and ethanol (0.3 ml) containing an appropriate amount of **1**. The ionic strength of kinetic solutions was maintained at 0.10 with KCl.

pH Measurements. pH Measurements were carried out with a Beckman expandomatic SS-2 pH meter equipped with a Metrohm EA-125 combined electrode after calibration with a combination of appropriate aqueous standard buffers.





Results and Discussion

The kinetic effect of a [20]paracyclophane bearing an imidazole moiety (**1**) on the deacylation of various *p*-nitrophenyl carboxylates was investigated in 10.9%(v/v)ethanol-1.0%(v/v)dioxane–water of μ 0.10 (KCl) at 40.0±0.1 °C. Since it is now well known that *p*-nitrophenyl carboxylates bearing a long alkyl chain tend to aggregate above the critical concentrations[9,10] (*e.g.*, 2.0×10^{-6} M for decanoate in 1.0%(v/v) aqueous dioxane at 40 °C),[10] the initial substrate concentrations were adjusted at 7.0×10^{-7} M for most kinetic runs. Although the present paracyclophane is not so much soluble in the above kinetic solvent system, the 5.0×10^{-6} M solution was confirmed to be completely homogeneous in the pH 7—10 range. *p*-Nitrophenyl esters of the present study may be classified into four categories in reference to the extent of rate acceleration brought about by **1** (5.0×10^{-6} M) at pH 8.33 (see Table 1): those having a long alkyl chain (**4**, **5**, and **6**), more than 240-fold acceleration; those having a cyclodecyl moiety or a cyclohexyl moiety with an additional methyl substituent or substituents (**9**, **10**, and **11**), 20—170-fold acceleration; those having a less hydrophobic acyl portion (**3**, **7**, **8**, and**13**), 4—8-fold acceleration; acetate (**2**) and phenylacetate (**12**), the catalytic effect was fairly detected. Thus, the hydrophobic interaction of **1** with an acyl portion of each substrate ester seems to be responsible for the catalytic efficiency. This is evidenced by the effect of ethanol content on the catalytic activity of **1**. As the ethanol content is raised the hydrophobic interaction between **1** and the substrate would be progressively reduced, and consequently the catalytic efficiency of **1** would be lowered. This is what is observed in the deacylation of decanoate (7.0×10^{-7} M) as effected by 5.0×10^{-6} M of **1**: apparent second-order rate constants; 970 in 10%(v/v), 123 in 15%(v/v), 20 in 20%(v/v), and 11 $M^{-1}\,s^{-1}$ in 30%(v/v) aqueous ethanol. Similar effects have been noted elsewhere.[1,10]

TABLE 1. PSEUDO-FIRST-ORDER RATE CONSTANTS FOR DEACYLATION OF *p*-NITROPHENYL CARBOXYLATES IN THE ABSENCE (k_{hyd}) AND PRESENCE (k_{obsd}) OF **1**[a)]

Substrate ester	$k_{\mathrm{hyd}}\times10^{5}/\mathrm{s}^{-1}$	$k_{\mathrm{obsd}}\times10^{4}/\mathrm{s}^{-1}$	$k_{\mathrm{obsd}}/k_{\mathrm{hyd}}$
2	7.8	8.2	1.0
3	3.2	20	6.2
4	2.0	480	240
5	1.4	590	420
6	0.73	680	930
7	1.5	8.3	5.5
8	2.1	9.7	4.6
9	0.18[b)]	3.7	20
10	2.0	69	35
11	1.0	170	170
12	17.3	24	1.4
13	1.0	7.9	7.9

a) At 40.0±0.1 °C, pH 8.33 (6.3×10^{-3} M KH_2PO_4—3.7×10^{-3} M $Na_2B_4O_7$), and μ 0.10 (KCl) in 10.9%(v/v) ethanol–1.0%(v/v) dioxane-water; initial substrate concentration, 7.0×10^{-7} M unless otherwise stated; initial concentration of **1**, 5.0×10^{-6} M. b) Initial substrate concentration, 1.0×10^{-5} M.

The kinetic pK_a value for the deacylation of decanoate (1.0×10^{-6} M) as effected by **1** (5.0×10^{-6} M) was estimated graphically as 6.8 from the pH-rate profile (Fig. 1), which is referred to the acid dissociation of the catalytically active group, imidazole. The facile reaction of **1** with a hydrophobic ester resulted in the

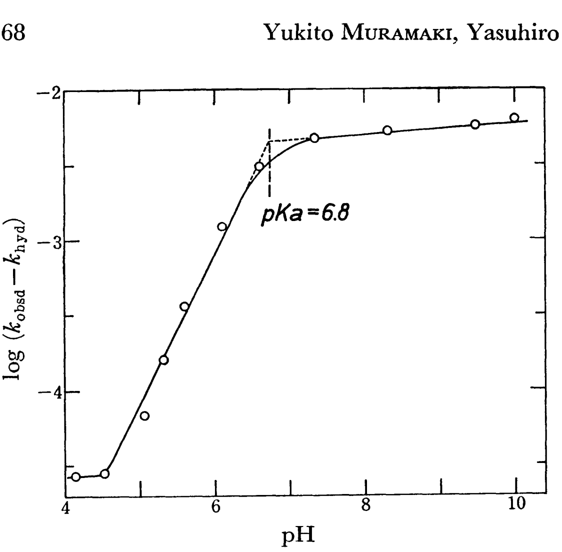


Fig. 1. pH-Rate profile for the deacylation of **4** in the presence of **1** (5.0×10^{-6} M) at 40.0±0.1 °C and μ 0.10 (KCl) in 10.9%(v/v) ethanol-1.0%(v/v) dioxane-water; initial concentration of **4**, 1.0×10^{-6} M. Both k_{obsd} and k_{hyd} are in s^{-1}.

accumulation of the N^{Im}-acyl derivative of **1** along the progress of reaction. The rate of regeneration of **1** (turnover) upon deacylation of the N^{Im}-acyl derivative was negligibly small under present experimental conditions compared to the corresponding acylation rate. With excess substrate ester, the amount of *p*-nitrophenol liberated in the course of reaction increased until it reached a maximal accumulation which exactly corresponds to the amount of **1** used.

Aggregation Behavior. The pseudo-first-order rate constants at pH 8.33 for deacylation of *active esters* are graphically shown in Figs. 2, 3, and 4 as a function of the initial concentration of **1**. In all cases the overall feature is similar to those observed for micelle-catalyzed reactions.[11] The linear portion in a rate-concentration correlation below the break point may correspond to the reaction of the monomeric species of **1**, and a sharp

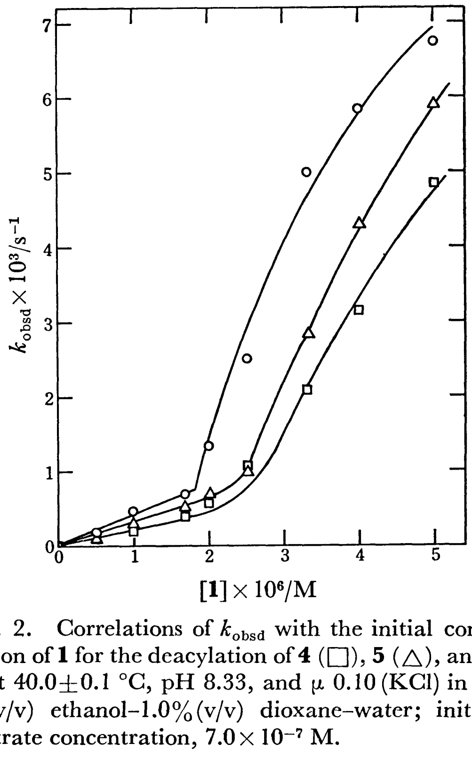


Fig. 2. Correlations of k_{obsd} with the initial concentration of **1** for the deacylation of **4** (□), **5** (△), and **6** (○) at 40.0±0.1 °C, pH 8.33, and μ 0.10 (KCl) in 10.9%-(v/v) ethanol-1.0%(v/v) dioxane-water; initial substrate concentration, 7.0×10^{-7} M.

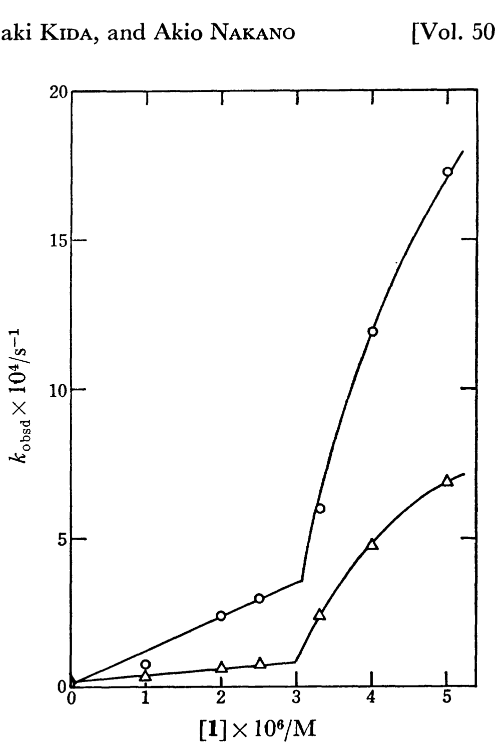


Fig. 3. Correlations of k_{obsd} with the initial concentration of **1** for the deacylation of **10** (△) and **11** (○) at 40.0±0.1 °C, pH 8.33, and μ 0.10 (KC) in 10.9%-(v/v) ethanol-1.0%(v/v) dioxane-water; initial substrate concentration, 7.0×10^{-7} M.

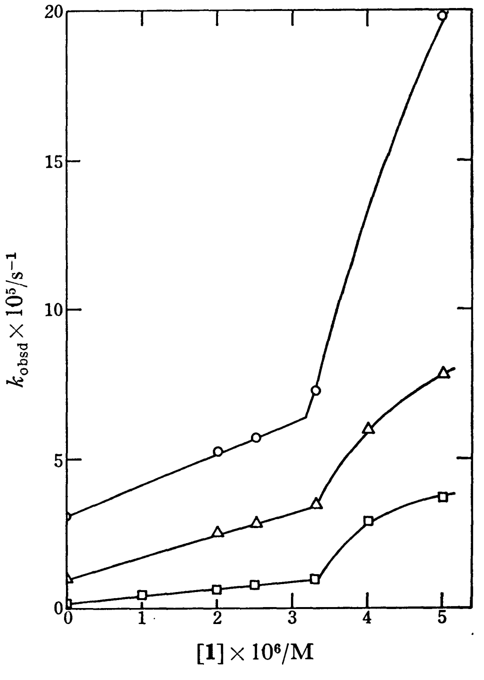


Fig. 4. Correlations of k_{obsd} with the initial concentration of **1** for the deacylation of **3** (○), **9** (□), and **13** (△) at 40.0±0.1 °C, pH 8.33, and μ 0.10 (KCl) in 10.9%(v/v) ethanol-1.0%(v/v) dioxane-water; initial substrate concentration, 7.0×10^{-7} M.

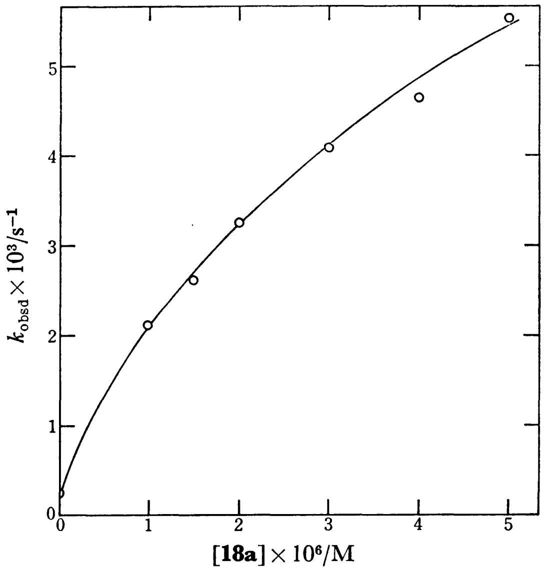
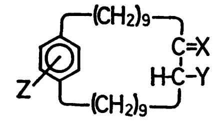


Fig. 5. Saturation-type kinetics for the deacylation of **6** in the presence of **18a** at 40.0±0.1 °C, pH 11.23, and μ 0.10 (KCl) in 10.9%(v/v) ethanol-1.0%(v/v) dioxane-water; initial substrate concentration, 7.0×10^{-7} M.

increase in rate above the break point may be due to aggregation or micellization of **1**. In marked contrast to [20]paracyclophane oximes which are believed to be monomeric under similar conditions,[1,2,4] such a kinetic behavior is rather surprising. Since the previous studies with paracyclophane oximes as catalysts in the deacylation reaction were carried out under conditions where the substrate esters were mostly in aggregated forms, we have re-examined the effect of 10-hydroxy-11-hydroxyimino[20]paracyclophane (**18a**) on the deacylation of *p*-nitrophenyl hexadecanoate at pH 11.23 under the same kinetic conditions as employed here; solvent system, temperature, and the initial substrate concentration (7.0×10^{-7} M) as shown in Fig. 5. The rate constant (k_{obsd}) is smoothly correlated with the initial concentration of **18a** without any break point in contrast to the **1**-catalyzed reaction shown in Fig. 2. Furthermore, the plot of $1/(k_{obsd}-k_{hyd})$ *vs.* $1/[\mathbf{18a}]$ yielded a straight line in a manner as observed previously.[1,2,4] It may be concluded, therefore, that there is no abrupt change in physical state of **18a** in the concentration range investigated and its critical micelle concentration (CMC), if any, would be greater than 5.0×10^{-6} M. The difference in CMC between the imidazole and oxime derivatives of [20]paracyclophane may arise at least in part from charge difference between the two catalysts: the anionic hydroxyimino group placed in the catalytically active cyclophane raises CMC significantly relative to the imidazole group in neutral form (**1**). It is generally true that the CMC's of ionic surfactants are larger than those of nonionic ones, both types having similar apolar structures. Comparison of the CMC's of surfactants having a dodecyl or dodecanoyl moiety[12] reveals this point: dodecylammonium chloride (cationic), 1.5×10^{-2} M in water at 25 °C; dodecyltrimethylammonium bromide (cationic), 1.5×10^{-2} M in water at 25 °C; sodium dodecanoate (anionic), 2.4×10^{-2} M in water at 25 °C; polyoxyethylene(6) dodecanol (nonionic), 8.7×10^{-5} M in water at 25 °C; *N*,*N*-dimethyldodecylamine oxide (nonionic), 2.1×10^{-3} M in water at 27 °C; dodecyldimethylphosphine oxide (nonionic), 5.7×10^{-4} M in water at 30 °C; sucrose dodecanoate (nonionic), 1.9×10^{-4} M in water at 20 °C. Steroid micelles which bear a better resemblance to our paracyclophanes from the structural viewpoint are subject to a charge effect even to a pronounced extent: cholic acid derivatives (anionic), 10^{-3}—10^{-2} M in water at 25 °C;[13-16] and cholesterol (nonionic), 2—4×10^{-8} M in water at 25 °C.[17]



18a X=NOH, Y=OH, Z=H
18b X=O, Y=H, Z=$CH_2N^+(CH_3)_3$ Cl^-

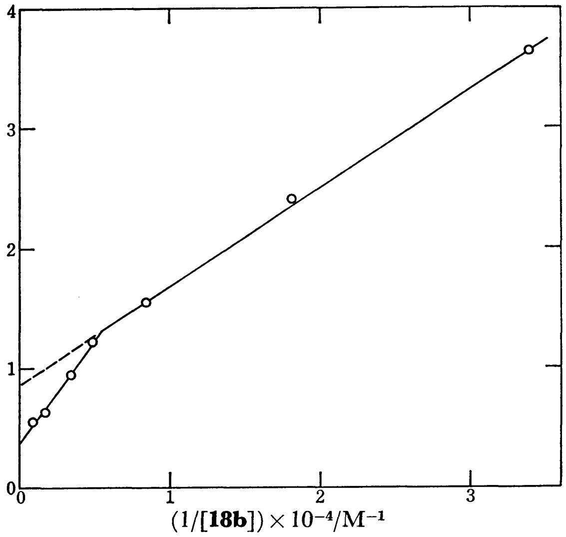


Fig. 6. Analysis of fluorescence spectral data for the interaction of **18b** with ANS (5.0×10^{-5} M) in a buffer solution of pH 8 at 20 °C by means of Eq. 1; I_f, in arbitrary scale.

The proposed charge effect on the CMC's of paracyclophanes is further exemplified by the micellization behavior of the cationic [20]paracyclophane bearing a quaternary ammonium group (**18b**) as investigated by the fluorescence dye technique with 1-anilinonaphthalene-8-sulfonate (ANS) as a probe: concentration of ANS, 5.0×10^{-5} M; excitation wavelength, 384 nm; fluorescence intensity, measured at 468 nm. The relative fluorescence intensity of ANS in a pH 8 buffer at 20 °C increased as the concentration of **18b** was raised in a concentration range of $2.95\times10^{-5}-1.18\times10^{-3}$ M. The reciprocal fluorescence intensity ($1/I_f$) is plotted in Fig. 6 as a function of reciprocal concentration of **18b** on the basis of the Benesi-Hildebrand type relationship[18] (Eq. 1) derived under the assumption that only the 1:1 interaction between **18b** and ANS takes place: I_f, observed fluorescence intensity; I_a, fluorescence intensity of ANS bound to the monomeric form of

TABLE 2. KINETIC PARAMETERS FOR DEACYLATION OF *p*-NITROPHENYL CARBOXYLATES AS CATALYZED BY **1** AND IMIDAZOLE[a)]

Substrate	k_2 ($M^{-1}s^{-1}$)	k_{Im} ($M^{-1}s^{-1}$)	k_2/k_{Im}[b)]	CMC[c)] (M)	k_m (s^{-1})	K/N (M^{-1})	k_mK/N ($M^{-1}s^{-1}$)	k_2(app)[d)] ($M^{-1}s^{-1}$)
2		0.79						0.8
3	10	0.57	18 (13)	3.1×10^{-6}			70	33
4	210	0.44	480 (270)	2.4×10^{-6}	≈0.02	1×10^5	2000	970
5	310	0.21	1500 (390)	2.3×10^{-6}	≈0.02	1.3×10^5	2600	1200
6	420	0.004	11000 (530)	1.8×10^{-6}	≈0.02	1.5×10^5	3000	1400
7		0.44						14
8		0.13						15
9	2.3	0.014	160	3.3×10^{-6}			30	7.0
10	22	0.093	240	3.0×10^{-6}	0.001	5×10^5	500	130
11	110	0.075	1500	3.0×10^{-6}	0.005	2×10^5	1000	340
12		1.6						14
13	7.5	0.033	230	3.3×10^{-6}			40	14

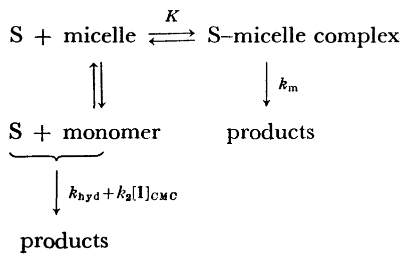
a) At 40.0±0.1 °C, pH 8.33 (6.3×10^{-3} M KH_2PO_4–3.7×10^{-3} M $Na_2B_4O_7$), and μ 0.10 (KCl) in 10.9%(v/v) ethanol–1.0%(v/v) dioxane–water; initial substrate concentration, 7.0×10^{-7} M. b) Values in parentheses, k_{Im} for acetate (**2**) was taken as the reference value. c) Critical micelle concentration of **1**. d) $k_2(\text{app})=(k_{obsd}-k_{hyd})/[\mathbf{1}]$, $[\mathbf{1}]=5.0\times10^{-6}$ M.

18b; K, binding constant for complex formation between the monomeric form of **18b** and ANS.

$$\frac{[\text{ANS}]}{I_f}=\frac{1}{I_a}+\frac{1}{I_aK[\mathbf{18b}]} \tag{1}$$

The break point, which corresponds to the CMC of **18b**, is observed at 1.8×10^{-4} M as shown in Fig. 6. This CMC value is 50—100 times as large as the substrate-dependent CMC's of **1** (Figs. 2, 3, and 4, and Table 2). The binding constant ($K=1.1\times10^4\ M^{-1}$) is 190 and 7 times as large as those for β-cyclodextrin and a water-soluble heterocyclophane, respectively.[19)]

The overall feature of deacylation reactions of *p*-nitrophenyl carboxylates in the presence of **1** is consistent with reaction pathways shown in Scheme 1.



Scheme 1.

$$\frac{1}{k_{obsd}-(k_{hyd}+k_2[\mathbf{1}]_{CMC})}=\frac{1}{k_m-(k_{hyd}+k_2[\mathbf{1}]_{CMC})}\times\left\{1+\frac{N}{K([\mathbf{1}]-[\mathbf{1}]_{CMC})}\right\} \tag{2}$$

The kinetic parameters can be determined by Eq. 2:[11)] k_{hyd}, rate constant for spontaneous hydrolysis; k_2, second-order rate constant for reaction of an ester with the monomeric form of **1**; k_m, first-order rate constant for reaction of an ester in micellar phase; k_{obsd}, observed pseudo-first-order rate constant; K, association constant for formation of the ester–micelle complex; N, aggregation number of the micelle; [**1**], total concentration of **1**; $[\mathbf{1}]_{CMC}$, CMC of **1** which is dependent on the nature of a particular substrate used. The kinetic data for reactions of **4**, **5**, **6**, **10**, and **11** were analyzed by the aid of Eq. 2. Because of the limited solubilities of the reactants as well as relatively higher kinetic CMC's of **1**, the critical evaluation of k_m and K/N values were not performed for reactions of **3**, **9**, and **13** and the apparent second-order rate constants per mole of **1** forming micelles (k_mK/N) were obtained from the rate profiles shown in Fig. 4 by the aid of Eq. 2. For esters **2**, **7**, **8**, and **12** which showed the least affinity with **1**, the apparent second-order rate constants defined by $k_2(\text{app})=(k_{obsd}-k_{hyd})/[\mathbf{1}]$ were evaluated for the purpose of comparison of reactivity with other esters. All the kinetic parameters are summarized in Table 2 along with the second-order rate constants for reactions of the esters with imidazole as a reference.

Catalytic Efficiency. All the esters except acetate (**2**) are subject to profound catalysis by **1**. The second-order rate constants (k_2) for reactions of the monomeric species of **1** with relatively hydrophobic esters exceed considerably the corresponding second-order rate constants for imidazole-catalyzed reactions (k_{Im}), suggesting the significant hydrophobic interaction between **1** and each hydrophobic ester. The k_2/k_{Im} value may be referred to a measure of the extent of hydrophobic interaction. Care should be exercised for the long-chain esters. The hydrophobic coiling of the alkyl chain, which results in masking of the ester carbonyl against an attacking nucleophile,[10)] seems to be responsible for the decrease in k_{Im} in going from decanoate through hexadecanoate. The geometry of the hydrophobic interaction between each of these esters and the monomeric species of **1** is not known at present. If the intramolecular coiling were retained in the transition state of acyl transfer from the ester to **1**, the k_2/k_{Im} values would provide a meaningful comparison of relative catalytic efficiency. However, the esters most plausibly undergo decoiling or elongation before the transition state is reached.[1—5)] The k_{Im} value for acetate which is free from the coiling effect may be cited as the plausible reference for the evaluation of catalytic efficiency of the monomeric form of **1** as shown in parentheses in Table 2. As expected from the viewpoint of hydrophobic affinity, the k_2/k_{Im} value increases as the alkyl chain of ester is lengthened (**3**<**4**<**5**<**6**). This value also increases in the order **9**<**10**<**11**. This fact may allow to draw the following conclusion; the extent of hydrophobic interaction between the monomeric species of **1** and an ester having an aliphatic ring moiety in its acyl portion may be primarily governed by the surface area20) or more simply by the number of carbon atoms in the acyl portion of the ester concerned. The aromatic ester, α-naphthoate (**13**), seems less hydrophobic relative to **10** and **11** in the light of k_2/k_{Im} values even though all three esters have comparable number of carbon atoms.

The binding constants for micellar complex formation with **4**—**6**, **10**, and **11** are greater than 10^5 M^{-1}. It may be a surprise to see the decrease in K/N in going from **10** to **11** since **11** is expected to be more hydrophobic than **10** judging from the k_2/k_{Im} values for the two esters. This would be cited as an example of substrate selectivity for the binding process. However, we are not certain whether or not the micelle of **1** has the same aggregation number (N) for different substrates. It is reasonable to assume that N is relatively small for hydrophobic macrocycles in a manner as observed for cholic acid derivatives (N=5—22).16) Under such circumstances, the aggregation number as well as CMC may be significantly dependent on the nature of a particular substrate employed.

The product of micellar reaction rate and binding constants ($k_{\mathrm{m}}K$) is referred to the second-order rate constant for reaction of a substrate and a micelle, and consequently the $k_{\mathrm{m}}K/N$ value corresponds to the second-order rate constant for the same reaction as evaluated per mole of **1** forming micelles. There is indeed a wide variation in $k_{\mathrm{m}}K/N$ as can be seen in Table 2, and the long-chain carboxylates (**4**—**6**) show the highest values in the 2000—3000 M^{-1} s^{-1} range. These values are the largest ever achieved for reactions of synthetic 4-substituted imidazoles with *p*-nitrophenyl esters,21) and may be comparable to or greater than the second-order rate constant for the acylation of chymotrypsin with *p*-nitrophenyl acetate (410 M^{-1} s^{-1} at 25 °C and pH 7.75).22) The profound catalytic activity of **1** is due primarily to its large binding ability toward the substrates (K>10^5 M^{-1}), since the micellar rate constant (k_{m}=0.02 s^{-1}) is less than one hundredth of the corresponding rate constant for acylation of the enzyme with the bound acetate (k_{m}=3.15 s^{-1} at 25 °C).

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