A Macrocyclic Enzyme Model System. Acyl Transfer from *p*-Nitrophenyl Carboxylates to Paracyclophane Oxime[1)]

Yukito MURAKAMI, Junzo SUNAMOTO,* and Koji KANO

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

(Received January 8, 1974)

The deacylation reactions of *p*-nitrophenyl carboxylates by 10-hydroxy-11-hydroxyimino[20]paracyclophane (Oxime-I) have been investigated in an alkaline aqueous acetone. Oxime-I was acylated by *p*-nitrophenyl laurate (PNPL) and decanoate (PNPD), but was not affected by *p*-nitrophenyl acetate (PNPA) and hexanoate (PNPH). Meanwhile, 2-hydroxycyclodecanone oxime (Oxime-II) and acetoxime (Oxime-III) did not undergo any reaction with all of the present carboxylic esters under the same conditions. These results suggest that the acylation reactions of Oxime-I with PNPD and PNPL proceed primarily through incorporation of the substrates into the cyclic oxime cavity. The formation of an intra-complex was also suggested by an evidence that the copper(II) ion markedly retarded the reaction rate of PNPL with Oxime-I below the catalyst-absence level. The binding constant for PNPL with Oxime-I was considerably large in comparison with the usual micelle-substrate or synthetic polymer-substrate complexes. On the basis of thermodynamic parameters for the binding and the subsequent acylation, the driving force for the incorporation and the geometry of the intra-complex have been discussed.

The three kinds of esterase model systems have been widely developed to establish some new catalytic systems for organic synthesis as well as to understand the enzymatic mechanisms from the bioorganic viewpoints: these are micellar surfactants,[2)] synthetic polymers,[3)] and macrocyclic compounds.[4)] Micellar surfactants and synthetic polymers have been known to construct the hydrophobic field through micelle formation and aggregation, respectively. The hydrophobic interaction between these enzyme models and substrates resulted in the formation of intra-complexes to provide the proximity effects due to plausible orientation and to raise the local concentrations of the attacking nucleophiles. The formation of hydrophobic binding sites in these cases is, however, in dynamic equilibria with the bulk phase and subject to the external medium effects consequently.[5)] Thus, these mobile structures may provide a limited substrate specificity. On the other hand, macrocyclic compounds may exhibit several novel and unique characters primarily due to the following effects.

1) The macrocyclic cavity provides a steady binding site due to a characteristic ring conformation of hydrophobic nature. This binding site can be little affected by the external factors such as temperature, hydrogen ion concentration, ionic strength, dielectric constant, and other medium properties.

2) A high substrate specificity can be brought about by the geometric requirements for binding of substrates into these macrocyclic cavities as well as by spatial geometries of substrate molecules incorporated into these cavities on the other. Utilization of cycloamyloses,[4a)] cyclic peptides,[4b)] and cyclic *N*-methylhydroxamic acid[4c)] have provided much fruitful results though they need to be further investigated.

In this work, we prepared a sizable cyclic compound, 10-hydroxy-11-hydroxyimino[20]paracyclophane (Oxime-I), and studied the acyl transfer from *p*-nitrophenyl carboxylates to the cyclic oxime. Oxime-I provides an appropriate hydrophobic binding site with the paracyclophane skeleton and one nucleophilic function with the oxime group. In reference to the Stuart molecular models shown in Fig. 1, Oxime-I has an inner diameter of about 6.5 Å and a depth of about 4.5 Å. 2-Hydroxycyclodecanone oxime (Oxime-II) and

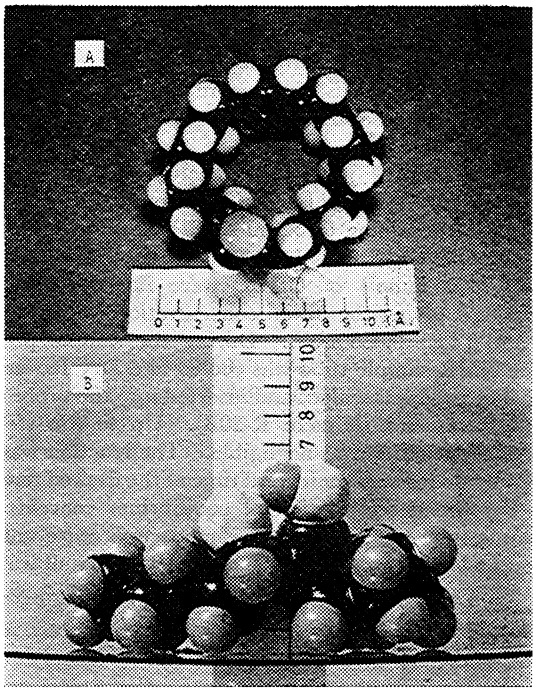
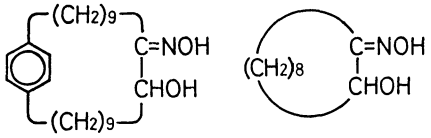


Fig. 1. Stuart molecular models of Oxime-I with scale: A, top view showing an inner diameter of *ca.* 6.5 Å; B, side view showing a depth of *ca.* 4.5 Å.



Oxime-I Oxime-II

* Present address: Department of Industrial Chemistry, Faculty of Engineering, Nagasaki University, Nagasaki 852.

acetoxime were also used in order to characterize the catalytic ability of Oxime-I.

Experimental

Spectroscopic measurements were run on a JASCO DS-403G grating IR spectrophotometer, a Varian A60 NMR spectrometer, a JEOL JMS-01SG mass spectrometer, and a Hitachi EPS-2 recording spectrophotometer. pH measurements were carried out with a TOA HM-9A pH-meter equipped with a Metrohm EA-125 combined electrode.

Acetoxime of reagent grade was commercially obtained (Nakarai) and used without further purification.

p-*Nitrophenyl Carboxylates.* *p*-Nitrophenyl acetate (PNPA), hexanoate (PNPH), decanoate (PNPD), and laurate (PNPL) were respectively prepared by the reaction of the corresponding carbonyl chlorides with *p*-nitrophenol in ether in the presence of pyridine. The crude esters were further purified and identified by elemental analyses and spectroscopic measurements before use.

The synthetic steps for Oxime-I are shown in Scheme 1.

Ethyl ω-Phenyldecanoate (1). Ethyl ω-phenyldecanoate was prepared by the esterification of ω-phenyldecanoic acid, which was synthesized according to the procedures described in the literature,[6] and purified by distillation *in vacuo*: bp 140—142 °C/0.075 mmHg, yield 82.3%.

1-(9-Carbethoxynonanyl)-4-(9-carbethoxy-1-oxononanyl) benzene (2). In a 100-ml three-necked flask was placed a mixture of 2.76 g (0.01 mol) of **1**, 4.0 g (0.03 mol) of anhydrous aluminum chloride, and 20 ml of carbon disulfide and the reaction flask was cooled in an ice bath with vigorous stirring. Then, 2.48 g (0.01 mol) of ω-carbethoxypelargonoyl chloride was added dropwise into the mixture in about 15 min. The reaction mixture was stirred for 30 min at room temperature and 3 hr under reflux, and then allowed to stand overnight at room temperature. The red oil obtained was treated with hydrochloric acid at ice-cooled temperature and extracted with carbon disulfide. The organic layer was separated, washed with water, and dried over sodium sulfate. After removal of the solvent, the pale yellow residue was recrystallized from petroleum ether (bp 30—70 °C): mp 56—58.5 °C, yield 2.4 g (49%).

Found: C, 73.74; H, 9.87%. Calcd for $C_{30}H_{18}O_5$: C, 73.73; H, 9.90%.

p-*Bis(9-carboxynonanyl)benzene (3).* A mixture of 6.0 g (0.012 mol) of **2**, 8.4 g (0.15 mol) of potassium hydroxide, 9.0 g (0.13 mol) of hydrazine hydrate, and 50 ml of diethylene glycol was heated under reflux for 3 hr. After removal of hydrazine and water, the mixture was refluxed for additional 10 hr. Into the resulting solution cooled to room temperature was added about 250 ml of water. This solution was neutralized with hydrochloric acid. The precipitates were recovered, washed with water, and recrystallized from methanol: mp 124—128.5 °C, yield 3.2 g (64%).

Found: C, 74.35; H, 10.16%. Calcd for $C_{26}H_{42}O_4$: C, 74.58; H, 10.13%.

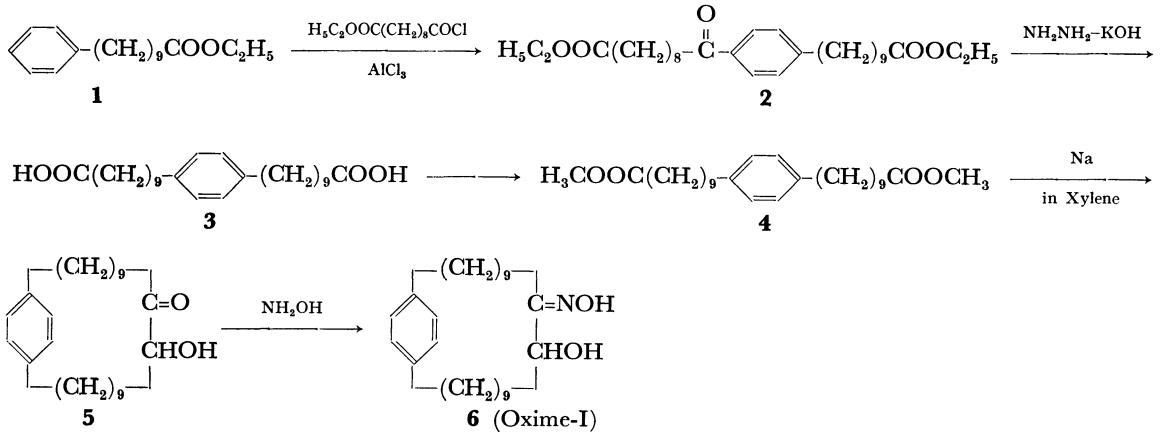
p-*Bis(9-carbomethoxynonanyl)benzene (4).* A mixture of 4.5 g (0.011 mol) of **3**, 5 ml of concentrated sulfuric acid, and 150 ml of methanol was heated under reflux for 12 hr. A half volume of methanol was distilled off. The residue was diluted with water and extracted with ether. The ethereal solution was washed with water and dried over sodium sulfate. After removal of ether, the crude material was recrystallized from methanol: mp 61—66.4 °C, yield 3.5 g (71.2%). NMR (CCl_4, TMS as an internal reference): δ 1.27—2.63 (36H, m, methylene envelope), 3.58 (6H, s, $-C\underline{H}_3$), and 6.96 (4H, br., $-C_6\underline{H}_4-$).

Found: C, 75.35; H, 10.31%. Calcd for $C_{28}H_{46}O_4$: C, 75.27; H, 10.39%.

10-Hydroxy-11-oxo[20]paracyclophane (5). A 3-liter creased flask of three-necked round-bottom was equipped with a mechanical vacuum stirrer of stainless steel and a reflux condenser, the top of which was connected to a dropping funnel furnished with a needle valve. In the flask were placed 500 ml of xylene and 1.0 g (0.043 g atom) of crust-free sodium metal. It was refluxed for 15 min with vigorous stirring under nitrogen atmosphere. Gaseous nitrogen was streamed throughout the apparatus during the course of reaction. Then, 3.0 g (6.7 mmol) of **4** dissolved in 250 ml of xylene was added dropwise for a period of 20 hr. Reflux was continued for additional 30 min after the complete addition of **4**. The reaction flask was then cooled in an ice bath. Glacial acetic acid (4 ml) dissolved in 6 ml of xylene was added dropwise in about 30 min while cooling and stirring were continued. The resulting mixture was transferred into a 2-liter separatory funnel together with 25 ml of water. The xylene layer was separated, washed with water, and dried over active calcium sulfate. After rapid removal of xylene, the crude material was recrystallized from *n*-pentane as white granular crystals: mp 46—51 °C, yield 1.4 g (53.7%). IR (KBr disk): ν_{OH}, 3400; $\nu_{C=O}$, 1704 cm^{-1}.

Found: C, 81.57; H, 11.48%; M^+, 386. Calcd for $C_{26}H_{42}O_2$: C, 80.75; H, 10.97%; mol wt, 386.68.

10-Hydroxy-11-hydroxyimino[20]paracyclophane (Oxime-I). Into a mixture of 500 mg (1.29 mmol) of **5**, 300 mg (4.32 mmol) of hydroxylamine hydrochloride, and 10 ml of methanol



1 **2** **3** **4** **5** **6** (Oxime-I)

Scheme 1. Synthesis of Oxime-I.

containing a small amount of water was added gradually 530 mg (13 mmol) of sodium hydroxide powder. The mixture was heated under reflux for 10 min, neutralized with dilute aqueous hydrochloric acid, and then extracted with about 200 ml of *n*-pentane. The organic layer was separated, washed with water, and dried over sodium sulfate. After a half volume of *n*-pentane was distilled off, the residue was cooled to about −10 °C. The colorless needles were recovered: mp 91.5—93 °C, yield 440 mg (84.8%). IR (KBr disk): ν_{OH}, 3300; $\nu_{C=N}$, 1650 cm^{-1}.

Found: C, 77.70; H, 11.28; N, 3.20%; M^+, 401. Calcd for $C_{26}H_{43}NO_2$: C, 77.73; H, 10.81; N, 3.49%; mol wt, 401.70.

2-Hydroxycyclodecanone (sebacoin). 2-Hydroxycyclodecanone was prepared according to the procedures described in the literature:[7] mp 36.5—39 °C (lit,[7] 38—39 °C), yield 47.3%.

2-Hydroxycyclodecanone Oxime (Oxime-II). Sebacoin oxime was prepared by a method similar to that used for the preparation of Oxime-I. The crude Oxime-II (mp 86—96 °C) was purified by means of column chromatographic technique with the combination of active alumina and absolute methanol: mp 101—103 °C, yield 1.2 g (50%). IR (KBr disk): ν_{OH}, 3220; $\nu_{C=N}$, 1650 cm^{-1}.

Found: C, 64.92; H, 10.58; N, 7.30%; M^+, 185. Calcd for $C_{10}H_{19}NO_2$: C, 64.81; H, 10.36; N, 7.56%; mol wt, 185.30.

Kinetic Measurements. The rate of formation of *p*-nitrophenoxide ion was measured at 400 nm. Reactions were initiated by the addition of 30 μl of 1×10^{-3}M *p*-nitrophenyl carboxylate in acetone to 3.0 ml of a reaction solution which was pre-equilibrated at an appropriate temperature in the thermostatted cell set in a Hitachi 124 recording spectrophotometer. The reaction solution was prepared in a 10 ml volumetric flask by placing 1.0 ml of $1-7\times10^{-4}$M Oxime-I in acetone–water (1: 1 by volume), 0.5 ml of acetone, 0.9 ml of 1.0 M aqueous potassium chloride, and an appropriate amount of 0.1 M sodium hydroxide to adjust the pH-value of medium in this order, and subsequently by filling up with distilled and deioninized water to the mark. The overall concentrations of *p*-nitrophenyl esters and the oxime in the reaction mixture were 1×10^{-5}M and $2-15\times10^{-6}$M, respectively. The acetone content in the above reaction mixture was about 10% of the total volume, and also varied in a 10—30% (v/v) range. The temperature of a reaction mixture was maintained constant with an accuracy of ±0.1 °C. A buffer system, sodium carbonate–sodium borate, was used for reactions at pH 10.

Product Analysis. *p*-Nitrophenyl laurate (82.9 mg, 0.26 mmol) underwent reaction at 20 °C with Oxime-I (98.8 mg, 0.24 mmol) in 50 ml of acetone–water (1: 5 by volume) containing 2.6 ml of 0.1 M sodium hydroxide. After the complete decomposition of the ester, the reaction mixture was extracted with *n*-hexane. The aqueous alkaline layer remained was carefully neutralized with dilute hydrochloric acid and extracted with ether. Upon evaporation of the ethereal layer, *p*-nitrophenol and lauric acid were detected by tlc as well as by UV and IR spectroscopy. On the other hand, tlc of the mixture obtained from *n*-hexane extracts showed the existence of three components. Two of them were identified as the unreacted Oxime-I and its hydrolyzate, 10-hydroxy-11-oxo[20]paracyclophane (**5**), though both were only in a trace amount. The third component, which was isolated from the mixture, showed a strong IR absorption band at 1710 cm^{-1} ($\nu_{C=O}$) and a UV spectrum having absorption maxima at 213, 220, 260, 267, and 274 nm in methanol. These results indicate that Oxime-I was acylated by *p*-nitrophenyl laurate (Eq. (1)) in a manner as seen for the acylation of aryl-oxime with *p*-nitrophenyl carboxylates.[8]

$$\text{>C=N-OH} + \text{Ph-O-C(O)-R} \longrightarrow \text{>C=N-O-C(O)-R} + \text{Ph-OH} \quad (1)$$

Meanwhile, Oxime-I was hydrolyzed to give the parent ketocyclophane (**5**) to an insignificant extent under the same reaction conditions. In addition, the degradation of Oxime-I did not show any meaningful effect on the reaction kinetics.

Results and Discussion

In the alkaline hydrolyses of PNPD and PNPL, pseudo-first-order plots deviated positively after 25—30% conversion of the esters. This phenomenon has been attributed to the self-aggregation of the substrates.[9] Meanwhile, in the presence of Oxime-I the rate of *p*-nitrophenol release from the esters started to deviate gradually downward from the first-order plot in the same conversion range. Therefore, a pseudo-first-order rate constant k_{obs} was obtained from the linear portion at the initial stage of reaction. Oxime-I did not, however, provide any effect on the *p*-nitrophenol release from esters of smaller alkyl chains, PNPA and PNPH (Table 1). Similarly, smaller cyclic oxime (Oxime-II) as well as acetoxime also did not show any effect on the reaction rate of all the substrates studied (Table 1). In the reaction of Oxime-I either with PNPD or PNPL, the usual saturation-type kinetics have been observed as illustrated for the PNPL–Oxime-I system in Fig. 2. These kinetic evidences and the product analysis suggest that there proceed two competetive reactions, the hydrolysis of an uncomplexed substrate and the acyl transfer reaction *via* an intra-complex formed between Oxime-I and either PNPD or PNPL, as shown by Eq. (2).

$$\begin{array}{ccc} S + \text{Oxime-I} & \overset{K}{\rightleftharpoons} & \text{S-Oxime-I} \\ \downarrow k_{hyd} & & \downarrow k_{acyl} \\ P_1 & & P_2 \end{array} \quad (2)$$

where k_{hyd} and k_{acyl} stand respectively for the rate

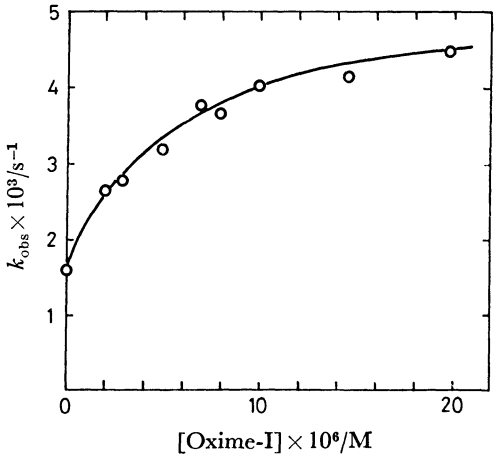


Fig. 2. Saturation-type kinetics for the reaction of PNPL (9.90×10^{-6}M) with Oxime-I in 10.9% (v/v) aqueous acetone at 34.1 °C, μ=0.10 (KCl), and pH 12.3 (1.00×10^{-2}M sodium hydroxide).

Table 1. First-order rate constants for the *p*-nitrophenol release from *p*-nitrophenyl carboxylates in the presence of oxime-I, -II, and acetoxime (oxime-III)

Carboxylate Species: 10^{-5}M	Oxime Species: M	NaOH, 10^{-2}M	Acetone content, % (v/v)	Ionic strength (KCl), M	Temp., °C	k_{obs}, $10^{-3}s^{-1}$
PNPA: 5.00	None	0.50	30.0	0.05	25.0	26.5
PNPA: 5.00	I: 1.00×10^{-4}	0.50	30.0	0.05	25.0	26.5
PNPH: 5.00	None	0.50	30.0	0.05	25.0	20.7
PNPH: 5.00	I: 1.00×10^{-4}	0.50	30.0	0.05	25.0	20.2
PNPH: 4.76	None	1.00	26.7	0.01	19.6	20.9
PNPH: 4.76	I: 4.76×10^{-5}	1.00	26.7	0.01	19.6	19.7
PNPH: 4.76	II: 4.76×10^{-5}	1.00	26.7	0.01	19.6	20.9
PNPD: 4.76	None	1.00	26.7	0.01	19.6	7.05
PNPD: 4.76	I: 4.76×10^{-5}	1.00	26.7	0.01	19.6	5.57
PNPD: 4.76	II: 4.76×10^{-5}	1.00	26.7	0.01	19.6	7.55
PNPD: 0.99	None	1.00	10.9	0.10	20.3	3.59
PNPD: 0.99	I: 9.90×10^{-6}	1.00	10.9	0.10	20.3	4.26
PNPL: 0.99	None	1.00	10.9	0.10	20.0	0.31
PNPL: 0.99	I: 9.90×10^{-6}	1.00	10.9	0.10	20.0	2.25
PNPL: 0.99	II: 9.90×10^{-6}	1.00	10.9	0.10	20.0	0.33
PNPL: 0.99	III:9.90×10^{-6}	1.00	10.9	0.10	20.0	0.33

Table 2. Kinetic parameters for the reactions of *p*-nitrophenyl decanoate and laurate with oxime-I at μ=0.10 (KCl)

Carboxylate [a,b]	Temp., °C	Acetone content, % (v/v)	pH	$k_{hyd}\times10^4$, s^{-1}	$k_{acyl}\times10^4$, s^{-1}	$K\times10^{-5}$, M^{-1}
PNPD	20.3	10.9	12.3	35.9	43.0	3.8
	30.3	10.0	9.3	0.88	3.42	14.6
	30.3	10.0	9.9	2.71	7.29	6.4
	30.3	10.0	11.0	20.9	44.1	5.4
PNPL	15.2	10.9	12.3	2.1	13.8	4.8
	24.5	10.9	12.3	5.1	33.7	1.5
	34.1	10.9	12.3	16.1	58.7	1.3
	42.1	10.9	12.3	37.1	137	0.6
	33.9	10.9	10.4	0.3	4.4	1.3
	43.1	10.9	10.4	1.4	8.9	1.3
	20.0	10.9	12.3	3.1	20.0	2.6
	20.1	20.8	12.5	14.1	54.9	1.2

a) Total initial concentration: PNPD, 1.03×10^{-5}M; PNPL, 9.90×10^{-6}M. b) Total initial concentration of Oxime-I: with PNPD, $6.03-48.2\times10^{-6}$M; with PNPL, $1.98-19.80\times10^{-6}$M.

constant of hydrolysis in the bulk phase and that of acylation due to the intra-complex; S and S–Oxime-I represent the substrate and the substrate—Oxime-I complex, respectively, and K is the binding constant. The observed pseudo-first-order rate constant k_{obs} is given by Eq. (3)

$$k_{obs} = (k_{hyd}+k_{acyl}K[\text{Oxime-I}])/(1+K[\text{Oxime-I}]) \quad (3)$$

which can be rearranged to

$$1/(k_{hyd}-k_{obs}) = 1/(k_{hyd}-k_{acyl}) + 1/(k_{hyd}-k_{acyl})K[\text{Oxime-I}] \quad (4)$$

A good linear relationship has been obtained by plotting the left-hand side of Eq. (4) against the reciprocal concentration of Oxime-I, as typically seen in Fig. 3. We calculated the rate constant for acylation and the binding constant from the intercept and the slope, respectively. Kinetic results are summarized in Table 2.

It is interesting to examine the difference in reactivity in terms of the correlations between the ring size of

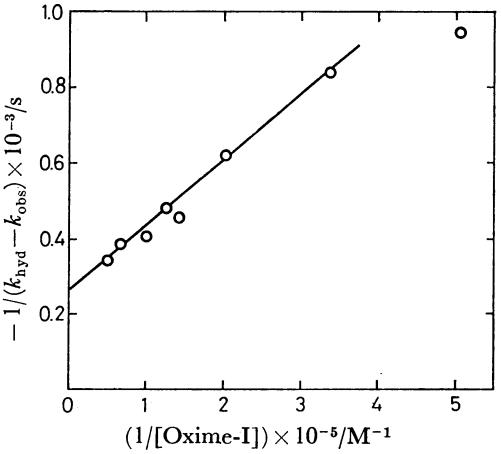


Fig. 3. Analysis of kinetic data (given in Fig. 2) for the decomposition of PNPL in the presence of Oxime-I by means of Eq. (4).

cyclic oximes and the molecular size (or geometry) of substrates. Table 1 shows that Oxime-I was acylated only by the esters having a long alkyl chain residue and not by the smaller esters such as PNPA or PNPH, while Oxime-II and acetoxime could not be acylated even by longer esters. It may be, therefore, reasonable to assume that the present acyl transfer reactions from PNPL and PNPD to Oxime-I must proceed through the intra-complex formation, which would be caused by an incorporation of a substrate into the cyclic cavity of Oxime-I and not by a simple hydrophobic entanglement.

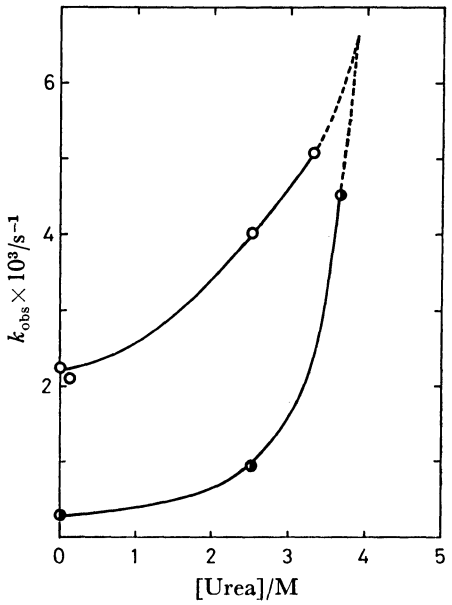


Fig. 4. Effects of added urea on the decomposition of PNPL (9.90×10^{-6}M) in 10.9% (v/v) aqueous acetone at 20.0 °C, μ=0.10 (KCl), and pH 12.3 (1.00×10^{-2}M sodium hydroxide): ○, with Oxime-I (9.90×10^{-6}M); ◑, without Oxime-I.

Driving Force for Incorporation. The increase in concentration of acetone appeared to decrease the incorporation of the substrate into the cyclic cavity of Oxime-I significantly (Table 2). Similar results have been obtained for the cycloamylose—phenyl acetate system,[4a] where the effect of acetonitrile content was examined. Moreover, Fig. 4 shows that the addition of urea to the reaction system also reduces the incorporation: as the concentration of urea increases, the overall pseudo-first-order rate constant (k_{obs}) and the rate constant for spontaneous hydrolysis increase and finally both become equal. Consequently, a driving force for the incorporation of a substrate into the cavity of Oxime-I should be attributed to the hydrophobicity of a substrate and the oxime as well. These results suggest that the self-aggregation of the substrate acts in favor of the incorporation but the resulting hindered nucleophilic center provides a steric effect against attacking hydroxide ion. The Stuart molecular model shows, in fact, that the folded substrate molecule can be inserted tightly into the Oxime-I cavity. If the elongated substrate molecule could be incorporated, PNPA and PNPH, having molecular head and tail similar to PNPD and PNPL, would also undergo the acyl transfer reaction with Oxime-I. It should be noted in the present study that urea or an organic solvent perturbs the hydrophobicity of the substrate esters only and not the cyclic oxime cavity.

Effect of the Copper(II) Ion. Oxime-I has three donor atoms, one nitrogen and two oxygens. It is expected that an appropriate metal ion may show somewhat a unique effect on the reactivity of Oxime-I. The copper(II) ion, which was used as nitrate, did not give any effect on the reaction of PNPL in the absence of Oxime-I, while it profoundly retarded the *p*-nitrophenol release in the presence of the oxime. Upon addition of over equimolar amount of copper ion relative to the ester and the oxime, the reaction rate was reduced below the Oxime-I absence level (Fig. 5). These results suggest that Oxime-I deactivated by the coordination of the copper(II) ion still holds a large ability of substrate-incorporation and consequently the ester decomposition becomes largely decelerated. Thus, we can say that the effect of copper(II) provides another evidence for the incorporation. Hershfield and Bender investigated the hydrolysis of PNPL catalyzed by a macrocyclic hydroxamic acid. Although the reaction rate was markedly accelerated upon addition of copper(II), they did not give any plausible elucidation for the catalytic mechanism.[4c] The copper-concerned reactions in both cases apparently proceed through somewhat different mechanisms.

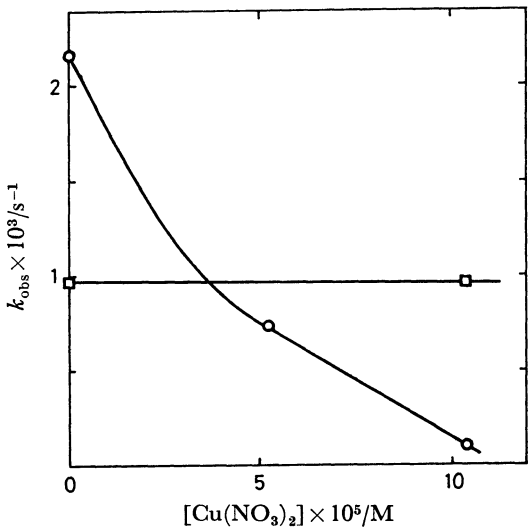


Fig. 5. Effects of added copper(II) ion on the decomposition of PNPL (4.76×10^{-5}M) in 26.7% (v/v) aqueous acetone at 20.0 °C and pH 12.3 (1.00×10^{-2}M sodium hydroxide): ○, with Oxime-I (4.76×10^{-5}M); □, without Oxime-I.

Catalytic Effect and Substrate Specificity. Oxime-II and acetoxime did not show any meaningful effect on the decomposition of PNPD and PNPL. This means that these two oximes undergo no acyl transfer reaction at least under the present conditions. It has been well known that acetoxime (pK_a 12.4) shows exceedingly large nucleophilicity due to α-effect.[10] Our present cyclic oximes, Oxime-I and -II, must have approximately the same nucleophilicity as that of acetoxime as

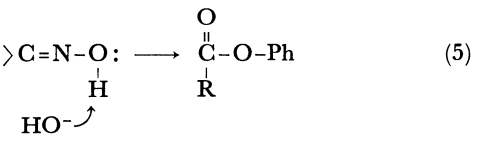
far as the oxime moiety concerns. The large reactivity of Oxime-I, therefore, can be attributed to the incorporation which brings about the proximity effect for the reaction. This effect is analogous to the cases of intramolecular catalysis. The inertness of Oxime-II, on the other hand, seems to be ascribed to its small ring size which inhibits such an incorporation.

Table 3. Catalytic specificity of oxime-I in the reactions with *p*-nitrophenyl carboxylates

Carboxylate	$k_{obs(Oxime-I)}/k_{obs(Oxime-II)}$	k_{acyl}/k_{hyd}
PNPA	*ca.* 1[a]	—
PNPH	*ca.* 1[a]	—
PNPD	0.7[b]; 1.2[c]	2.4[c]
PNPL	2.3[b]; 7.4[c]	14.7[c]

a) At 25.0 °C in 30% (v/v) aqueous acetone with pH 11.5. b) At 19.6 °C in 26.7% (v/v) aqueous acetone with pH 12. c) At 20.0 °C in 10.9% (v/v) aqueous acetone with pH 10.

Clearly from the preceding comments, the overall rate constant in the presence of Oxime-II, $k_{obs(Oxime-II)}$, is nearly equal to the rate constant for spontaneous hydrolysis of esters. We define the catalysis specificity of Oxime-I in terms of either $k_{obs(Oxime-I)}/k_{obs(Oxime-II)}$ or $k_{obs(Oxime-I)}/k_{hyd}$, each rate constant being obtained under the same conditions. These are listed in Table 3, along with the specific rate ratio, k_{acyl}/k_{hyd}. The specificity of Oxime-I further increased at lower pH region where the nucleophilicity of the oxime group must become smaller. This fact, as well as the product analysis, suggests that the acyl transfer reaction in the intra-complex may proceed through the direct nucleophilic attack of the oxime to the carbonyl carbon, not through the general base catalysis. In addition, the acylation exhibits the linear dependence on pH in a 9—12 pH range (Fig. 6). This is consistent with a mechanism shown by Eq. (5). Deceleration of the decomposition rate of PNPD in the presence of Oxime-I at higher pH (Table 3) is not clear at



(5)

present. However, this is predictive of the presence of substrate specificity for Oxime-I. Our results are, therefore, apparently different from those obtained by Hershfield and Bender,[4c] in which the rate enhancement was gained only by the extension of alkyl-chain length without substrate specificity. They did not indicate clearly which effect, simple entanglement or

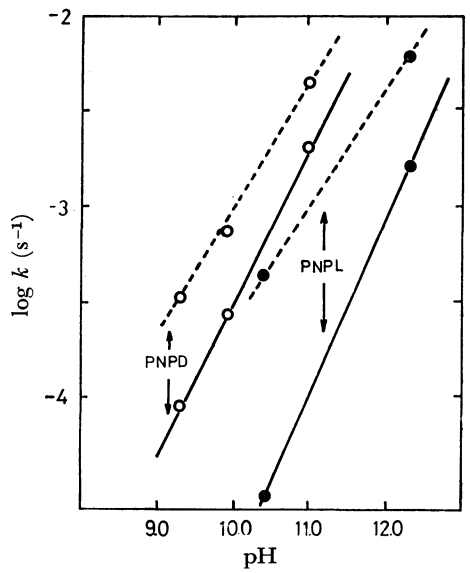


Fig. 6. pH-rate correlations for alkaline hydrolyses (solid lines) of PNPD (1.00×10^{-5}M, ○) in 10.0% (v/v) aqueous acetone with μ=0.10 (KCl) at 30.0 °C and PNPL (9.90×10^{-6}M, ●) in 10.9% (v/v) aqueous acetone with μ=0.10 (KCl) at 33.9 °C; and acyl transfer reactions of these carboxylates (broken lines) with an equimolar concentration of Oxime-I under the same conditions employed for the corresponding alkaline hydrolyses.

incorporation, contributed to the interaction of the cyclic compound with substrates.

Binding Property. Table 2 shows that the binding constants for the incorporation of PNPD and PNPL into Oxime-I are considerably larger ($K\approx10^5$ M^{-1}) than those for phenyl acetates into cycloamyloses ($K\approx10^3$ M^{-1}).[11] In the present study, it became clear that large hydrophobic guest molecules lead to the formation of relatively stable inclusion complexes and the interior of the cyclic oxime provides a better hydrophobic binding site. In order to understand the nature of incorporation, the temperature dependency of binding constant for PNPL–Oxime-I was examined using kinetic method. Thermodynamic parameters were obtained from a plot of $\ln K$ *vs.* $1/T$(K) by the least-squares method: $\Delta G°$(307.3 K) = −7.2 kcal mol^{-1}, $\Delta H°$ = −9.4 kcal mol^{-1}, and $\Delta S°$ = −7.3 e.u. at pH 12 in 10.9 vol% aqueous acetone. The binding constant is comparatively temperature dependent in a similar trend as observed for the combination of *m*-ethylphenyl acetate and cyclohexaamylose.[11a] The thermodynamic data apparently show that the stabilization due to binding is mostly caused by a favorable enthalpy change. Bender and his co-workers have proposed

Table 4. Activation parameters for alkaline hydrolysis and acylation reaction of PNPL[a]

Reaction	E_a kcal mol^{-1}	$\Delta S^{\ddagger}$ e.u.	$\Delta H^{\ddagger}$ kcal mol^{-1}	$\Delta G^{\ddagger}$ kcal mol^{-1}
Hydrolysis	18.5±0.7	−13.2±2.3	17.9±0.7	22.0±1.5
Acylation	14.9±0.3	−22.5±0.8	14.3±0.3	21.3±0.6

a) Experimental conditions: pH, 12.3; μ, 0.10 (KCl); temp., 307.3 K; medium, 10.9% (v/v) acetone–water.

that the sizable enthalpy change in the formation of cycloamylose complex was brought about by liberating water molecules, which were loosely bound to the cycloamylose cavity through hydrogen bonding, upon inclusion of a guest molecule.[11a] In the paracyclophane cavity, however, the van der Waals forces would provide such a favorable enthalpy effect. Further investigation is in progress in our laboratories.

Activation Parameters for Hydrolysis and Acyl Transfer Reactions. Activation parameters for the hydrolysis of PNPL and the acylation of Oxime-I with the same ester are listed in Table 4. A decrease of activation enthalpy ($\Delta\Delta H^{\ddagger}=4$ kcal mol^{-1}), which plays the major role in the stabilization of transition state, may be caused by the difference in nucleophilicity between hydroxide ion and oximate. Furthermore, the proximity effect brought about by the intra-complex formation seems to be responsible for such a smaller activation enthalpy. A much larger loss of activation entropy ($\Delta\Delta S^{\ddagger}=-10$ e.u.) for the acyl transfer reaction would be rather unexpected: the intramolecular catalysis is entropically favored.[12] This fact suggests that the base-catalyzed nucleophilic reaction results in the formation of a polar transition state and consequently the hydrophobic interaction is partly destroyed.

Conclusion

The sizable paracyclophane oxime demonstrated several unique behaviors as expected. An appropriate cavity size can give out the substrate specificity: this is associated with bulkiness, geometry, and molecular volume of a substrate as well as hydrophobicity of both substrate and paracyclophane. Most significantly, the incorporation of a substrate, having an appropriate geometrical character, into the cyclic cavity was confirmed by the kinetic method. It has been also shown that Oxime-I has a large binding ability toward *p*-nitrophenyl decanoate and laurate, as can be compared to some native enzyme systems.[13] The present study provides a rare and unique example among the enzyme model systems so far studied,[2-5] and may be predictive of the possibility for preparation of a more reactive esterase models. Upon substitution with appropriate functional groups, the paracyclophane skeleton may be modified to yield higher deacylation ability.

Thanks are due to Miss Chieko Gondo for her assistance in part of the rate measurements, and also to the Research Institute of Yoshitomi Pharmaceutical Co., Ltd. for the mass spectral measurements.

References

1) Contribution No. 318 from the Department of Organic Synthesis, Faculty of Engineering, Kyushu University. Preliminary communication: Y. Murakami, J. Sunamoto, and K. Kano, *Chem. Lett.*, **1973**, 223.

2) E. J. Fendler and J. H. Fendler, "Advances in Physical Organic Chemistry," Vol. 8, ed. by V. Gold, Academic Press, New York, N. Y. (1970), p. 271.

3) M. L. Bender, "Mechanisms of Homogeneous Catalysis from Protons to Proteins," Wiley-Interscience of John Wiley & Sons, Inc., New York, N. Y. (1971), pp. 382—390.

4) a) Ref. 3, pp. 373—382; b) J. C. Sheehan, G. B. Bennett, and J. A. Schneider, *J. Amer. Chem. Soc.*, **88**, 3455 (1966); K. Nakajima and K. Okawa, This Bulletin, **46**, 1811 (1973); c) R. Hershfield and M. L. Bender, *J. Amer. Chem. Soc.*, **94**, 1376 (1972).

5) E. Cordes, Ed., "Reaction Kinetics in Micelles," Plenum Press, New York, N. Y. (1973).

6) R. Huisgen, W. Rapp, I. Ugi, H. Walz, and I. Glogger, *Ann. Chem.*, **586**, 52 (1954).

7) N. L. Allinger, "Organic Syntheses," Coll. Vol. IV, p. 840 (1963).

8) A. K. Yatsimirski, K. Martinek, and I. V. Berezin, *Tetrahedron*, **27**, 2855 (1971).

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

10) J. O. Edwards and R. G. Pearson, *ibid.*, **84**, 16 (1962).

11) a) R. L. VanEtten, J. F. Sebastian, G. A. Clowes, and M. L. Bender, *ibid.*, **89**, 3242 (1967); b) R. L. VanEtten, G. A. Clowes, J. F. Sebastian, and M. L. Bender, *ibid.*, **89**, 3253 (1967).

12) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. 1, W. A. Benjamin, Inc., New York, N. Y. (1966), Chapt. 1.

13) D. M. Chipman and N. Sharon, *Science*, **165**, 454 (1969).