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A MACROCYCLIC ENZYME-MODEL SYSTEM. AN EVIDENCE FOR INCORPORATION OF A SUBSTRATE INTO A SIMPLE CYCLIC CAVITY[1)]

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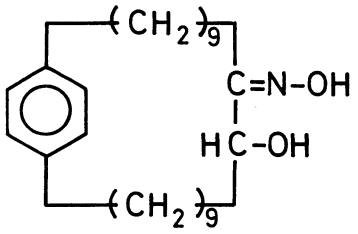
The release of *p*-nitrophenol from *p*-nitrophenyl hexanoate (PNPH), decanoate (PNPD), and laurate (PNPL) in the presence of 10-hydroxy-11-hydroxyimino[20]paracyclophane (Oxime-I) or 2-hydroxycyclodecanone oxime (Oxime-II) has been investigated in alkaline aqueous acetone at 19.6°C and pH 12.1. For the decomposition of PNPL, the specificity constant (k_r), defined as the ratio of the apparent first-order rate constant for the reaction of Oxime-I to that for the reaction of Oxime-II, sharply increased below 26.7 % of acetone-content, while the rate constant for the simple alkaline hydrolysis without added catalyst species decreased with the decrease of acetone concentration. The copper(II) ion largely inhibited the reaction of PNPL with Oxime-I to reduce the rate below the catalyst-absence level. These results manifest the incorporation of the substrate into the cyclic cavity of the catalyst. The presence of substrate specificity was also indicated for the reaction of Oxime-I with *p*-nitrophenyl carboxylates.

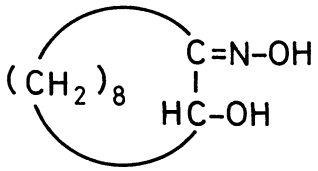
Macrocyclic compounds, such as cycloamilose,[2)] cyclopeptides,[3)] and a macrocyclic hydroxamic acid,[4)] have been utilized as enzyme models for the ester hydrolysis. The hydrophobic binding site geometrically constructed with a characteristic ring conformation in these macrocyclic compounds may be maintained relatively steady independent of the external factors such as hydrogen ion concentration, salt concentration, temperature, and medium. On the other hand, polymer compounds or detergents, which were used for various ester hydrolyses as enzyme models, have been known to construct the hydrophobic field through aggregation or micelle formation. The formation of hydrophobic binding site in these cases is in equilibrium with bulk solution and subject to the medium effects consequently. Thus, a higher substrate specificity may be provided by preparing a designed macrocyclic compound having some characteristic conformation with respect to the ring size and the molecular geometry.

In this communication, we report an evidence for the substrate-incorporation into the macrocyclic cavity of the catalyst, Oxime-I,[5)] in the decomposition of *p*-nitrophenyl carboxylates in alkaline aqueous acetone. In order to prove the characteristic feature of Oxime-I, the reaction of Oxime-II[5)] with the carboxylate esters was also carried out under the same conditions. In these systems with Oxime-I, acyl transfer from the ester to the oxime[6)] may occur and the resulting deactivation of



OXIME-I

10-Hydroxy-11-hydroxyimino-[20]paracyclophane



OXIME-II

2-Hydroxycyclodecanone oxime

Table 1. Comparison of the effect of Oxime-I and -II on *p*-nitrophenol release from *p*-nitrophenyl carboxylates at 19.6°C and pH 12.1[a]

Ester	$k_0\times10^3$[b] (sec^{-1})	$k_I\times10^3$[c] (sec^{-1})	$k_{II}\times10^3$[c] (sec^{-1})	k_r[d]
PNPH[e]	20.9	19.7	20.9	0.94
PNPD	7.05	5.57	7.55	0.74
PNPL	0.97	2.17	0.97	2.26

[a] In 26.7 vol% aqueous acetone, $[oxime]_0 = [ester]_0 = 4.76\times10^{-5}$M and $[NaOH]_0 = 1.10\times10^{-2}$ M. [b] Apparent first-order rate constants for the alkaline hydrolysis of the esters. [c] k_I and k_{II} stand for the apparent first-order rate constants for the reactions in the presence of Oxime-I and -II, respectively, in alkaline aqueous acetone. [d] k_r is the ratio of k_I to k_{II}. [e] *p*-Nitrophenyl hexanoate.

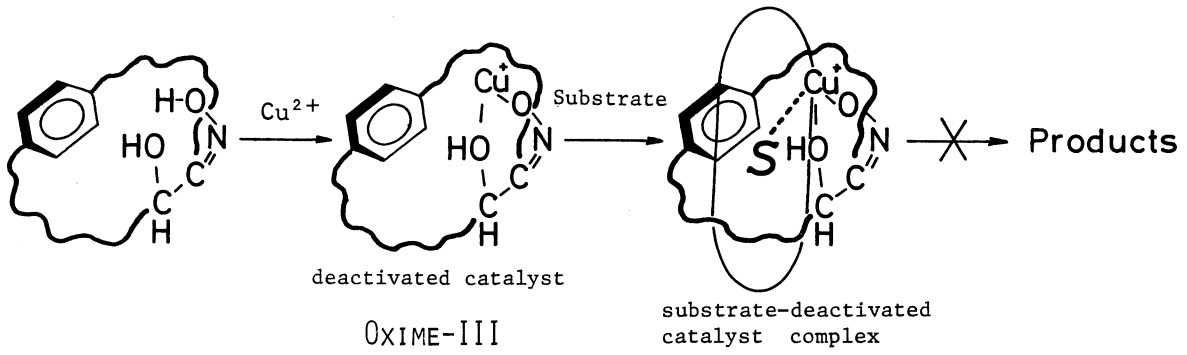
the catalyst is expected.

The release of *p*-nitrophenol from the esters was followed spectrophotometrically at 400 nm. In all cases, the apparent first-order kinetics was observed in the initial stage of reaction. The kinetic data are summarized in Table 1. Oxime-II did not provide any meaningful catalysis in the hydrolysis of the three carboxylates. The inertness seems to be attributed to its small ring size. Oxime-I gave out the rate enhancement in the *p*-nitrophenol release from PNPL on one hand, and the rate retardation in that from PNPD on the other.

Incorporation of the Substrate Into the Catalyst. In the alkaline hydrolyses of PNPD and PNPL, the reaction rate increased gradually with the progress of reaction. The deaggregation of the substrate in the course of reaction seems to be responsible for it.[7] Meanwhile, in the presence of Oxime-I the rate of *p*-nitrophenol release from both esters started to deviate gradually downward from the first-order plot after the first half-life. This retardation phenomenon seems to be an evidence for deactivation of catalyst which was brought about by the acylation of the oxime group. No turnover reaction was observed in the present cases as a result. The significant

rate enhancement in *p*-nitrophenol release from PNPL was observed, nevertheless, in the presence of Oxime-I (Table 1). It may be reasonable to consider that the incorporation of the substrate into the cyclic compound (Oxime-I) certainly occurs.

Another evidence for the incorporation was seen in the inhibition reaction due to the copper(II) ion. Although the copper(II) ion did not show any effect on the alkaline hydrolysis of the esters, it markedly reduced the reaction rate of PNPL with Oxime-I below the catalyst-absence level (Table 2). Judging from these results, it is quite certain that the copper(II) ion behaves as an effective inhibitor through the complex formation, Oxime-III, and that the deactivated catalyst species still holds the ability of binding the substrate, which causes the large decerelation of the ester decomposition. The most plausible reaction process for the inhibition by



SCHEME 1

Table 2. Effect of the copper(II) ion on the *p*-nitrophenol release from the carboxylates in the presence of Oxime-I[a]

Ester	$[Cu^{2+}]\times10^{5}$,M	$k_{obs}\times10^{3}$,sec^{-1}[b]
PNPH	10.37	19.7
PNPD	10.37	7.55
PNPL	5.19	0.74
PNPL	10.37	0.11

[a] $[ester]_0 = [Oxime\text{-}I]_0 = 4.76\times10^{-5}$M, at 19.6°C and pH 12.1 in 26.7 vol% aqueous acetone. The copper(II) ion was added as the nitrate.

[b] k_{obs} should be compared with k_0 and k_I values in Table 1.

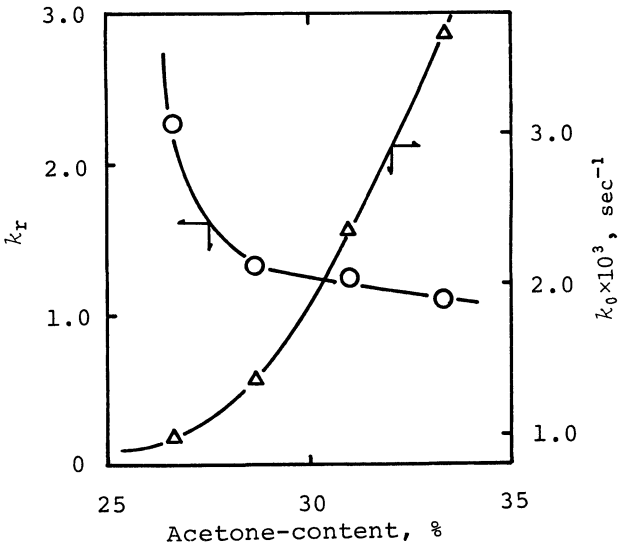


Fig. 1. Influences of the acetone-content on the *p*-nitrophenol release from PNPL.

the copper(II) ion can be given schematically in Scheme 1. From the fact that the solubility of Oxime-I in water increased in the presence of the copper(II) ion, the complex formed between the oxime and the metal ion seems to be a 1:1 charged complex.

Driving Force for the Incorporation of the Substrate. Under the present reaction conditions, the rate of the ester decomposition was largely affected by the medium. The content of organic solvent may be reflected on the dielectric nature of the medium, and consequently on the hydrophobic conformation of the substrate. Fig. 1 shows the effect of the acetone-content on the release of *p*-nitrophenol from PNPL. The decrease of the rate of hydrolysis without added catalyst at lower acetone concentration must be again due to the self-aggregation of the substrate molecules.[7] On the other hand, the fact that the k_r-value increased sharply below 27 % of the acetone concentration, suggests that the hydrophobic interaction of PNPL with Oxime-I is the major driving force for the incorporation of the substrate.

Substrate Specificity. Table 1 shows that the presence of Oxime-I resulted in the acceleration of the release of *p*-nitrophenol from PNPL, while retarded the rate of phenol-release from PNPD and hardly affected that from PNPH. This is predictive of the presence of the substrate specificity for Oxime-I. Thus, our present results are apparently different from those obtained by Hershfield and Bender,[4] in which the rate acceleration was gained only with the extension of alkyl-chain length and no substrate specificity was observed.

REFERENCES AND NOTES

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3) For example, J. C. Sheehan, G. B. Bennett, and J. A. Schneider, *ibid.*, 88 3455 (1966).

4) R. Hershfield and M. L. Bender, *ibid.*, 94, 1376 (1972).

5) Oxime-I: mp 91.5-93.0°C. Mass spectrum *m/e*: 401 (M^+). Found: C, 77.70; H, 11.28; N, 3.20%. Calcd for $C_{26}H_{43}O_2N$: C, 77.73; H, 10.81; N, 3.49%. IR (KBr disk): 3280 (ν_{O-H}), 1650 ($\nu_{C=N}$), and 942 cm^{-1} (ν_{N-O}).

Oxime-II: mp 101-103°C. Mass spectrum *m/e*: 185 (M^+). Found: C, 64.92; H, 10.58; N, 7.30%. Calcd for $C_{10}H_{19}O_2N$: C, 64.81; H, 10.36; N, 7.56%. IR (KBr disk): 3220 (ν_{O-H}), 1650 ($\nu_{C=N}$), and 956 cm^{-1} (ν_{N-O}).

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