Macrocyclic Enzyme Model Systems. Unusual Nucleophilic Reactivity of Un-ionized [20]Paracyclophane Oximes provided by Hydrophobic Effects

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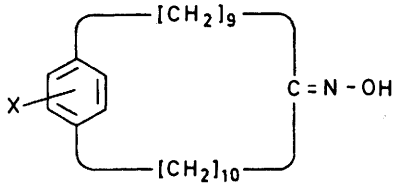
p-Nitrophenyl decanoate and palmitate were deacylated in a neutral to acidic pH region by the nucleophilic and hydrophobic catalysis of nearly equimolar 10-hydroxyimino[20]paracyclophane (1) and 10(11)-hydroxyimino-[20]paracyclophane-22-carboxylic acid (2) in aqueous dioxan or aqueous methanol. The direct nucleophilic participation of an un-ionized hydroxyimino-group was confirmed by the product analysis. The unusual nucleophilic reactivity of (1) and (2) was consistent with the following three-step mechanism: (i) pre-equilibrium complex formation between the acyl substrate and the cyclic oxime; (ii) nucleophilic attack of the neutral hydroxyimino-group of the cyclic oxime on the substrate carbonyl as effected by a proximity effect; and (iii) abstraction of the hydroxyimino-proton by the structured water molecules around the inclusion complex. The last step was rate determining on the basis of an appreciable deuterium solvent isotope effect (k_{H_2O}/k_{D_2O} 4.5—3.0). {10(11)-Hydroxyimino[20]paracyclophan-22-ylmethyl}trimethylammonium chloride did not show any remarkable kinetic effect in deacylation due to the structure breaking ability of the positively charged ammonium group.

It is remarkable to note that an acyl acceptor in many hydrolytic enzymes is considered to be a weak nucleophile unless it is converted to the corresponding deprotonated form by an appropriate mechanism. The hydroxy-group of a serine residue placed in an active site of various serine esterases may be cited as the best example. The activation mechanism for these particular groups of poor reactivity in enzymatic processes has been the subject of many investigations and a few attractive postulates such as charge relay mechanism [1]

[1] P. B. Sigler, D. M. Blow, B. W. Matthews, and R. Henderson, *J. Mol. Biol.*, 1968, **35**, 143.

and orbital steering theory [2] have been advanced. Thus, it is quite interesting and necessary to constitute an adequate model system in which some activation mechanism is operative so that a particular functional group is to be converted to an effective nucleophile. Such model systems would provide means of clarifying the activation process which takes place in real enzymatic reactions.

Recently, we have been studying the catalytic effects of [20]paracyclophane oximes (1)—(3) on the deacylation of *p*-nitrophenyl carboxylates bearing a long alkyl chain.[3-8] The reaction kinetics in alkaline solutions has been consistent with the Michaelis–Menten type mechanism: a pre-equilibrium complexation of the substrate with the cyclic oxime is followed by a pseudo-intramolecular acyl transfer to give the acylated paracyclophane derivative. In these reaction conditions, the incorporated substrate underwent acyl transfer effectively to the anionic hydroxyimino-group of [20]paracyclophanes and the catalytic reactivity of these macrocycles



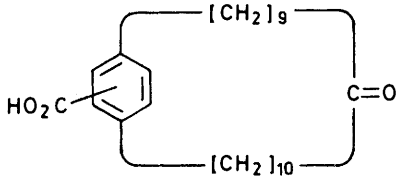
(1) X = H
(2) X = CO_2H
(3) X = $CH_2\overset{+}{N}(CH_3)_3$ Cl^-

was much dependent on the nature of substituents placed in the benzene ring.[8]

Along the line of complete characterization of these [20]paracyclophane oximes, we have observed in the present work the unusual reactivity of the macrocyclic oximes even in a relatively low pH region where the oxime group is completely un-ionized. That is, *p*-nitrophenyl decanoate (PNPD) and palmitate (PNPP) were deacylated effectively at pH 4—7 either in aqueous dioxan or in aqueous methanol by an equimolar amount of un-ionized [20]paracyclophane oximes: 10-hydroxyimino-[20]paracyclophane (1) and 10(11)-hydroxyimino[20]-paracyclophane-22-carboxylic acid (2).

EXPERIMENTAL

Materials.—10-Hydroxyimino[20]paracyclophane (1), 10(11)-hydroxyimino[20]paracyclophane-22-carboxylic acid (2), (10(11)-hydroxyimino[20]paracyclophan-22-ylmethyl)-trimethylammonium chloride (3), and 10(11)-oxo[20]paracyclophane-22-carboxylic acid (4) were prepared as described previously.[7,8] *p*-Nitrophenyl esters of aliphatic carboxylic acids were prepared by condensation of the corresponding acid chlorides with *p*-nitrophenol. The crude esters were further purified and identified by elemental analyses and spectral measurements before use. Deuterium oxide



(4)

(>99.7%) was the product of Carl Roth OHG, Karlsruhe, West Germany.

pH(pD) Measurements.—A Beckmann Expandomatic SS-2 pH meter equipped with a Metrohm EA-125 combined electrode was used to measure pH values of reaction media. pH Values were read on the pH meter which was calibrated by using a combination of appropriate aqueous standard buffers. The pH meter readings for deuterium oxide solutions, which were adjusted with acetate–acetic acid (undeuteriated) buffer system, were converted to pD values with the aid of equation (1).

$$pD = pH + 0.4 \quad (1)$$

Kinetic Measurements.—The rates of *p*-nitrophenol liberation from *p*-nitrophenyl acetate (PNPA), hexanoate (PNPH), and decanoate (PNPD) were measured spectrophotometrically at 320 nm. Each kinetic run was initiated by adding a stock solution (30 μl) of an ester substrate dissolved in dioxan (1.00×10^{-3}M) to a reaction medium (3.0 ml) pre-equilibrated at 40.0 ± 0.1° in a thermostatted cell set in a Hitachi 124 recording spectrophotometer. The reaction medium was prepared by mixing a stock solution (30 μl; in dioxan) of a paracyclophane oxime with an aqueous buffer solution (CH_3CO_2H–CH_3CO_2Na) (2.7 ml) and dioxan (0.27 ml). The ionic strength of each reaction mixture was maintained at 0.20 with KCl. The pseudo-first-order rate constants of *p*-nitrophenol liberation (k_{obs}) were determined according to equation (2), where A_i and A_t are the absorbances (320 nm) at time 0 and t respectively, while A_∞ is the infinity absorbance (320 nm).

$$\log \frac{A_\infty - A_i}{A_\infty - A_t} = \frac{k_{obs}}{2.303} \cdot t \quad (2)$$

The A_∞ value was determined for a solution prepared as follows. A stock solution of an ester substrate (30 μl) was added to a mixture of phosphate buffer (1.7 ml; pH 11.0) and dioxan (0.3 ml), and the ester underwent complete hydrolysis. Hydrochloric acid (1.0 ml, 0.1M) was added to acidify the solution (pH 5.5) which was subjected to spectroscopic measurement (320 nm) at 40.0°. The A_∞ value thus obtained was confirmed to be identical within 3% with that evaluated independently under kinetic conditions. This result indicates that the absorbance increase at 320 nm in kinetic runs can be referred exactly to the amount of *p*-nitrophenol liberated. The presence of

[2] D. R. Storm and D. E. Koshland, jun., *J. Amer. Chem. Soc.*, 1972, **94**, 5805, 5815.
[3] Y. Murakami, J. Sunamoto, and K. Kano, *Chem. Letters*, 1973, 223.
[4] Y. Murakami, J. Sunamoto, and K. Kano, *Bull. Chem. Soc. Japan*, 1974, **47**, 1238.
[5] Y. Murakami, J. Sunamoto, H. Okamoto, and K. Kawanami, *Bull. Chem. Soc. Japan*, 1975, **48**, 1537.
[6] J. Sunamoto, H. Okamoto, H. Kondo, and Y. Murakami, *Tetrahedron Letters*, 1975, 2761.
[7] Y. Murakami, Y. Aoyama, K. Ohno, K. Dobashi, T. Nakagawa, and J. Sunamoto, *J.C.S. Perkin I*, 1976, 1320.
[8] Y. Murakami, Y. Aoyama, and K. Dobashi, preceding paper.

an isosbestic point at 302 nm for all kinetic runs also supports the conclusion that the liberation of *p*-nitrophenol is accompanied only by the corresponding acyl transfer without any other side reactions.

In order to increase the analytical reliability, the following alternative analytical method has been adopted for measurements of the *p*-nitrophenol liberation from *p*-nitrophenyl palmitate (PNPP) in neutral and acidic media [pH <7 for (1) and pH $\lesssim 8$ for (2)] in the presence of either (1) or (2). In a reaction vessel (100 ml) was placed a buffer solution (27 ml), dioxan (or methanol) (2.7 ml), and a stock solution of (1) (0.3 ml) in dioxan [or (2) either in dioxan or methanol]. The mixture was maintained at 40.0 ± 0.1° by the external jacket through which thermostatted water was circulated. The reaction was initiated by adding 1.00×10^{-2}M-PNPP (30 μl) in acetone. Samples were withdrawn from the mixture at appropriate intervals (2—5 min initially and 10—15 min in the later stages). The pH of each sample was adjusted to 9.5—10.5 by the addition of an aqueous sodium hydroxide. The amounts of *p*-nitrophenoxide ion in the resulting alkaline solutions were rapidly determined spectrophotometrically at 400 nm. The analytical reliability for this procedure was confirmed by independent measurements. This method is applicable only for the PNPP system since the other substrates of the present study undergo significant hydrolysis under the analytical conditions. The rates of *p*-nitrophenol liberation from PNPP in neutral and alkaline media were evaluated by following directly the absorbance change at 400 nm, whereas the liberation rates in the presence of (3) in acidic media (pH 5.5 and 4.0) were measured by following the absorbance change at 320 nm.

All the pseudo-first-order rate constants were obtained from the early stage of reactions.

Product Analysis.—A mixture of (1) (50 mg) and PNPD (100 mg) in an aqueous acetate buffer (800 ml, 0.04M; pH 5.4) containing KCl (13 g) and dioxan (200 ml) was stirred at 20° for 40 h to complete the acyl transfer reaction. The mixture was then extracted with ether (5 × 150 ml). The ether extract was washed sufficiently with aqueous sodium hydroxide (5%; 7 × 100 ml) to remove *p*-nitrophenol, dried over sodium sulphate, and evaporated *in vacuo*. The chromatographic separation of the residue on a column (2.4 × 10 cm) of silica gel (Wako gel C-100) with benzene as eluant afforded unchanged PNPD (35 mg), the acylated oxime, *i.e.* 10-decanoyloxyimino[20]paracyclophane (5) (20 mg), and a mixture of both components (*ca.* 40 mg). Compound (5) had $\nu_{max.}$ (neat) 1 764 cm^{-1} (C=O stretch of an acylated oxime [9]), δ (CCl_4; tetramethylsilane as internal reference) 6.98 (4 H, s, aromatic), 2.57 (4 H, t, benzyl methylene), 2.4—1.95 (6 H, m, methylene adjacent to C=NOR and methylene adjacent to C=O), and 1.95—0.8 (47 H, m).

TABLE 1

Second-order rate constants for the deacylation of *p*-nitrophenyl carboxylates in the presence and absence of cyclic oximes: 40.0 ± 0.1°, and μ = 0.20 (KCl) in acetate buffer

Catalyst	Conc. (M)	Substrate [a]	Medium	pH(pD)	Buffer conc. (M)	k/l mol^{-1} s^{-1} [b]
(1)	0.834×10^{-5}	PNPP	*c*	3.98	0.04	44
(1)	0.834×10^{-5}	PNPP	*c*	4.03	0.006	36
(1)	0.834×10^{-5}	PNPP	*c*	5.47	0.04	37
(1)	0.834×10^{-5}	PNPP	*c*	5.53	0.006	36
(1)	0.834×10^{-5}	PNPP	*c* (D_2O)	5.64	0.006	8.0
(1)	0.834×10^{-5}	PNPD	*d*	3.98	0.04	85
(1)	0.834×10^{-5}	PNPD	*d*	4.03	0.006	72
(1)	0.834×10^{-5}	PNPD	*d*	4.70	0.04	70
(1)	0.834×10^{-5}	PNPD	*d*	4.74	0.006	68
(1)	0.834×10^{-5}	PNPD	*d*	5.47	0.04	56
(1)	0.834×10^{-5}	PNPH	*d*	5.53	0.006	11
(1)	0.834×10^{-5}	PNPA [e]	*d*	5.53	0.006	<4.5
(2)	0.879×10^{-5}	PNPP	*c*	4.03	0.006	33
(2)	0.879×10^{-5}	PNPP	*c*	5.53	0.006	31
(2)	0.879×10^{-5}	PNPP	*c* (D_2O)	5.64	0.006	6.2
(2)	0.879×10^{-5}	PNPD	*d*	3.98	0.04	81
(2)	0.879×10^{-5}	PNPD	*d*	4.03	0.006	67
(2)	0.879×10^{-5}	PNPD	*d*	5.47	0.04	71
(2)	0.879×10^{-5}	PNPD	*d*	5.53	0.006	64
(3)	8.52×10^{-5}	PNPP	*d*	5.53	0.006	<2.2
(4)	1.26×10^{-5}	PNPP	*c*	5.53	0.006	~0
None		PNPP	*c*	5.53	0.006	~0
None		PNPA	*d*	5.53	0.006	~0
Acetoxime	0.10	PNPA	*d*	5.53	0.006	~0

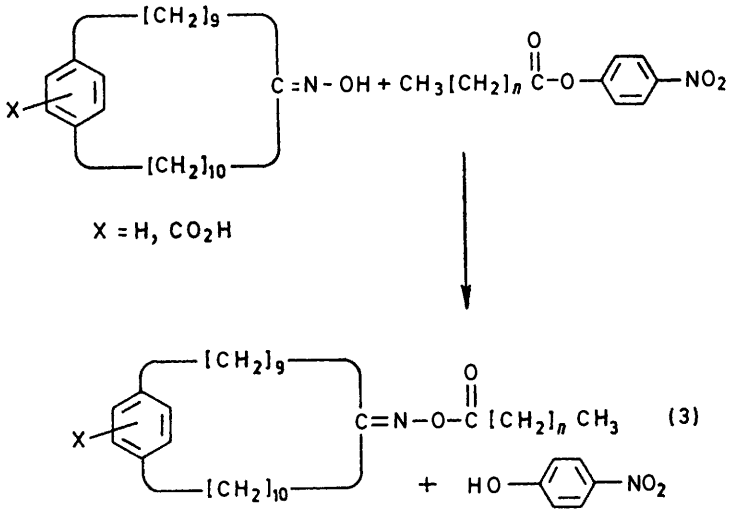
[a] Initial concentration, 1.00×10^{-5}M unless otherwise indicated. [b] $k = k_{obs}$/[Catalyst]. [c] 10% (v/v) Aqueous dioxan containing 0.1% (v/v) acetone. [d] 10.9% (v/v) Aqueous dioxan. [e] Initial concentration, 3.00×10^{-5}M.

RESULTS

p-Nitrophenyl carboxylates were deacylated in the presence of a nearly equimolar amount of either (1) or (2) under the following reaction conditions: 40.0 ± 0.1°, pH 4.0—5.5 (acetate buffer), and μ = 0.20 (KCl) in aqueous dioxan. The apparent second-order rate constants are listed in Table 1 and the reaction products were identified as *p*-nitrophenol and the acylated oxime (5) [equation (3)]. The deacylation reactivity of the carboxylic esters is much dependent on their alkyl-chain length and follows in the decreasing order: PNPD > PNPP > PNPH > PNPA. In the absence of (1) [or (2)], the *p*-nitrophenyl carboxylates proved to be inactive in the pH region investigated. A large excess of acetoxime (0.1M) failed to deacylate PNPA to any detectable extent under the same reaction con-

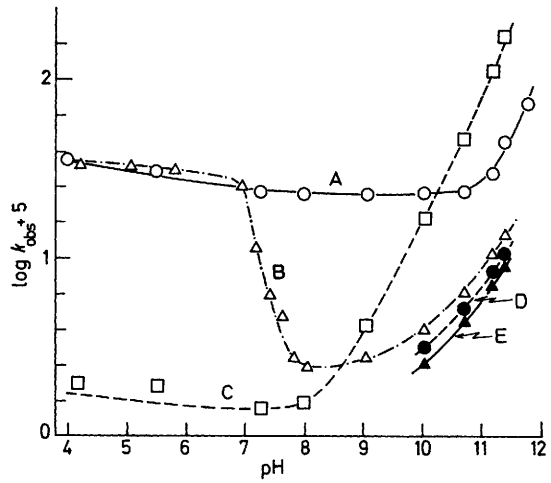
[9] Reported carbonyl frequencies: pentanone oxime acetate 1762 and benzophenone oxime acetate 1768 cm^{-1}, J. P. Freeman, *J. Amer. Chem. Soc.*, 1958, **80**, 5954; acetophenone oxime acetate 1763 and acetophenone oxime benzoate 1754 cm^{-1}, L. A. Cappino, C. A. Giza, and B. A. Cappino, *ibid.*, 1959, **81**, 955.

ditions. In the marked contrast to (1) and (2), (3) which bears a quaternary ammonium group on its benzene ring did not exhibit any remarkable reactivity toward PNPP in an acidic medium, although (3) catalysed the deacylation of the same substrate in a higher pH region [8] (Figure and Table 2). 10(11)-Oxo[20]paracyclophane-22-carboxylic acid (4), which is closely related to (2) from the structural viewpoint but lacks in a nucleophilic centre, also failed to deacylate PNPP. The catalysed deacylation of PNPP was subjected to an appreciable deuterium solvent isotope effect (Table 1). On the other hand, the changes



(5)

in pH of the medium and in concentration of the buffer species at constant ionic strength in the acidic region resulted in only minor effects on the deacylation rates



pH–Rate profiles for the deacylation of *p*-nitrophenyl palmitate in the presence and absence of various [20]paracyclophanes at 40.0 ± 0.1° and $\mu = 0.20$ (KCl): A, (1) (0.834×10^{-5}M); B, (2) (0.879×10^{-5}M); C, (3) (0.852×10^{-5}M); D, no catalyst; E, (4) (1.26×10^{-5}M); $[PNPP]_0$, 1.00×10^{-5}M. k_{obs} Values are in s^{-1}

(Table 1). The pH effects on the deacylation rate of PNPP as catalysed by various paracyclophanes were investigated extensively as shown in the Figure and Table 2. The kinetic pK_a value for (2) was graphically estimated as 7.1, which corresponds to the dissociation of the carboxy-proton.

DISCUSSION

Unusual Nucleophilic Reactivity of Un-ionized Paracyclophane Oximes.—The hydroxyimino-group of the paracyclophane macrocycles certainly provides an effective nucleophilic centre in the present deacylation reactions in reference to the following results: replacement of the hydroxyimino group in (2) by an oxo-group, which affords the keto-acid (4), resulted in the complete disappearance of reactivity toward PNPP, and (1) was acylated with PNPD at its hydroxyimino-group as the product analysis indicates. The latter result is the direct evidence for the effective nucleophilic catalysis by the hydroxyimino-group. The pK_a values of oximes generally fall in the neighbourhood of 12. The present cyclic oximes are not exceptions in this respect. The kinetically determined pK_a values for (1)—(3) are in a range of 11.2—11.7.[8] Thus, the hydroxyimino-group placed in the macrocyclic skeleton exists in a completely protonated form in the neutral to acidic pH range. Although the nucleophilicity of anionic oxygen groups is generally large, protonation acts to reduce their nucleophilic activity appreciably. A factor of 10^9 has been reported for the difference in nucleophilicity between water and hydroxide toward PNPA.[10] The situation may be similar for the hydroxyimino-nucleophile. The intrinsic nucleophilicity of an un-ionized hydroxyimino-group is so small that even a large excess of acetoxime failed to react with PNPA at a measurable rate, even though the bimolecular reaction between acetoxime and PNPA does not suffer from the steric

[10] J. O. Edwards and R. G. Pearson, *J. Amer. Chem. Soc.*, 1962, **84**, 16.

hindrance effect which would be expected in the deacylation reactions of carboxylic esters bearing a long alkyl chain with oximes.

In the alkaline region where deacylation of carboxylic esters takes place by nucleophilic attack of the anionic hydroxyimino-group of the paracyclophane macrocycles, (3) shows a profound rate enhancement. This effective catalysis was partly attributed to an electrostatic effect, which acts to stabilize a negative charge developed on the carbonyl oxygen of the ester substrate through tight ion-pair association with the positively charged ammonium group in the transition state.[8] The reactivity of (3) toward PNPP is markedly reduced in the neutral to acidic region in a manner similar to that of normal oximes.* On the other hand, the unfavourable electrostatic effect of the negatively charged carboxylate group of (2) reduced the nucleophilic reactivity of the anionic hydroxyimino-group in the alkaline region.[8] The pH–rate profile (Figure) indicates that (2) is reactivated in a lower pH region where its carboxylate group is completely protonated. The kinetic pK_a value of 7.1 must be referred to the dissociation of the carboxy-proton.† The unit slope observed for the log k_{obs}–pH correlation in the pH 7—8 range seems to be the evidence for this conclusion. The large pK_a value for the carboxy-group is presumably attributed to the hydrophobic field effect provided by the inclusion complex.

Among paracyclophane oximes (1)—(3), the catalytic reactivity towards carboxylic esters was observed to decrease in an alkaline region in the sequence: (3) ≫ (1) > (2).[8] On the other hand, (1) and (2) showed a comparable catalytic efficiency but (3) exhibited a markedly low reactivity in the neutral to acidic region.

* The reactivity of (3) is so small in the acidic region that the participation of the hydroxyimino-group is not expected. The decomposition of PNPP most likely proceeds through the normal hydrolytic pathway after incorporation into the cyclic cavity of (3).

It should be emphasized, therefore, that some novel reaction mechanism must take place in the lower pH region to afford the unusual nucleophilic reactivity of the neutral paracyclophane oximes (1) and (2), which is different from those predicted for the alkaline region.[8]

Reaction Mechanism.—As discussed in previous papers,[4,5,8] the acylation of a paracyclophane oxime takes place by a two-step mechanism: the pre-equilibrium formation of an inclusion complex between an acyl substrate and a cyclic oxime is effected by hydrophobic interaction and subsequent intracomplex acyl transfer occurs from the bound substrate to the hydroxyimino-group of a paracyclophane. The full reaction scheme for acylation of the present cyclic oximes in the neutral to acidic region is shown in equation (4) where

$$S + \text{Oxime} \overset{K_b}{\rightleftharpoons} [S\text{–Oxime}] \xrightarrow{k_{acyl}} \text{Acyloxime} + \text{Phenol} \quad (4)$$

S and Oxime stand for an acyl substrate and a paracyclophane oxime, respectively. Because of the limited solubility of the cyclic oximes under our reaction conditions, the complete kinetic measurements for the evaluation of binding constant (K_b) and acyl transfer rate constant (k_{acyl}) were not performed in the present study. Nevertheless, the kinetic reactivity of the *p*-nitrophenyl esters of carboxylic acids with the paracyclo-

TABLE 2

Pseudo-first-order rate constants for the *p*-nitrophenol release from PNPP in the presence and absence of paracyclophanes: 40.0 ± 0.1°, μ = 0.20 (KCl), $[PNPP]_0 = 1.00 \times 10^{-5}$M [a]

(1) (0.834×10^{-5}M) [b]		(2) (0.879×10^{-5}M) [c]		(3) (0.852×10^{-5}M) [d]		(4) (1.26×10^{-5}M) [d]		None [d]	
pH	k_{obs}/s^{-1}	pH	k_{obs}/s^{-1}	pH	k_{obs}/s^{-1}	pH	k_{obs}/s^{-1}	pH	k_{obs}/s^{-1}
11.75	7.3×10^{-4}	11.37	1.3×10^{-4}	11.37	1.7×10^{-3}	11.37	8.9×10^{-5}	11.37	1.1×10^{-4}
11.37	4.5×10^{-4}	11.17	1.1×10^{-4}	11.17	1.1×10^{-3}	11.17	7.0×10^{-5}	11.17	8.4×10^{-5}
11.17	3.0×10^{-4}	10.69	6.4×10^{-5}	10 69	4.6×10^{-4}	10.69	4.5×10^{-5}	10.69	5.2×10^{-5}
10.69	2.3×10^{-4}	10.02	4.0×10^{-5}	10.02	1.7×10^{-4}	10.02	2.6×10^{-5}	10.02	3.2×10^{-5}
10.02	2.3×10^{-4}	9.03	2.8×10^{-5}	9.03	4.2×10^{-5}				
9.03	2.2×10^{-4}	8.03	2.5×10^{-5}	7.98	1.5×10^{-5} [e]				
7.98	2.2×10^{-4}	7.81	2.8×10^{-5}	7.26	*ca.* 1.4×10^{-5} [e]				
7.26	2.3×10^{-4}	7.62	4.8×10^{-5}	5.47	$<1.9 \times 10^{-5}$ [e,f]				
5.47	3.1×10^{-4}	7.40	6.2×10^{-5}	3.98	$<2.0 \times 10^{-5}$ [e,f]				
3.98	3.7×10^{-4}	7.17	1.1×10^{-4}						
		6.95	2.6×10^{-4}						
		5.85	3.2×10^{-4}						
		5.07	3.3×10^{-4}						
		4.18	3.3×10^{-4}						

[a] pH, buffer species: 11.75, NaOH; 11.4—11.2, phosphate–NaOH; 10.7—10.0, borate–carbonate; 9.05—5.9, borate–phosphate; 5.5, 4.2, and 4.0, acetate; 5.1, borate–succinate. [b] In 10% (v/v) aqueous dioxan containing 0.1% (v/v) acetone (pH < 6) or 10.9% (v/v) aqueous dioxan (pH > 7). [c] In 10.9% (v/v) aqueous dioxan (pH ≥ 9.03) or 10% (v/v) aqueous methanol containing 0.1% (v/v) acetone (pH ≤ 8.03). [d] In 10.9% (v/v) aqueous dioxan. [e] The first-order rate constant in the presence of an excess amount of (3) (8.52×10^{-5}M) was divided by 10 to obtain the value. [f] Measured by following the absorbance change at 320 nm. Uncertainties are due to the low molar extinction coefficient of *p*-nitrophenol (320 nm) compared with that of *p*-nitrophenoxide (400 nm).

† *Note added in proof:* The kinetic pK_a value obtained for the carboxy-group of (2) is significantly large relative to those for the ordinary carboxylic acids. It would be desirable to determine the thermodynamic pK_a value independently. However, the solubility of (2) in acidic media is too low (*ca.* 1×10^{-5}M) to allow a reliable determination of such pK_a values by conventional methods. The solubility, which is *ca.* 1×10^{-3}M in the alkaline region, sharply decreases below pH 8 until it reaches a minimal value of *ca.* 1×10^{-5}M at pH 6. No further depression of the solubility is observed below this pH. This solubility behaviour seems to indicate qualitatively that the pK_a value of the carboxy-group is about 7 in agreement with the kinetically determined value.

phane oximes is very much dependent on their alkyl-chain lengths as shown in Table 1. Judging from the fact that the deacylation of PNPA is much less affected by the cyclic oximes, the hydrophobic affinity of the substrates to the cyclic oximes is undoubtedly the major factor determining the kinetic reactivity.

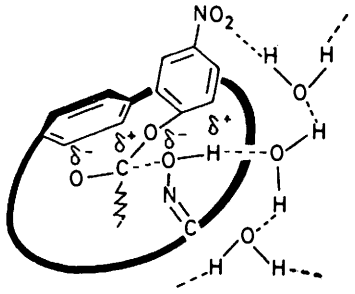
The plausible origin of the unusual nucleophilic reactivity of (1) and (2) in their neutral forms may be a favourable spatial orientation of the substrate carbonyl and the hydroxyimino-group of the macrocycles in the inclusion state (proximity effect). However, the proximity effect would not be the sole factor for the enhanced reactivity. If it were the case, we should observe much less reactivity of (1) and (2) due to the poor nucleophilicity of the neutral hydroxyimino-group. Furthermore, this effect alone does not explain why only (1) and (2), and not (3), are unusually reactive. The participation of concerted specific acid catalysis as well as general acid or base catalysis by buffer species is ruled out in reference to the kinetic data shown in Table 1. The deacylation rates of PNPP and PNPD in the presence of either (1) or (2) show a general tendency to increase with increasing hydrogen ion and buffer concentrations. Nevertheless, the extents of rate enhancements are only 1.5-fold for the 40-fold change in hydrogen ion concentration and 1.2-fold for the seven-fold change in buffer concentration. Thus, the novel acylation of (1) and (2) does not seem to be explained reasonably by any familiar concepts. The primary factor which differentiates the reaction behaviour of (1) and (2) from that of normal oximes such as acetoxime is the hydrophobic field effect provided by the former species. The special feature of water molecules surrounding the hydrophobic paracyclophane skeleton seems to facilitate unusually the nucleophilic attack of the neutral hydroxyimino-group on the incorporated substrate. On the basis of the concept of hydrophobic interaction, water molecules in the neighbourhood of a non-polar species would be highly structured.[11,12] This phenomenon is often referred to as ' iceberg ' formation. Hertz and Zeidler [13] have obtained an experimental evidence for ' iceberg ' formation from the rates of water reorganization in aqueous solutions of tetra-alkyl-ammonium salts. It is reasonable to expect that such is the case for the present systems of paracyclophane oximes. Nucleophilic attack of a neutral nucleophile on the carbonyl group of an acyl substrate in aqueous media develops a dipolar transition state which should be highly hydrated as reflected on a large negative entropy of activation.[14,15] Since the hydrophobic inclusion complex formed between an acyl substrate bearing a long alkyl chain and a paracyclophane oxime would be surrounded by highly structured water molecules, further structure making by water should not be required to any noticeable extent in the dipolar transition state of acyl transfer.[16] In other words, the stabilization (salting-in) of the transition state would be more readily achieved in a solvent of greater structure where the reorganization of water molecules is least needed. The salting-in effects by structure making additives have also been observed for various dipolar species.[17] Consequently, the enhanced reactivity of the hydroxyimino-group of (1) and (2) seems to be achieved partly by an entropy effect.

The flickering cluster model of liquid water is provided by the extensive hydrogen bonding interaction. According to the view put forward by Frank and Wen,[12] water molecules become ordered around the non-polar organic molecule with an increase in hydrogen bonding in this region. They also hypothesized that the hydrogen bonding interaction enhances both acidity and basicity of water molecule. This view was supported by *ab initio* MO–SCF calculations on linear water trimer.[18] Furthermore, the enhanced basicity of structured water molecules has been confirmed by an n.m.r. study for the chloroform–water system [19] and by kinetic investigation of water-catalysed detritiation of malononitriles.[16] On the basis of these views, the water molecules around the present hydrophobic inclusion complexes must be highly structured and act as favourable proton acceptors due to the enhanced basicity. The direct involvement of water molecules in the transition state of acyl transfer is confirmed by the appreciable solvent isotope effects (Table 1). The k_{H_2O}/k_{D_2O} values observed for reactions of PNPP with (1) and (2) are in the range 4.5—5.0. These values are even larger than those reported for some water-catalysed hydrolyses of reactive esters: acetic anhydride (2.9),[20] *p*-nitrophenyl dichloroacetate (3.15),[15] *p*-methoxyphenyl dichloroacetate (3.24),[15] and bis-*p*-nitrophenyl carbonate (2.88).[14]

In conclusion, the present acyl transfer reaction proceeds most plausibly by a three-step mechanism: (i) the pre-equilibrium complex formation takes place between an acyl substrate bearing a long alkyl chain and a paracyclophane oxime (1) or (2); (ii) the bound substrate is subjected to nucleophilic attack by the neutral hydroxyimino-group of a paracyclophane oxime under the influence of the proximity effect, and consequently an active tetrahedral intermediate (6), which bears a resemblance to the ground state structure, is formed; and (iii) the hydroxyimino-proton is abstracted by the structured water molecules around the inclusion complex, as the rate-determining step.

[11] W. P. Jencks, ' Catalysis in Chemistry and Enzymology,' McGraw-Hill, New York, 1969, ch. 8.
[12] H. S. Frank and W.-Y. Wen, *Discuss. Faraday Soc.*, 1957, **24**, 133.
[13] H. G. Hertz and M. D. Zeidler, *Ber. Bunsengesellschaft Phys. Chem.*, 1964, **68**, 821.
[14] T. H. Fife and D. M. McMahon, *J. Amer. Chem. Soc.*, 1969, **91**, 7481.
[15] J. F. J. Engbersen and J. B. F. N. Engberts, *J. Amer. Chem. Soc.*, 1975, **97**, 1563.
[16] A similar explanation has been advanced: F. Hibbert and F. A. Long, *J. Amer. Chem. Soc.*, 1972, **94**, 7637.
[17] S. Davidson and W. P. Jencks, *J. Amer. Chem. Soc.*, 1969, **91**, 225.
[18] D. Hankins, J. W. Moskowitz, and F. H. Stillinger, *J. Chem. Phys.*, 1970, **53**, 4544.
[19] J. E. Gordon, *J. Amer. Chem. Soc.*, 1972, **94**, 650.
[20] A. R. Butler and V. Gold, *J. Chem. Soc.*, 1961, 2305.

The much lower reactivity of (3) relative to (1) and (2) must be due to the presence of the positively charged ammonium group on its benzene ring. Since the structure-breaking ability of this group must disrupt the hydrogen bonded structure of water molecules surrounding the hydrophobic paracyclophane skeleton, novel water catalysis may not be expected in the acyl transfer reaction. On the other hand, the enhanced reactivity of (2) observed for the pH region lower than its kinetic pK_a (7.1) is gained by the protonation of the carboxylate group, so that the effective hydrophobicity of the catalyst is attained.



(6)

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