Macrocyclic Enzyme Model Systems. Concurrent Nucleophilic–Electrostatic Bifunctional Catalysis by [20]Paracyclophanes in Deacylation of *p*-Nitrophenyl Carboxylates

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Deacylation of *p*-nitrophenyl laurate (PNPL) and palmitate (PNPP) was accelerated in alkaline solution by the presence of a 10-hydroxyimino[20]paracyclophane derivative. A [20]paracyclophane which bears a quaternary ammonium group on the benzene ring and a nucleophilic hydroxyimino-group on the polymethylene bridge (2a) greatly enhanced the reaction rate due to an electrostatic effect provided by the positively charged substituent, whereas a 10-hydroxyimino[20]paracyclophane derivative bearing a carboxylate group on the benzene group (2c) enhanced the rate only slightly and the parent oxime (2b) showed the intermediate catalytic activity. The kinetics of deacylation are consistent with a reaction mechanism which involves pre-equilibrium complex formation between an ester substrate and a paracyclophane oxime, followed by pseudo-intramolecular acyl transfer from the incorporated substrate to the deprotonated hydroxyimino-group of a paracyclophane macrocycle. The paracyclophane oximes exhibited comparatively large binding constants (10^3—10^5 l mol^{-1}) in the decreasing sequence (2b) > (2c) > (2a). The reactivity of the subsequent intracomplex acyl transfer was characterized by the nature of an electric charge on the benzene group and decreases in the sequence (2a) ≫ (2b) > (2c). The apparent second-order rate constant for acylation of (2a) with PNPL exceeds that for the reaction of chymotrypsin with *p*-nitrophenyl acetate. The ammonium salt (2a) is thus claimed as a novel enzyme model which exhibits both favourable hydrophobic binding ability towards an appropriate ester substrate and nucleophilic–electrostatic bifunctional catalysis giving rise to significant rate acceleration in its decomposition.

VARIOUS investigations have been carried out in recent years on synthetic catalysts which show enzyme-like behaviour.[1] Micellar surfactants and water-soluble polymers bearing various functional groups have shown phenomenological similarities with enzyme systems.[2] However, these systems suffer from intrinsic deficiencies as enzyme models primarily due to their dynamic nature in forming catalytic fields.[2] Macrocylic systems are expected to have some potentiality as superior enzyme models for the following reasons. (i) The macrocyclic cavity provides a stable binding site which is little affected by external medium factors such as pH, temperature, and ionic strength. (ii) The stable binding site shows a high substrate specificity due to its intrinsic geometrical requirements for host–guest interactions. (iii) Introduction of two or more catalytically active groups into the macrocyclic skeleton at a fixed, favourable spatial orientation provides an effective reaction site of cooperative polyfunctional nature. The most thoroughly investigated macrocycles recently reported are cycloamyloses [1c] although their hydrophobic binding ability is not sufficient for them to serve as enzyme models. The catalytic functions of cyclic peptides [3] and cyclic hydroxamic acid [4] can be noted as other examples.

We have recently prepared a sizable cyclic compound, 10-hydroxy-11-hydroxyimino[20]paracyclophane (1a).[5]

[1] (*a*) T. C. Bruice and S. J. Benkovic, 'Bioorganic Mechanisms,' Benjamin, New York, 1966; (*b*) W. P. Jencks, 'Catalysis in Chemistry and Enzymology,' McGraw-Hill, New York, 1969; (*c*) M. L. Bender, 'Mechanisms of Homogeneous Catalysis from Protons to Proteins,' Wiley–Interscience, New York, 1971.

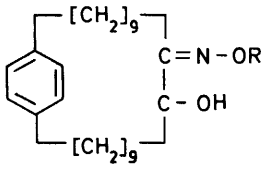
[2] (*a*) E. Cordes, 'Reaction Kinetics in Micelles,' Plenum Press, New York, 1973; (*b*) J. H. Fendler and E. J. Fendler, 'Catalysis in Micellar and Macromolecular Systems,' Academic Press. New York, 1975.

[3] (*a*) J. C. Sheehan, G. B. Bennett, and J. A. Schneider, *J. Amer. Chem. Soc.*, 1966, **88**, 3455; (*b*) K. Nakajima and K. Okawa, *Bull. Chem. Soc. Japan*, 1973, **46**, 1811; (*c*) Y. Murakami, J. Sunamoto, K. Nishida, and A. Nakano, unpublished result.

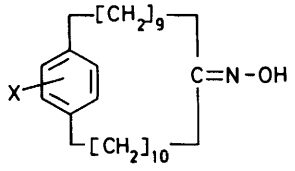
[4] R. Hershfield and M. L. Bender, *J. Amer. Chem. Soc.*, 1972, **94**, 1376.

[5] (*a*) Y. Murakami, J. Sunamoto, and K. Kano, *Chem. Letters*, 1973, 223; (*b*) Y. Murakami, J. Sunamoto, and K. Kano, *Bull. Chem. Soc. Japan*, 1974, **47**, 1238; (*c*) Y. Murakami, J. Sunamoto, H. Okamoto, and K. Kawanami, *ibid.*, 1975, **48**, 1537; (*d*) J. Sunamoto, H. Okamoto, H. Kondo, and Y. Murakami, *Tetrahedron Letters*, 1975, 2761.

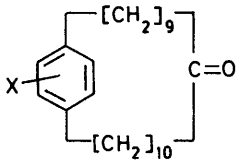
The cyclic oxime (1a) has been designed to give both a hydrophobic binding site and a nucleophilic centre on the same macrocyclic skeleton. In the presence of (1a), the deacylation of *p*-nitrophenyl carboxylates with a long alkyl chain was significantly accelerated. The reaction kinetics were consistent with a Michaelis–Menten-type mechanism, pre-equilibrium complexation of the substrate with (1a), followed by pseudo-intramolecular acyl transfer to give the acylated paracyclophane derivative (1b). For further characterization of the intrinsic structural features of this unique macrocyclic system, a number of [20]paracyclophane derivatives have been prepared and investigated from the structural viewpoint.[6]



(1) a ; R = H
b ; R = $CO[CH_2]_8CH_3$



(2) a ; X = $CH_2\overset{+}{N}(CH_3)_3Cl^-$
b ; X = H
c ; X = CO_2H



(3) a ; X = H
b ; X = CH_2Cl
c ; X = $CH_2\overset{+}{N}(CH_3)_3Cl^-$

In order to develop [20]paracyclophanes into bifunctional catalysts, we have prepared {10(11)-hydroxyimino-[20]paracyclophan-22-ylmethyl}trimethylammonium chloride (2a) and have investigated the catalytic efficiencies of (2a), 10-hydroxyimino[20]paracyclophane (2b), and of 10(11)-hydroxyimino[20]paracyclophane-22-carboxylic acid (2c). The favourable enzyme-like behaviour of (2a) is discussed in terms of nucleophilic–electrostatic catalysis.

EXPERIMENTAL

I.r. spectra were recorded on a JASCO DS-403G grating spectrophotometer. ^1H N.m.r. spectra were obtained with either a Varian A-60 or a Bruker WH-90 Fourier transform spectrometer with tetramethylsilane as internal standard. High speed liquid chromatography was performed on a Hitachi 635 liquid chromatograph by employing Hitachi gel 3010 and 3019 for component analysis and preparative purposes, respectively. Methanol was used as eluant and the eluting components were detected by u.v. absorption at 254 nm.

Materials.— {10(11)-*Hydroxyimino*[20]*paracyclophan*-22-*ylmethyl*}*trimethylammonium chloride* (2a) was prepared by a three-step procedure from [20]paracyclophan-10-one (3a).[6] Into a solution of (3a) (1.5 g) and chloromethyl methyl ether (4.0 g) in dichloromethane (100 ml) was added dropwise with stirring at 5° over 1 h a dichloromethane solution (30 ml) of anhydrous stannic chloride (4.0 g). The mixture was stirred for an additional 1 h and then poured into ice–water (200 g), and the aqueous layer was extracted with dichloromethane (3 × 50 ml). The extract and the organic layer were combined and washed with water (7 × 50 ml), dried ($MgSO_4$), and evaporated to give 22(23)-*chloromethyl*[20]-*paracyclophan*-10-*one* (3b) as an oil (1.2 g, 53%), $\nu_{max.}$ (neat) 1 718 (C=O str.), 1 260 (CH bend. in CH_2Cl), and 737 cm^{-1} (C–Cl str. in CH_2Cl); δ(CCl_4) 6.98 (3 H, distorted s, aromatic), 4.53 (2 H, s, CH_2Cl), 2.63 (4 H, m, benzyl methylene), 2.25 (4 H, m, methylene adjacent to C=O), and 1.20 (30 H, m, methylene).

Gaseous trimethylamine, which was generated by the addition of aqueous trimethylamine (40%; 10 g) to solid sodium hydroxide (50 g), was dried over soda lime and introduced over 1.5 h into a benzene solution (30 ml) of (3b) (180 mg) with stirring at room temperature. The mixture was stirred for 2 h, and left overnight at room temperature. The solvent and excess of trimethylamine were removed *in vacuo* and the residue was purified by preparative liquid chromatography to give {10(11)-*oxo*[20]-*paracyclophan*-22-*ylmethyl*}*trimethylammonium chloride* (3c) as a glassy, hygroscopic material, $\nu_{max.}$ (neat) 1 718 cm^{-1} (C=O str.); δ([$^2H_6$]DMSO) 7.7—7.2 (3 H, m, aromatic), 4.65br (2 H, s, CH_2Cl), 3.07 (9 H, s, CH_3), 2.9—2.1 (benzyl methylene and methylene adjacent to C=O), and 1.11 (30 H, m, methylene) (Found: C, 73.25; H, 10.9; N, 2.45. $C_{30}H_{52}ClNO$ requires C, 75.35; H, 10.95; N, 2.95. $C_{30}H_{52}ClNO,H_2O$ requires C, 72.6; H, 10.55; N, 2.8%).

A mixture of (3c) (200 mg), hydroxylamine hydrochloride (200 mg), and powdered potassium hydroxide (400 mg) in methanol (40 ml) was stirred under reflux for 1 h. The theoretical amount of hydrogen chloride in methanol was then added to neutralise the solution. After evaporation, the residue was poured into saturated aqueous sodium chloride (300 ml). An oily material was collected, dried at 80° *in vacuo*, and re-dissolved in dichloromethane. Upon evaporation of the solvent, crude (2a) was obtained as a hygroscopic glass free of sodium chloride. A pure sample of (2a) was obtained as the monohydrate by preparative liquid chromatography, $\nu_{max.}$ ($CHCl_3$) 3 663 and 3 600 (OH str. of H_2O)[7] and 3 270 cm^{-1} (OH str. of hydroxyimino); δ ($CDCl_3$) 7.25 (3 H, distorted s, aromatic), 4.78 [2 H. s, $(CH_3)_3N^+CH_2$], 3.36 [9 H, s, $(CH_3)_3N^+$], 2.70 (4 H, m, benzyl methylene), 2.20 (4 H, m, methylene adjacent to C=NOH), and 1.23 (30 H, m, methylene) (Found: C, 70.2; H, 10.65; N, 5.65. $C_{30}H_{53}ClN_2O,H_2O$ requires C, 70.5; H, 10.85; N, 5.5%).

10-*Hydroxyimino*[20]*paracyclophane* (2b) was prepared by oximation of (3a)[6] (Found: C, 80.65; H, 11.2; N, 3.45. $C_{26}H_{43}NO$ requires C, 81.0; H, 11.25; N, 3.65%).

10(11)-*Hydroxyimino*[20]*paracyclophane*-22-*carboxylic acid* (2c) was obtained by carboxylation of (3a) followed by oximation [6] (Found: C, 74.95; H, 10.0; N, 3.15. $C_{27}H_{43}NO_3$ requires C, 75.5; H, 10.1; N, 3.25%).

p-Nitrophenyl laurate (PNPL) and palmitate (PNPP) were prepared by the reaction of the corresponding acid chlorides

[6] Y. Murakami, Y. Aoyama, K. Ohno, K. Dobashi, T. Nakagawa, and J. Sunamoto, *J.C.S. Perkin I*, 1976, 1320.

[7] Reported vibrational frequencies, 3 760 and 3 650 cm^{-1}: C. C. Ferriso and D. F. Horning, *J. Chem. Phys.*, 1955, **23**, 1464; J. T. Mullhaupt and D. F. Horning, *ibid.*, 1956, **24**, 169; K. B. Wiberg, ' Physical Organic Chemistry,' Wiley, New York, 1966, p. 276.

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.

Kinetic Measurements.—Rates of *p*-nitrophenoxide liberation were measured at 400 nm. Each run was initiated by adding an acetone solution (30 μl) of *p*-nitrophenyl carboxylate (1.0×10^{-3}M) to the reaction medium (3.0 ml) which was pre-equilibrated at an appropriate temperature in a thermostatted cell set in a Hitachi 124 recording spectrophotometer. The reaction medium was prepared by mixing an appropriate amount of a stock solution of paracyclophane oxime with a buffer solution (Na_2HPO_4–NaOH). An organic solvent was further added if necessary, so that the resulting medium contained 1% (v/v) methanol [for (2a)] or 10% (v/v) ethanol [for (2b and c)]. The final ionic strength of each mixture was maintained at 0.10 with KCl and temperature was maintained constant to ±0.1° for reactions at ≤40° and to ±0.2° for those carried out at 48°.

pH Measurements.—For a reaction system of 1% (v/v) methanol–1% (v/v) acetone–water with μ 0.10 (KCl), the −log[H$^+$] value was determined by using equation (1) with the assumption that the calibration method for an aqueous system of μ 0.10 (KCl) [8] can be adopted. The pH value is

$$-\log[H^+] = pH + \Delta pH \qquad (1)$$

the pH-meter reading after calibration with a combination of appropriate standard buffers and ΔpH is the correction factor which was established from the pH-meter reading in the presence of a known amount of hydroxide ion and referring to the ionization constant of water at μ 0.10 (KCl) reported in the literature.[8] pH Measurements were carried out with a Beckman Expandomatic SS-2 pH meter equipped with a Metrohm EA-125 combined electrode. For a solvent system of 10% (v/v) ethanol–1% (v/v) acetone–water with μ 0.10 (KCl), the ΔpH value was evaluated as follows. (i) The ionization constant of water at a desired temperature in 11% (v/v) aqueous ethanol was obtained by interpolation of the literature values.[9] (ii) The activity coefficient for water was assumed to vary on changing the ionic strength of the medium in the same way as observed for the aqueous system. (iii) The ionization constant of water in aqueous ethanol was assumed to show the same temperature dependence as observed in the aqueous system.

RESULTS AND DISCUSSION

Alkaline Hydrolysis and Acyl Transfer Reactions of p-*Nitrophenyl Carboxylates.*—The liberation of *p*-nitrophenoxide from *p*-nitrophenyl laurate (PNPL) and palmitate (PNPP) was accelerated by the paracyclophane oximes (2a—c) relative to spontaneous alkaline hydrolyses under the same conditions, *i.e.*, initial concentration of substrate esters (1.0×10^{-5}M), ionic strength (0.10 with KCl), and solvent systems [1% (v/v) methanol–1% (v/v) acetone for (2a); 10% (v/v) ethanol–1% (v/v) acetone for (2b and c)]. The apparent catalytic first-order rate constants (k_{obs}) determined from the initial stage of deacylation reactions of PNPL and PNPP are summarized in Tables 1—4, along with the corresponding rates of alkaline hydrolyses (k_{hyd}). Saturation-type kin-

TABLE 1

First-order rate constants for *p*-nitrophenol release from PNPL in the presence and absence of (2a) at μ 0.10 (KCl) [a]

20°			25°			30°		
−log[H$^+$]	10^5[(2a)]/M	k_{obs}/s^{-1}	−log[H$^+$]	10^5[(2a)]/M	k_{obs}/s^{-1}	−log[H$^+$]	10^5[(2a)]/M	k_{obs}/s^{-1}
11.11	8.52	5.91×10^{-3}	10.97	8.52	7.50×10^{-3}	10.82	8.52	1.04×10^{-2}
	5.68	4.90×10^{-3}		5.68	6.65×10^{-3}		5.68	7.73×10^{-3}
	4.26	3.80×10^{-3}		4.26	5.08×10^{-3}		4.26	5.73×10^{-3}
	2.84	2.81×10^{-3}		2.84	3.57×10^{-3}		2.84	4.60×10^{-3}
	0	3.45×10^{-5}		0	4.55×10^{-5}		0	6.00×10^{-5}
11.26	8.52	1.08×10^{-2}	11.20	8.52	1.37×10^{-2}	11.03	8.52	1.97×10^{-2}
	5.68	7.59×10^{-3}		5.68	1.04×10^{-2}		5.68	1.47×10^{-2}
	4.26	7.00×10^{-3}		4.26	8.80×10^{-3}		4.26	1.13×10^{-2}
	2.84	5.45×10^{-3}		2.84	6.09×10^{-3}		2.84	8.43×10^{-3}
	0	5.07×10^{-5}		0	7.41×10^{-5}		0	1.10×10^{-4}
11.65	8.52	1.95×10^{-2}	11.56	8.52	2.76×10^{-2}	11.43	8.52	4.29×10^{-2}
	5.68	1.62×10^{-2}		5.68	1.94×10^{-2}		5.68	3.11×10^{-2}
	4.26	1.30×10^{-2}		4.26	1.69×10^{-2}		2.84	2.07×10^{-2}
	2.84	9.76×10^{-3}		2.84	1.27×10^{-2}		1.42	1.04×10^{-2}
	0	1.29×10^{-4}		0	1.61×10^{-4}		0	2.75×10^{-4}

[a] $[PNPL]_0 = 1.00 \times 10^{-5}$M; in 1% (v/v) methanol–1% (v/v) acetone–water.

TABLE 2

First-order rate constants for *p*-nitrophenol release from PNPP in the presence and absence of (2a) at μ 0.10 (KCl) [a]

20°			25°			30°		
−log[H$^+$]	10^5[(2a)]/M	k_{obs}/s^{-1}	−log[H$^+$]	10^5[(2a)]/M	k_{obs}/s^{-1}	−log[H$^+$]	10^5[(2a)]/M	k_{obs}/s^{-1}
11.26	2.84	4.79×10^{-3}	11.20	2.84	5.90×10^{-3}	11.03	2.84	8.97×10^{-3}
						11.03	0	6.00×10^{-5}
11.65	2.84	7.71×10^{-3}	11.56	2.84	1.00×10^{-2}	11.43	2.84	2.02×10^{-2}
						11.43	0	1.07×10^{-4}

[a] $[PNPP]_0 = 1.00 \times 10^{-5}$M; in 1% (v/v) methanol–1% (v/v) acetone–water.

[8] H. S. Harned and B. B. Owen, ' The Physical Chemistry of Electrolytic Solutions,' Reinhold, New York, 1950, 2nd edn., pp. 480—579.

[9] E. M. Woolley, D. G. Hurkot, and L. G. Hepler, *J. Phys. Chem.*, 1970, **74**, 3908.

TABLE 3

First-order rate constants for *p*-nitrophenol release from PNPP in the presence and absence of (2b) at μ 0.10 (KCl) [a]

33.4°			40°			48°		
−log[H+]	10^5[(2b)]/M	k_{obs}/s^{-1}	−log[H+]	10^5[(2b)]/M	k_{obs}/s^{-1}	−log[H+]	10^5[(2b)]/M	k_{obs}/s^{-1}
11.02	2.50	1.92×10^{-4}	10.73	2.50	3.27×10^{-4}	10.62	2.50	5.84×10^{-4}
	1.67	2.11×10^{-4}		1.67	3.38×10^{-4}		1.67	5.43×10^{-4}
	0.834	1.32×10^{-4}		0.834	2.11×10^{-4}		0.834	3.86×10^{-4}
	0.556	1.04×10^{-4}		0.556	1.68×10^{-4}		0.556	2.83×10^{-4}
	0.278	7.78×10^{-5}		0.278	1.25×10^{-4}		0.278	1.95×10^{-4}
	0	2.66×10^{-5}		0	4.82×10^{-5}		0	6.40×10^{-5}
11.53	2.50	3.68×10^{-4}	11.37	2.50	6.41×10^{-4}	11.23	2.50	1.53×10^{-3}
	1.67	3.41×10^{-4}		1.67	5.84×10^{-4}		1.67	1.13×10^{-3}
	0.834	2.68×10^{-4}		0.834	4.41×10^{-4}		0.834	8.90×10^{-4}
	0.556	2.17×10^{-4}		0.556	3.37×10^{-4}		0.556	6.40×10^{-4}
	0.278	1.49×10^{-4}		0.278	2.58×10^{-4}		0.278	4.61×10^{-4}
	0	5.49×10^{-5}		0	1.15×10^{-4}		0	2.23×10^{-4}

[a] $[PNPP]_0 = 1.00 \times 10^{-5}$M; in 10% (v/v) ethanol–1% (v/v) acetone–water.

TABLE 4

First-order rate constants for *p*-nitrophenol release from PNPP in the presence and absence of (2c) at μ 0.10 (KCl) [a]

33.4°			40°			48°		
−log[H+]	10^5[(2c)]/M	k_{obs}/s^{-1}	−log[H+]	10^5[(2c)]/M	k_{obs}/s^{-1}	−log[H+]	10^5[(2c)]/M	k_{obs}/s^{-1}
11.02	8.79	4.60×10^{-5}	10.73	8.79	8.52×10^{-5}	10.62	8.79	1.35×10^{-4}
	5.86	4.20×10^{-5}		5.86	7.31×10^{-5}		5.86	1.12×10^{-4}
	2.93	3.83×10^{-5}		2.93	6.63×10^{-5}		2.93	9.53×10^{-5}
	1.76	3.45×10^{-5}		1.76	5.96×10^{-5}		1.76	8.33×10^{-5}
	0	2.66×10^{-5}		0	4.82×10^{-5}		0	6.40×10^{-5}
11.53	8.79	1.02×10^{-4}						
	5.86	9.66×10^{-5}						
	2.93	8.35×10^{-5}						
	1.76	7.43×10^{-5}						
	0	5.49×10^{-5}						

[a] $[PNPP]_0 = 1.00 \times 10^{-5}$M; in 10% (v/v) ethanol–1% (v/v) acetone–water.

etics were observed as exemplified by the correlation between concentration of cyclic oxime (2b) and rate constant for the deacylation of PNPP (Figure 1). These

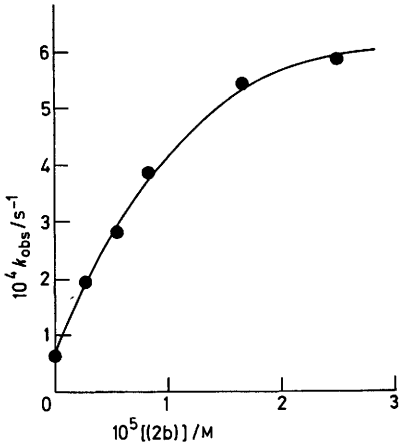


FIGURE 1 Saturation-type kinetics for the deacylation of PNPP in the presence of cyclic oxime (2b) in 10% (v/v) ethanol–1% (v/v) acetone–water at 48°, μ 0.10 (KCl), and −log[H+] 10.62; $[PNPP]_0 = 1.00 \times 10^{-5}$M

results are consistent with a reaction mechanism in which pre-equilibrium complexation of the cyclic oxime and the substrate at a 1 : 1 molar ratio is followed by pseudo-intramolecular acyl transfer from the bound substrate to the hydroxyimino-group of the cyclic oxime. The primary product of a similar deacylation reaction of *p*-nitrophenyl carboxylates catalysed by cyclic oxime (1a) has been identified as the acylated paracyclophane (1b).[5b] Even a large excess (0.1M) of acetoxime, which has no hydrophobic binding site, did not accelerate the present deacylation reactions to any significant extent. The catalytic effectiveness of paracyclophane oximes is characterized as follows on the basis of the kinetic results. (i) The oximes accelerate the reaction significantly even when present in relatively low concentration, indicating

TABLE 5

Apparent catalytic efficiency of cyclic oximes for *p*-nitrophenol release from *p*-nitrophenyl carboxylates [a,b]

Oxime	Concentration (M)	Substrate	−log[H+]	t/°C	k_c [b]/l mol^{-1} s^{-1}	r_e [c]
(2a) [d]	2.84×10^{-5}	PNPP	11.03	30	313	150
(2a) [d]	2.84×10^{-5}	PNPP	11.43	30	708	188
(2a) [d]	2.84×10^{-5}	PNPL	10.82	30	160	77
(2a) [d]	2.84×10^{-5}	PNPL	11.43	30	719	75
(2b) [e]	2.50×10^{-5}	PNPP	11.02	33.4	6.6	7.2
(2b) [e]	2.50×10^{-5}	PNPP	11.53	33.4	12.5	6.7
(2c) [e]	2.93×10^{-5}	PNPP	11.02	33.4	0.40	1.4
(2c) [e]	2.93×10^{-5}	PNPP	11.53	33.4	0.98	1.5

[a] $[PNPL]_0 = [PNPP]_0 = 1.00 \times 10^{-5}$M. [b] See equation (2) for definition. [c] See equation (3) for definition. [d] Measured in 1% (v/v) methanol–1% (v/v) acetone–water at μ 0.10 (KCl). [e] Measured in 10% (v/v) ethanol–1% (v/v) acetone–water at μ 0.10 (KCl).

comparatively large ability to bind the substrates. (ii) The catalytic efficiencies of the cyclic oximes are much dependent on the nature of the substituent placed

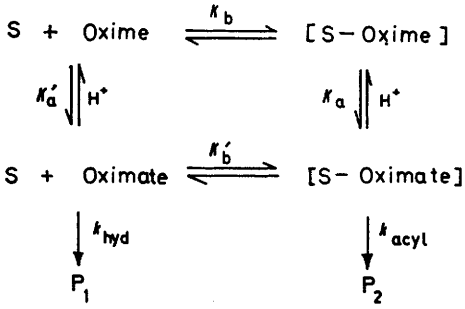
in the benzene ring. The apparent catalytic constants (k_c) and the catalytic efficiency factors (r_e) defined by equations (2) and (3), respectively, are listed in Table 5 for selected cases to provide the means of evaluating the catalytic ability of paracyclophane oximes. The high

$$k_c = (k_{obs} - k_{hyd})/[\text{Oxime}] \quad (2)$$

$$r_e = k_{obs}/k_{hyd} \quad (3)$$

activity of (2a) must be attributed to the electrostatic field effect exerted by the positively charged ammonium group on the benzene ring.

Since the acyl transfer rate is dependent on pH of the medium (Tables 1—4), the reaction sequence is shown by the Scheme. This means that the real nucleophile is



SCHEME P_1 = carboxylate and *p*-nitrophenoxide ion; P_2 = acylated paracyclophane oxime and *p*-nitrophenoxide ion

not the neutral oxime but the anionic oximate group of the paracyclophane as observed for catalysis by (1a).[5] Such being the case, the observed first-order rate constant (k_{obs}) is given by equation (4) [5b] where $[S]_T$ and $[C]_T$ stand

$$k_{obs} = k_{hyd} + \left(\frac{1}{\gamma}k_{acyl} - k_{hyd}\right) \frac{K_b[C]_T}{\left(\frac{1+\beta}{1+\alpha}\right) + K_b[S]_T + K_b[C]_T} \quad (4)$$

for the total concentration of substrate and cyclic oxime, respectively, and α, β, and γ are defined as $K_a/[H^+]$, $K_a'/[H^+]$, and $([H^+]/K_a) + 1$, respectively.

Rearrangement of equation (4) gives equation (5).

$$\frac{1}{k_{obs} - k_{hyd}} = \frac{\left(\frac{1+\beta}{1+\alpha}\right) + K_b[S]_T}{\left(\frac{1}{\gamma}k_{acyl} - k_{hyd}\right)K_b[C]_T} + \frac{1}{\frac{1}{\gamma}k_{acyl} - k_{hyd}} \quad (5)$$

Thus, a linear relationship is obtained by plotting the left-hand side of equation (5) against the reciprocal initial concentration of cyclic oxime, when the initial concentration of substrate and pH of the reaction medium are maintained constant (see Figure 2). If a reasonable assumption is made that $K_a \simeq K'_a$, $(1/\gamma)k_{acyl}$ and K_b are obtained by equation (5). Upon evaluation of $(1/\gamma)k_{acyl}$

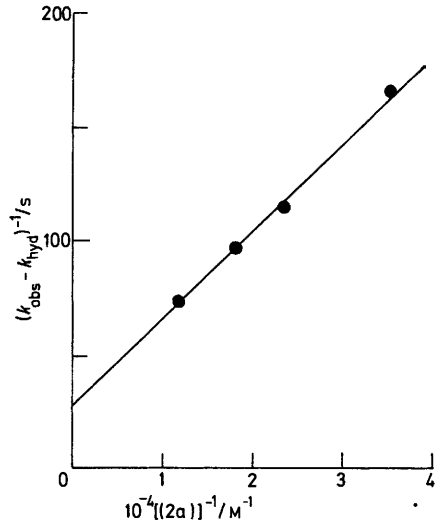


FIGURE 2 Analysis of kinetic data for the deacylation of PNPL in the presence of cyclic oxime (2a) by means of equation (5): kinetic measurements were carried out in 1% (v/v) methanol–1% (v/v) acetone–water at 25°, μ 0.10 (KCl), and $-\log[H^+]$ 11.20; $[PNPL]_0 = 1.00 \times 10^{-5}$M

values at various hydrogen ion concentrations, k_{acyl} is determined according to equation (6) (see Figure 3). All

$$\frac{\gamma}{k_{acyl}} = \frac{[H^+]}{K_a k_{acyl}} + \frac{1}{k_{acyl}} \quad (6)$$

the K_b and k_{acyl} values * are listed in Tables 6 and 7, respectively, for systems of (2a)–PNPL, (2b)–PNPP, and

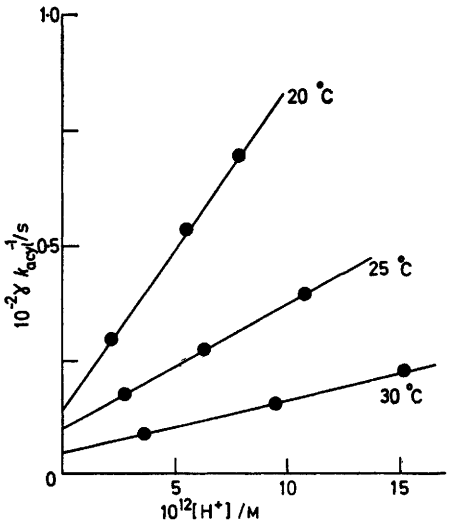


FIGURE 3 Analysis of kinetic data for the deacylation of PNPL in the presence of cyclic oxime (2a) by means of equation (6)

(2c)–PNPP. The pK_a values (Table 7) for the oxime proton are in a good agreement with those reported for (1a).[5c]

* Since the k_{obs} values for deacylation of PNPL were found to be rather insensitive to the variation of the initial concentrations of (2b and c), we failed to evaluate the respective binding and rate constants by the use of equations (5) and (6). The application of equation (5) to the kinetic analysis of the (2a)–PNPP system also failed to provide reliable constants: even though a straight line correlation was obtained, the intercept at the vertical axis was very close to the origin. This result qualitatively indicates that the rate constant for acyl transfer from the bound PNPP to (2a) is exceedingly large relative to that for the bound PNPL to the same cyclic oxime. A similar trend has been observed for catalysis by (1a) [5c] (Table 7).

TABLE 6

Kinetic parameters for reactions of *p*-nitrophenyl carboxylates with [20]paracyclophane oximes at μ 0.10 (KCl) [a]

Oxime	Substrate [b]	−log[H+]	*t*/°C	$\frac{1}{2}k_{acyl}$/s^{-1}	K_b/l mol^{-1}
(2a) [c]	PNPL	11.11	20	1.5×10^{-2}	8.8×10^{3}
		10.97	25	2.5×10^{-2}	5.8×10^{3}
		10.82	30	4.4×10^{-2}	4.0×10^{3}
		11.26	20	1.9×10^{-2}	1.4×10^{4}
		11.20	25	3.6×10^{-2}	7.7×10^{3}
		11.03	30	6.7×10^{-2}	5.2×10^{3}
		11.65	20	3.0×10^{-2}	2.0×10^{4}
		11.56	25	5.6×10^{-2}	1.1×10^{4}
		11.43	30	1.1×10^{-1}	6.7×10^{3}
(2b) [d]	PNPP	11.02	33.4	2.8×10^{-4}	6.5×10^{5}
		10.73	40	4.8×10^{-4}	3.2×10^{5}
		10.62	48	9.7×10^{-4}	1.4×10^{5}
		11.53	33.4	6.1×10^{-4}	2.6×10^{5}
		11.37	40	1.2×10^{-3}	1.2×10^{5}
		11.23	48	2.5×10^{-3}	6.7×10^{4}
(2c) [d]	PNPP	11.02	33.4	5.7×10^{-5}	2.5×10^{4}
		10.73	40	1.1×10^{-4}	1.5×10^{4}
		10.62	48	2.5×10^{-4}	7.4×10^{3}
		11.53	33.4	1.3×10^{-4}	2.9×10^{4}

[a] Parameters were obtained from the data in Tables 1, 3, and 4 with the aid of equation (5). [b] $[PNPL]_0 = [PNPP]_0 = 1.00 \times 10^{-5}$M. [c] Measured in 1% (v/v) methanol–1% (v/v) acetone–water. [d] Measured in 10% (v/v) ethanol–1% (v/v) acetone–water.

Binding Properties of Paracyclophane Oximes.—The binding constants (K_b, Table 6) for the present systems ($K_b \simeq 10^3$—10^5 l mol^{-1}) are considerably larger than those for the complexes formed between phenyl esters [10–12] or azo-dyes [13] and cycloamyloses ($K_b \simeq 10^2$—10^3 l mol^{-1}). The primary force which holds substrate and cyclic oxime together must be hydrophobic in origin as became evident from the effects of added urea and organic solvent on the kinetic behaviour of the (1a)–PNPL system.[5b] The binding constant for each system decreases by raising the reaction temperature (negative enthalpy change) as observed for cycloamyloses [10–13] and chymotrypsin.[14,15] The formation of an appreciably stable complex between the cyclic oxime and PNPL or PNPP is attributed to a favourable enthalpy change which is large enough to compensate for an unfavourable entropy change (Table 8). When the molecules which are largely hydrocarbon in nature are dissolved in water, some of the structured water molecules surrounding each solute molecule will be transferred to the bulk solvent upon association of solute molecules. Thus, the hydrophobic interaction will be accompanied by an increase in entropy and possibly by a positive enthalpy change.[1b,16] This thermodynamic behaviour has been observed experimentally in the polymerization of tobacco mosaic virus protein,[17] the aggregation of non-ionic detergent molecules into micelles,[18,19] and the association of small aromatic molecules with polyvinylpyrrolidone.[20] There are, however, a number of examples of hydrophobic association which result in negative enthalpy and entropy changes. The examples include the association of dyes [21,22] and purines.[23–25] In such cases, several factors other than the behaviour of structured water molecules should be in force, attractive van der Waals dispersion forces, conformational changes of interacting molecules, and so on. There are good reasons to expect that *p*-nitrophenyl carboxylates bearing a relatively long alkyl chain prefer to exist in a folded conformation [26,27] or a so-called self-

TABLE 7

Kinetic and activation parameters for acyl transfer reactions between bound *p*-nitrophenyl carboxylates and [20]-paracyclophane oximes [a]

Oxime	Substrate	*t*/°C	K_a/l mol^{-1}	pK_a	k_{acyl}/s^{-1}	$\Delta G^{\ddagger}_{303\ K}$/ kcal mol^{-1}	$\Delta H^{\ddagger}$/ kcal mol^{-1}	$\Delta S^{\ddagger}$/ cal mol^{-1} K^{-1}
(1a) [b]	PNPL	34	4.29×10^{-12}	11.4	7.80×10^{-3}	20.9	17.7	−10.6
	PNPP	20.2	7.62×10^{-13}	12.1	9.44×10^{-3}	20.2	17.6	−8.6
		39.9	2.14×10^{-12}	11.7	4.45×10^{-2}			
(2a)	PNPL	20	2.1×10^{-12}	11.68	6.7×10^{-2}			
		25	3.6×10^{-12}	11.44	1.0×10^{-1}	18.8	19.5	2.4
		30	4.3×10^{-12}	11.36	2.1×10^{-1}			
(2b)	PNPP	33.4	2.4×10^{-12}	11.63	1.4×10^{-3}			
		40	5.0×10^{-12}	11.30	2.3×10^{-3}	21.9	16.8	−16.9
		48	5.9×10^{-12}	11.23	5.0×10^{-3}			
(2c)	PNPP	33.4	2.18×10^{-12}	11.66	3.0×10^{-4}			

[a] Kinetic parameters were obtained from the data in Table 6 the with aid of equation (6). [b] Cited in ref. 5*c*; measured in 10.9% (v/v) aqueous acetone at μ 0.10 (KCl).

[10] R. L. VanEtten, J. F. Sebastian, G. A. Clowes, and M. L. Bender, *J. Amer. Chem. Soc.*, 1967, **89**, 3242.
[11] R. L. VanEtten, G. A. Clowes, J. F. Sebastian, and M. L. Bender, *J. Amer. Chem. Soc.*, 1967, **89**, 3253.
[12] D. L. VanderJagt, F. L. Killian, and M. L. Bender, *J. Amer. Chem. Soc.*, 1970, **92**, 1016.
[13] F. Cramer, W. Saenger, and H.-Ch. Spatz, *J. Amer. Chem. Soc.*, 1967, **89**, 14.
[14] D. G. Doherty and F. Vaslow, *J. Amer. Chem. Soc.*, 1952, **74**, 931.
[15] F. Vaslow and D. G. Doherty, *J. Amer. Chem. Soc.*, 1953, **75**, 928.
[16] W. B. Danklicker and V. A. de Saussure in ' The Chemistry of Biosurfaces,' ed. M. L. Hair, Marcel Dekker, New York, 1971, ch. 1.
[17] M. A. Lauffer, *Biochemistry*, 1966, **5**, 2440.
[18] J. M. Corkill, J. F. Goodman, and J. R. Tate, *Trans. Faraday Soc.*, 1964, **60**, 996.
[19] M. J. Schick, *J. Phys. Chem.*, 1963, **67**, 1796.
[20] P. Molyneux and H. P. Frank, *J. Amer. Chem. Soc.*, 1961, **83**, 3169.
[21] E. Rabinowitch and L. F. Epstein, *J. Amer. Chem. Soc.*, 1941, **63**, 69.
[22] Y. Tanizaki, T. Hoshi, and N. Ando, *Bull. Chem. Soc. Japan*, 1965, **38**, 264.
[23] A. Munck, J. F. Scott, and L. L. Engel, *Biochim. Biophys. Acta*, 1957, **26**, 397.
[24] P. R. Stoesser and S. J. Gill, *J. Phys. Chem.*, 1967, **71**, 564.
[25] S. J. Gill, M. Downing, and G. F. Sheats, *Biochemistry*, 1967, **6**, 272.
[26] T. Maugh, II and T. C. Bruice, *J. Amer. Chem. Soc.*, 1971, **93**, 6584.
[27] C. A. Blyth and J. R. Knowles, *J. Amer. Chem. Soc.*, 1971, **93**, 3021.

aggregated form.[28-30] Such being the case, the binding process may consist of two steps, unfolding or deaggregation of a free ester (step 1) followed by complexation of the unfolded ester with a macrocyclic oxime (step 2). The thermodynamic parameters associated with the binding process are determined by the relative importance of the two steps, and the unfolding process of

TABLE 8

Thermodynamic parameters for binding of *p*-nitrophenyl carboxylates with [20]paracyclophane oximes [a]

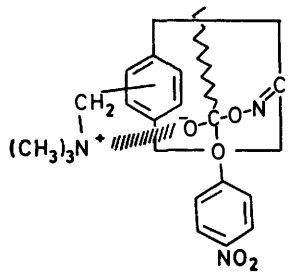
Oxime	Substrate	$-\log[H^+]$	$\Delta G°_{303\ K}$/ kcal mol^{-1}	$\Delta H°$/ kcal mol^{-1}	$\Delta S°$/ cal mol^{-1} K^{-1}
(1a) [b]	PNPL	12.37	−7.50	−14.4	−22.8
	PNPP	10.71	−7.65	−16.2	−28.2
		11.78	−7.32	−16.1	−28.9
(2a) [c]	PNPL	11.00	−5.1	−7.2	−6.8
		11.50	−5.4	−15.6	−33.8
(2b) [c]	PNPP	11.00	−8.2	−26.5	−60.1
		11.50	−7.8	−28.8	−69.4
(2c)	PNPP	10.73 [d]	−6.3	−16.0	−32.1

[a] Thermodynamic parameters were evaluated on the basis of K_b values listed in Table 6. [b] Cited in ref. 5*c*; measured in 10.9% (v/v) aqueous acetone at μ 0.10 (KCl). [c] Binding constants at $-\log[H^+]$ 11.00 and 11.50 were estimated by interpolation of the corresponding data listed in Table 6 at 20, 25, and 30°. [d] For kinetic reasons, an average value is given.

PNPL and PNPP would be responsibe for the observed large negative entropy change. The binding efficiency follows the decreasing order: (2b) > (1a) > (2c) > (2a) (Tables 6 and 8). The lower binding efficiencies of (2a and c) are presumably related to the nature of their charged substituents (ammonium and carboxylate, respectively), which are located in the vicinity of the hydrophobic binding site. Since the hydrophobic interaction is accompanied by the formation of a special structure of liquid water,[1b,16] the effects of added salts have been interpreted in terms of their disruption of the water structure.[16] The hydrophobic interaction is inhibited by highly chaotropic ions[16] and the effect is reflected in the disordering of the water structure, the extent of which is governed by the nature of chaotropic ions [16] and their concentrations. Consequently, the structure-breaking ability of the charged substituents of (2a and c) is primarily responsible for their weaker binding behaviour. It is interesting to note that the binding constant for the (2a)–PNPL system slightly increases with increasing pH of the medium in contrast to those for the (2b)–PNPP, (1a)–PNPL, and (1a)–PNPP systems (Table 6). The trend observed for the (2a) system may be attributed to the counterion effect. At lower pH values, the primary counterion of the ammonium group may be chloride which was added in large excess to maintain the ionic strength of the reaction medium constant. As the pH increases, chloride ions surrounding the ammonium groups are gradually replaced by hydroxide ions. Hydroxide ions around the binding site of (2a) at high pH may strengthen the hydrophobic interaction between (2a) and PNPL, since the hydroxide ion has been suggested to be structure-making.[16]

[28] F. M. Menger and L. E. Portnoy, *J. Amer. Chem. Soc.*, 1968, **90**, 1875.

Acyl Transfer Mechanism.—The acid dissociation constants for the oxime group of [20]paracyclophanes are in a range of 2—6 × 10^{-12} l mol^{-1} (Table 7), and are rather insensitive to the nature of an additional substituent placed on the benzene ring. The acyl transfer rate (k_{acyl}), on the other hand, is dependent on the nature of such charged substituents. The extremely high activity of (2a) in the acyl transfer reaction is evidently related to the super-acid nature of the quaternary ammonium group. Two alternative mechanisms explain these effects. (i) The hydroxide ion, the nucleophile which attacks the substrate, is concentrated around the ammonium group due to electrostatic attraction, and consequently a concentration effect facilitates reaction. (ii) The hydroxyimino and ammonium groups, a nucleophile and a super-acid, respectively, co-operate in the catalytic process (bifunctional catalysis), and a negative charge developing on the carbonyl oxygen of the substrate in the transition state, which is formed by nucleophilic attack of the deprotonated hydroxyimino-group on the acyl carbon of the substrate, is stabilized through tight ion-pair formation with the positive ammonium group as shown in (4). These two mechanisms differ



(4)

from each other in the way in which the hydroxyimino-group participates in the transition state. However, the former mechanism may be ruled out on the basis of kinetic data obtained in the presence of ammonium salt (3c) which lacks a nucleophilic centre. The observed first-order rate constant for deacylation of PNPP at 30° in the presence of 2.60 × 10^{-5}M-(3c) is 9.89 × 10^{-4} s^{-1}. Even though this value is somewhat larger than that for the spontaneous hydrolysis (1.07 × 10^{-4} s^{-1}), the catalytic efficiency of (3c) is negligible [*cf.* the corresponding rate constant observed in the presence of 2.84 × 10^{-5}M-(2a), 2.02 × 10^{-2} s^{-1}, Table 9]. The local concentration effect of hydroxide ion plays at most only a minor role. The absence of catalytic activity by (3c) in the present reaction seems to indicate that both (2a) and (3c) may exist primarily in a monomeric form different from typical cationic surfactants for which aggregation behaviour is generally observed.[2]

Electrostatic attraction between the ammonium and hydroxyimino-groups of (2a) may facilitate nucleophilic attack of the latter group on the bound substrate on one

[29] J. P. Guthrie, *Canad. J. Chem.*, 1973, **51**, 3494.
[30] F. M. Menger and C. E. Portnoy, *J. Amer. Chem. Soc.*, 1967, **89**, 4698.

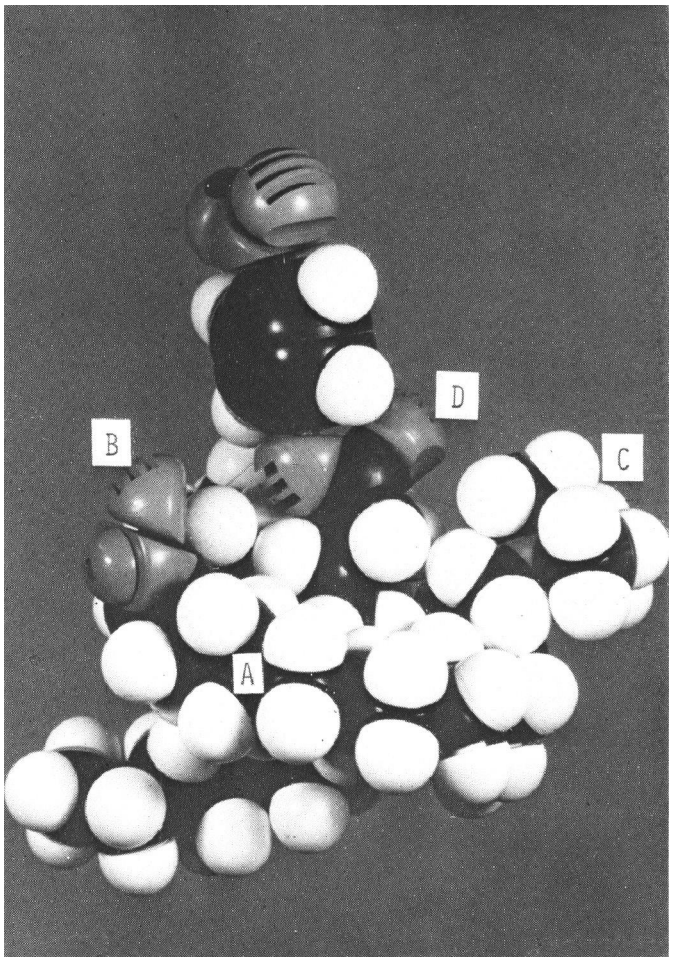


FIGURE 4 Corey–Pauling–Koltum molecular model of the inclusion complex formed between cyclic oxime (2a) and PNPL: A, paracyclophane skeleton; B, oximate oxygen; C, ammonium group; D, carbonyl oxygen of PNPL

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hand, and the former group may stabilize the transition state by its electrostatic field effect on the other. These effects may act to reduce the activation free energy appreciably. The opposite situation obtains for the (2c) system since this cyclic oxime bears a negatively charged carboxylate group and, therefore, destabilizes the negatively charged transition state. Compound (2b) which has no charged substituent on the benzene ring exhibits intermediate reactivity (Table 7). This type of electrostatic charge effect is rarely observed in common bimolecular ionic reactions in aqueous media. This is partly because of the exceptional charge-solvating ability of water [1b] and partly and more significantly because the interaction of a charged species with a dipolar neutral substrate is not large enough to compensate for an appreciable entropy loss which would be brought about by a bimolecular association. To make this point clear, catalytic activities of some quaternary ammonium species in the deacylation of PNPP were examined (Table 9). The addition of 0.1M-tetramethylammonium chloride to the (2b) system did not result in any significant effect on the reaction rate because of the absence of an intrinsic affinity for PNPP. On the other hand, hexadecyltrimethylammonium bromide (CTAB) showed a noticeable catalytic effect even in a concentration range below its c.m.c. It is important, however, to note that no co-operative acceleration is detected by the combination of CTAB (an electrostatic catalyst) and (2b) (a nucleophilic catalyst). It should be emphasized that the significant, bifunctional catalysis played by (2a) is brought about by the fixation of the ammonium and hydroxyimino-groups in a favourable orientation for simultaneous interaction with the bound substrate. A plausible structure of the (2a)–PNPL complex is illustrated in Figure 4. It has been also recognized recently that the rates of some amide hydrolyses,[31] lactonizations,[32] and esterifications [32] which go through intramolecular processes are surprisingly sensitive to the orientation of reacting groups.

TABLE 9

Effects of some quaternary ammonium species on the deacylation of PNPP [a]

Catalyst (M)	Medium [b]	$-\log[H^+]$	t/°C	k_{obs}/s^{-1}
	MAW	11.43	30	1.07×10^{-4}
(2a) (2.84×10^{-5})	MAW	11.43	30	2.02×10^{-2}
(3c) (2.60×10^{-5})	MAW	11.43	30	9.89×10^{-4}
(2b) (8.34×10^{-6})	EAW	11.53	33.4	2.68×10^{-4}
(2b) (8.34×10^{-6}) + $Me_4\overset{+}{N}Cl^-$ (0.1)	EAW	11.53	30	2.15×10^{-4}
CTAB [c] (4×10^{-5})	EAW	11.53	30	1.63×10^{-3}
(2b) (8.34×10^{-6}) + CTAB (4×10^{-5})	EAW	11.53	30	1.48×10^{-3}

[a] $[PNPP]_0 = 1.00 \times 10^{-5}$M. [b] MAW = 1% (v/v) methanol–1% (v/v) acetone–water, μ 0.10 (KCl). EAW = 10% (v/v) ethanol–1% (v/v) acetone–water, μ 0.10 (KCl). [c] CTAB = Hexadecyltrimethylammonium bromide.

Nucleophilic attack of the hydroxyimino-group on the substrates results in a transition state in which the substrate–carbonyl develops a partial negative charge. This polar transition state would then require appreciable solvation by water molecules as characterized by a negative entropy change (Table 7). On the other hand, the developed negative charge in the transition state of (2a)–PNPL system would be neutralised through a tight ion-pair association with the positively charged ammonium group [see (4)]. This transition state thus requires less solvation by water molecules, relative to that for the (2b)–PNPP system as reflected in the activation entropy change ($\Delta S^{\ddagger}$). Similar desolvation effects due to charge neutralisation have been indicated for metal-catalysed hydration of phenanthrolinecarbonitrile.[33] The acyl transfer rate for (1a)–PNPP is much greater than that for (2b)–PNPP (Table 7). The greater catalytic effectiveness of (1a) is apparently related to the entropy effect: $\Delta\Delta S^{\ddagger} = \Delta S^{\ddagger}_{(1a)} - \Delta S^{\ddagger}_{(2b)} = 8.3$ cal mol^{-1} K^{-1}. By extending the above argument for the characterization of the transition state of (2a)–PNPL, the hydroxy-group of (1a) would serve to neutralise in part a negative charge on the carbonyl oxygen of the substrate in the transition state through hydrogen bonding interaction.

Second-order Rate Constants.—If the condition is imposed that the hydroxyimino-group of the paracyclophane oximes is completely ionized, acylation of the cyclic oximes can be given simply by reaction (7). The overall

$$S + \text{Oximate} \overset{K_b'}{\rightleftharpoons} [\text{Oximate–S}] \xrightarrow{k_{acyl}} P_2 \qquad (7)$$

acylation rate given by equation (8) is consistent with this reaction. At sufficiently low concentrations of the

$$\frac{d[P_2]}{dt} = \frac{k_{acyl}K_b'[\text{Oximate}]_T[S]_T}{1 + K_b'[\text{Oximate}]} \qquad (8)$$

paracyclophane oximes, the reaction follows second-order kinetics with rate constant k_2 [$= k_{acyl}K_b$ with the reasonable assumption $K_b' \simeq K_b$ (see Scheme)]. The effectiveness of the present cyclic oximes is evident if their k_2 values are compared with that for reaction of acetoxime with PNPA (Table 10). Since the bimolecular reaction between acetoxime and PNPA does not suffer from the steric hindrance expected for acylation reactions of carboxylic esters bearing a long alkyl chain with oximes investigated here, the former reaction can be used as a reference. It should be noted that the reactivity of bifunctional catalyst (2a) in deacylation of PNPL is greater than that of chymotrypsin in deacylation of PNPA. The recently developed micellar systems with hydroxamate nucleophiles show catalytic reactivities comparable with that of (2a) (Table 10). However, the catalytic effectiveness of (2a) is superior to those of the micellar systems on the basis of the following facts. (i) CTAB is the indispensable cofactor for catalytic activity of the micellar systems, and a large excess of CTAB (by 10-fold relative to the hydroxamate species) were used and

[31] A. J. Kirby and P. W. Lancaster, *J.C.S. Perkin II*, 1972, 1206.

[32] D. R. Storm and D. E. Koshland, jun., *J. Amer. Chem. Soc.*, 1972, **94**, 5805, 5815.

[33] R. Breslow, R. Fairweather, and J. Keana, *J. Amer. Chem. Soc.*, 1967, **89**, 2135.

and (ii) the concentration of CTAB was not taken into account for the evaluation of k_2 values.

TABLE 10

Second-order rate constants for reactions of *p*-nitrophenyl carboxylates with cyclic oximes and other catalysts

Catalyst	Substrate	t/°C	k_2/l mol^{-1} s^{-1}	Ref.
(2a) [a]	PNPL	30	*ca.* 1.4×10^3	This work
(2b) [a]	PNPP	33.4	*ca.* 3.6×10^2	This work
(2c) [a]	PNPP	33.4	*ca.* 8.7	This work
OH^-	PNPP	33.4	7.3×10^{-3} [b]	This work
OH^-	PNPL	30	4.3×10^{-2} [b]	This work
OH^-	PNPA	25	1.5×10	*h*
$(CH_3)_2C{=}NO^-$	PNPA	25	6×10	*h*
LImHA [c] + CTAB [d]	PNPA	30	1.59×10^2 (pH 8.10)	*i*
			3.6—11.7×10^3 [f]	*i*
MLHA [e] + CTAB [d]	PNPA	22	2.06×10^3 (pH 9.99)	*j*
α-Chymotrypsin	PNPA	25.1	5.63×10^2 [g] (pH 7.49)	*k*

[a] The nucleophilic oxime group is in the deprotonated form. The K_b value used for evaluation of k_2 is that obtained at the highest pH investigated in the present study. [b] Obtained from the data listed in Tables 1 and 3. [c] *N*-Laurylimidazole-4-carbohydroxamic acid. [d] Hexadecyltrimethylammonium bromide. [e] *N*-Methyl-laurohydroxamic acid. [f] Estimated value for the completely deprotonated hydroxamate group. [g] For acylation step. [h] W. P. Jencks and J. Carriuolo, *J. Amer. Chem. Soc.*, 1960, **82**, 1778. [i] T. Kunitake, Y. Okahata, and T. Sakamoto, *Chem. Letters*, 1975, 459. [j] I. Tabushi, Y. Kuroda, and S. Kita, *Tetrahedron Letters*, 1974, 643. [k] M. L. Bender and N. Nakamura, *J. Amer. Chem. Soc.*, 1962, **84**, 2577.

Conclusions.—Two important points should be made on the basis of the present study.

(i) Despite the generally accepted view that electrostatic effects are small and variable in aqueous solution,[1b] a proper spatial orientation of nucleophile (base), positive charge (acid), and substrate results in a profound electrostatic catalysis, as observed in the present system. This finding may throw light on the unsettled problems of lysozyme catalysis. The suggested mechanism involves proton transfer from the un-ionized carboxy-group of glutamic acid-35 to the oxygen of the incipient leaving group, yielding a transition state bearing a carbonium ion which would be stabilized by the deprotonated carboxylate of aspartic acid-52 through electrostatic interaction. A subject of recent studies on the lysozyme catalysis is whether or not the carboxylate ion of Asp-52 is involved in the catalytic process.[34] We by no means suggest any critical mechanism for lysozyme catalysis at this stage, but it seems reasonable that the electrostatic interaction may be a potential candidate for the origin of catalytic activities of the enzyme.

(ii) The ammonium salt (2a) seems to be the best synthetic enzyme model yet produced for the acyl transfer reaction. This model provides a favourable hydrophobic binding site with binding constant as large as 10^4 l mol^{-1}, and a co-operative bifunctional catalysis site which acts to accelerate the acyl transfer reaction to an extent comparable with or even greater than that in enzymatic systems.

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[34] G. M. Loudon, C. K. Smith, and S. E. Zimmerman, *J. Amer. Chem. Soc.*, 1974, **96**, 465.