Syntheses of Macrocyclic Enzyme Models. Part 3.† Preparation and Properties of Water-soluble Azaparacyclophanes

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Azaparacyclophanes involving quaternary nitrogens in the cyclic skeleton and bearing long alkyl chains as branches of the skeleton were prepared to investigate their ability as macrocyclic enzyme models to incorporate various hydrophobic substrates. Water-soluble azaparacyclophanes, *NNN'N'N''N''N''' N''' '*-octamethyl-2,11,20,29-tetra-aza[3.3.3.3]paracyclophane tetraiodide (8), *NNN'N'N''N''N''' N''' 'N'' 'N''' 'N'''' N'''' 'N''''' N''''' '*-dodecamethyl-2,11,20,29,-38,47-hexa-aza[3.3.3.3.3.3]paracyclophane hexaiodide (9), and *NN'N''N'''*-tetrakis-(10-carboxydecyl)-2,11,20,-29-tetra-aza[3.3.3.3]paracyclophane-3,10,21,28-tetraone (13), incorporate a hydrophobic spin-labelled probe, 2,2,6,6-tetramethyl-1-oxyl-4-piperidyl cyclohexlyacetate (14). The aggregation behaviour of (13) was observed in a mixed aqueous–organic solvent, the critical micelle concentration (CMC) being 3.15×10^{-4}M. The azacyclophane (13) may provide an efficient hydrophobic field by the co-operation of the macrocyclic skeleton and the four alkyl chains due to its octopus-like structure and incorporate substrates of an appropriate molecular size with positive or neutral charge as confirmed by electronic spectroscopy using organic dyes. The hydrophobic binding ability of (13) was also acknowledged by the kinetic method using *p*-nitrophenyl 3,5-dimethylcyclohexylacetate as a substrate.

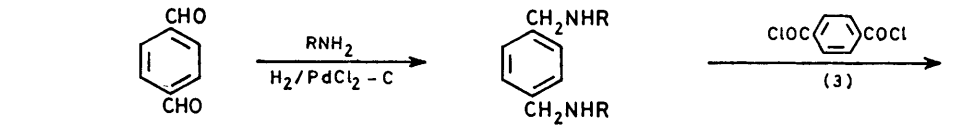
As an aid to the understanding of the mechanism of enzyme action, it would be useful to search for relatively simple model systems which exhibit some of the critical features of enzymatic reactions. The three kinds of esterase model systems, micellar surfactants,[1] synthetic polymers,[2] and macrocyclic compounds,[3] have been widely developed so far. We have recently prepared various paracyclophanes as macrocyclic compounds which have some potential as enzyme models, superior to others, due to the following reasons.[4] (i) The macrocyclic skeleton may provide a stable binding site which is little affected by external medium factors. (ii) The stable binding site shows a high substrate specificity due to its intrinsic geometrical requirement for host–guest interaction. Moreover, we have observed the two modes of hydrophobic interaction of our paracyclophanes with various hydrophobic substrates: inclusion of the substrate into the macrocyclic cavity and substrate-binding by face-to-face interaction.[4h] These results tempted us to investigate the variation of substrate-binding features by introduction of quaternary nitrogens into the paracyclophane skeleton. The most interesting and novel aspect of this work would be the substrate-binding behaviour of an azaparacyclophane which has a deeper hydrophobic cavity provided by substitution of long alkyl branches on the macrocyclic skeleton (octopus-like structure).

RESULTS AND DISCUSSION

Preparation.—Azaparacyclophane derivatives obtained in this work are summarized in the Scheme; (7), (8),‡ (9), (11), (12), and (13) were isolated for the first time in this work. *NN'N''N'''*-Tetramethyl-2,11,20,29-tetra-aza[3.3.3.3]paracyclophane (6) was prepared by the condensation of terephthaloyl dichloride (3) and *NN'*-dimethyl-*p*-xylylenediamine (2a) under highly dilute conditions and subsequent reduction with lithium aluminium hydride (LAH).[5] For the synthesis of tetra-azaparacyclophane (6) and *NN'N''N''' 'N''' ''-N''' '''*-hexamethyl-2,11,20,29,38,47-hexa-aza[3.3.3.3.3.3]-paracyclophane (7), the condensation method was modified in this work to maintain constant reaction conditions. Solutions in benzene of the diacid chloride (3) and of the diamine (2a) were individually added dropwise to a large amount of benzene at the same rate and therefore the high dilution condition was maintained throughout the reaction. This procedure gave a mixture of the tetra-one (4) and the hexa-one (5). The quaternization of (6) and (7) by methyl iodide gave *NNN'N'N''N''N''' 'N''' '*-octamethyl-2,11,20,29-tetra-aza[3.3.3.3]paracyclophane tetraiodide (8) and *NNN'N'-N''N''N''' 'N''' 'N''' ''N''' '' N''' '' 'N''' '' '*-dodecamethyl-2,-11,20,29,38,47-hexa-aza[3.3.3.3.3.3]paracyclophane hexaiodide (9), respectively. The reaction was followed by infrared spectroscopy with attention to the disappearance of the characteristic N–Me band at 2 770 cm^{-1}. The azacyclophane (8), in which four benzene rings are placed facing each other, is rather rigid in its conformation, with an estimated cavity size of 4.5—5.5 Å. On the other hand, (9) is flexible in its conformation with an estimated maximum cavity size of 8—10 Å. These quaternized compounds (8) and (9) are soluble in aqueous media and may be used as enzyme models. *NN'N''-N''' '*-tetraoctyl-2,11,20,29-tetra-aza[3.3.3.3]paracyclophane (11) was obtained by the condensation of the diacid chloride (3) with *NN'*-dioctyl-*p*-xylylenediamine (2b) and subsequent reduction. The quaternization of (11) was attempted by several procedures using methyl iodide or methyl fluorosulphonate, but the desired product was not obtained. The low reactivity of (11) may be attributed to steric hindrance by the bulky alkyl moieties. *NN'N''N''' '*-Tetrakis-(10-carboxydecyl)-2,11,-20,29-tetra-aza[3.3.3.3]paracyclophane-3,10,21,28-tetra-one (13) was obtained by alkaline hydrolysis of *NN'-N''N''' '*-tetrakis-(10-methoxycarbonyldecyl)-22,11,20,9-

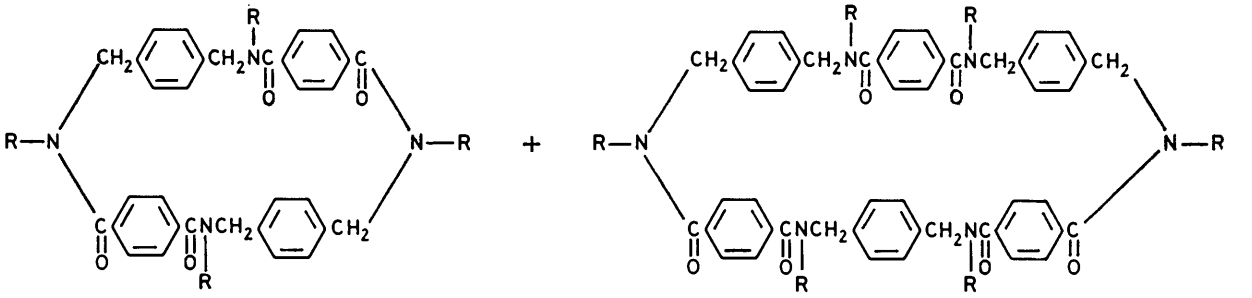
† Part 2, ref. 4*h*.

‡ The tetrafluoroborate salt of (8) has recently been prepared (I. Tabushi, Y. Kimura, and K. Yamamura, *J. Amer. Chem. Soc.*, 1978, **100**, 1304).



(1)

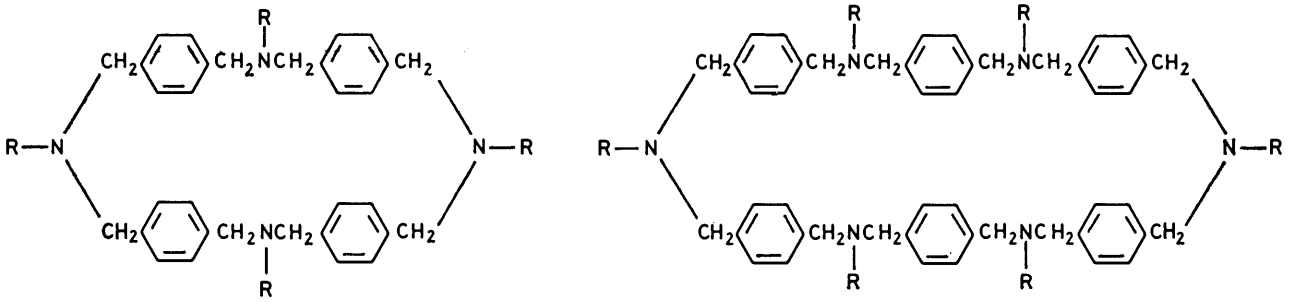
(2) a; R = Me
b; R = Me[CH$_2$]$_6$CH$_2$
c; R = MeO$_2$C[CH$_2$]$_{10}$



(4) R = Me
(10) R = Me[CH$_2$]$_6$CH$_2$
(12) R = MeO$_2$C[CH$_2$]$_{10}$
(13) R = HO$_2$C[CH$_2$]$_{10}$

(5) R = Me

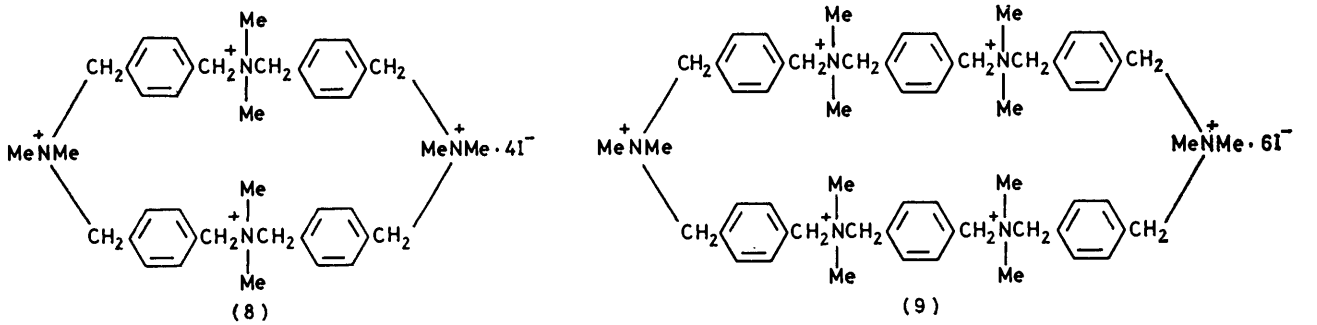
LAH



(6) R = Me
(11) R = Me[CH$_2$]$_6$CH$_2$

(7) R = Me

MeI



(8)

(9)

SCHEME

tetra-aza[3.3.3.3]paracyclophane-3,10,21,28-tetraone (12) which was prepared by the condensation of diacid chloride (3) with *NN'*-bis-(10-methoxycarbonyldecyl)-*p*-xylylenediamine (2c). The physical and analytical data for the azaparacyclophanes prepared in this work are listed in Table 1.

TABLE 1

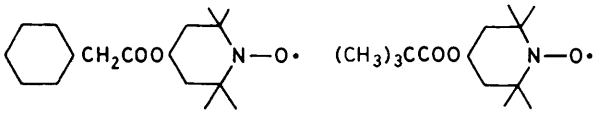
Physical and analytical data for azaparacyclophane derivatives

Cyclophane	Formula	Calc. (%) C	Calc. (%) H	Calc. (%) N	Found (%) C	Found (%) H	Found (%) N	M^+ (M. wt.)	M.p./°C	Crystal state
(6)								533 (532.77)	199—199.6	Needles
(7)	$C_{54}H_{66}N_6$	81.15	8.3	10.5	81.05	8.3	10.5	799 (799.16)	202.4—202.8	Needles
(8)	$C_{40}H_{56}N_4I_4 \cdot H_2O$	42.95	5.2	5.0	43.0	5.2	5.05		>280 (decomp.)	Prisms
(9)	$C_{60}H_{84}N_6I_6 \cdot 2H_2O$	42.7	5.0	5.0	42.5	5.25	5.0		>280 (decomp.)	Prisms
(11)	$C_{64}H_{100}N_4$	83.1	10.9	6.05	83.0	10.9	6.1	925 (925.53)	84.5—85.5	Needles
(12)	$C_{80}H_{116}N_4O_{12}$	72.4	8.8	4.25	72.5	8.75	4.25	1 326 (1 325.82)	184.2—185.7	Needles
(13)	$C_{76}H_{108}N_4O_{12}$	71.9	8.55	4.4	71.35	8.5	4.35		165—166.5	White powder

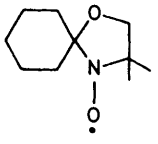
Incorporation of Spin-labelled Probes.—The interaction between synthetic azaparacyclophane and suitably bulky substrate was studied by using spin-labelled e.s.r. probes. Paton and Kaiser [6] have shown that rotational correlation time τ_c in solution is greater when a nitroxide radical is covalently bonded to β-cyclodextrin than when non-covalently associated. The rotational correlation time for the motion of a paramagnetic molecule can be obtained by equation (1): [7] $\Delta H_{(m=+1)}$ is the peak-to-peak line width in gauss of the derivative of the low field absorption line; $I_{(m=+1)}$ and $I_{(m=-1)}$ are the corresponding peak-to-peak heights for the low- and high-field lines, respectively. For the calculation of τ_c, A = 6.6 ×

$$\tau_c = A \cdot \Delta H_{(m=+1)}[(I_{(m=+1)}/I_{(m=-1)})^{1/2} - 1] \quad (1)$$

10^{-10}. This was obtained for di-t-butyl nitroxide,[8] and was used because it does not change much among various radicals. 2,2,6,6-Tetramethyl-1-oxyl-4-piperidyl cyclohexylacetate (14) and 2,2,6,6-tetramethyl-1-oxyl-4-



(14) (15)



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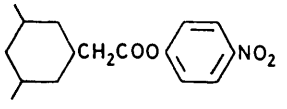

(17)

piperidyl 2,2-dimethylpropionate (15) were used as hydrophobic spin-labelled probes, and the corresponding data are listed in Table 2. These results suggest that Michaelis-type complexes are definitely formed between azacyclophanes and substrate (14). The extent of increase in rotational correlation time is comparable to that for the combination of spiro[1,2'-cyclohexane-(4',4'-dimethyloxazolidin-3'-oxyl)] (16) and α-cyclodextrin.[9] Since (15) is rather weakly incorporated into the cavity of azaparacyclophanes, the hydrophobicity of a substrate seems to be an important factor for the formation of a stable Michaelis-type complex. In spite of the difference in ring size between (8) and (9), the binding ability of (9) is almost similar in magnitude to that of (8), presumably because of the flexibility of the macrocyclic skeleton of (9) in aqueous media. In order that a substrate be effectively incorporated into the macrocyclic cavity, the host–guest interaction must be tight. Thus, (9) seems to undergo an appropriate conformational change so that the substrate is tightly incorporated into the hydrophobic cavity. This phenomenon can be referred to as the 'induced-fit' effect

TABLE 2

Isotropic nitrogen hyperfine splitting constants (A_N) and rotational correlation times (τ_c) for nitroxide radicals at room temperature

E.s.r. probe [a]	Host	Medium [b]	A_N/G	$10^{11}\tau_c$/s
(14), 5.0 × 10^{-5}M	None	MW	16.98	3.84
(14), 5.0 × 10^{-5}M	(8), 5.0 × 10^{-4}M	MW	17.12	10.86
(14), 5.0 × 10^{-5}M	(9), 5.2 × 10^{-4}M	MW	16.96	7.07
(14), 5.0 × 10^{-5}M	None	DEW	17.03	10.8
(14), 5.0 × 10^{-5}M	(13), 1.0 × 10^{-3}M	DEW	17.12	21.2
(15), 5.0 × 10^{-5}M	None	MW	16.91	5.90
(15), 5.0 × 10^{-5}M	(8), 5.0 × 10^{-4}M	MW	17.07	9.01
(15), 5.0 × 10^{-5}M	(9), 5.0 × 10^{-4}M	MW	17.06	9.07
(16), 3.0 × 10^{-5}M	None	Water		5.00
(16), 3.0 × 10^{-5}M	α-CD, 1.0 × 10^{-3}M	Water		10.0

[a] Alkaline hydrolysis of (14) and (15) was not detected throughout the measurements. [b] MW, methanol–water (1 : 9 v/v); DEW, dimethyl sulphoxide–ethanol–water (10 : 1 : 89 v/v) at pH 10.29 (5.2 × 10^{-3}M-sodium borate–3.5 × 10^{-2}M-sodium carbonate) and μ 0.15 (KCl).

postulated by Koshland for enzymatic reactions.[10] On the other hand, the isotropic nitrogen hyperfine splitting constants (A_N) in the presence of the azacyclophanes were not changed in any meaningful sense from those observed in their absence. Thus, the octopus cyclophane (13) may incorporate only the hydrophobic portion of the substrate into its deeper cavity while the nitroxide moiety remains exposed to the bulk phase.

Aggregation Behaviour of Octopus Cyclophane.—Since (13) is composed of a hydrophobic skeleton and four long alkyl branches with negative charges on their ends, it is expected to aggregate beyond a certain range of concentration in aqueous media. The critical micelle concentration (CMC) of (13) was determined by surface

tension measurements for solutions containing (13) at various concentrations in dimethyl sulphoxide–water (1 : 9 v/v) at pH 10.29, μ 0.15 (KCl), and 16 °C. A clear break point was observed at 3.15×10^{-4}M as shown in Figure 1. The value is lower than those for

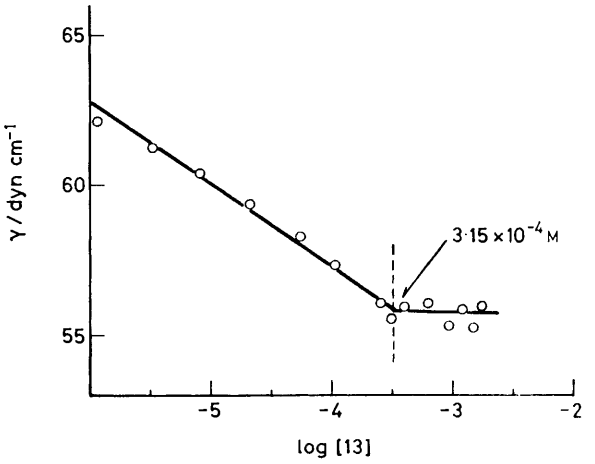

FIGURE 1 Correlation of surface tension γ with concentration of (13) in dimethyl sulphoxide–water (1 : 9 v/v) at 16 °C, pH 10.29 (9.8×10^{-3}M-sodium borate–3.0×10^{-2}M-sodium carbonate), and μ 0.15 (KCl)

the ionic surfactants bearing a long hydrocarbon group (10^{-2}—10^{-3}M) [1] and for cholic acid derivatives which bear a better resemblance to (13) from the structural viewpoint (10^{-2}—10^{-3}M) [11-14] in water at 25 °C. In a previous paper,[4g] we suggested that CMCs of ionic surfactants are larger than those of the corresponding non-ionic ones having similar apolar structure, and further confirmed the charge effect on CMC for paracyclophane derivatives: cationic [20]paracyclophane, 1.8×10^{-4}M in an aqueous buffer at pH 8.0 and 20 °C; neutral [20]paracyclophane, 1.8—3.3×10^{-6}M in ethanol–dioxan–water (10.9 : 1 : 88.1 v/v) at pH 8.33, μ 0.10 (KCl), and 40 °C. Thus, the CMC of (13) is comparable to that of the cationic [20]paracyclophane. Even though (13) bears four negative charges, the CMC value does not increase much beyond that for the paracyclophane bearing only one positive charge presumably due to its bulky rigid structure.

Substrate-binding Ability of Octopus Cyclophane.—Four hydrophobic alkyl chains in (13) may gather in the same direction by hydrophobic interaction between them in aqueous media in a similar manner to that observed for anionic micelles. Such hydrophobic branches may deepen the hydrophobic cavity of a paracyclophane. The substrate-binding ability of (13) was studied spectrophotometrically using several organic dyes as guest molecules. Host compounds such as cycloamyloses have been found to perturb the electronic spectra of various guest molecules upon complex formation.[15] A typical example of the spectral changes observed in this work is shown in Figure 2; the appearance of an isosbestic point is referred to the formation of a 1 : 1 complex. Rhodamine 6G may be incorporated into the cavity constructed by four alkyl chains and one cyclic skeleton of (13) to form a 1 : 1 complex and the binding behaviour is not dependent on the micelle formation. The dye was also confirmed to be incorporated into the usual anionic micelle (sodium dodecyl sulphate), although the spectral change was complicated probably due to the formation of complexes of more than one species in a CMC region. The absorbancy changes resulting from the complex formation between (13) and an organic dye in aqueous media were treated according to the Benesi–Hildebrand equation (2):[16] [E] and [S] stand for the initial stoicheiometric concentrations of (13) and dye, respectively, ΔA is the extent of absorbance change upon addition of (13), $\Delta\varepsilon$ represents the difference in molar extinction coefficient between bound and free dye, and K_d is the dissociation constant for a 1 : 1 complex formed between (13) and dye.

$$\frac{[E][S]}{\Delta A} = \frac{K_d}{\Delta\varepsilon} + \frac{([E] + [S])}{\Delta\varepsilon} \qquad (2)$$

The dissociation constants for combination of (13) and several guest compounds determined in this manner are shown in Table 3. Cationic dyes such as Rhodamine 6G and Quinaldine Red and a suitably bulky neutral dye, 1-(2-pyridylazo)-2-naphthol (PAN), undergo complex formation with (13) in 1 : 1 stoicheiometry. On the other hand, an anionic dye, Methyl Orange, and sodium *p*-nitrophenolate did not show any spectral change upon addition of (13). The results indicate that the cationic dyes are incorporated into the cavity of (13) more tightly than the neutral one of similar size and the smaller neutral substrate, and that the anionic substrates do not undergo any significant host–guest interaction. It is now interesting to compare the binding ability of cyclo-

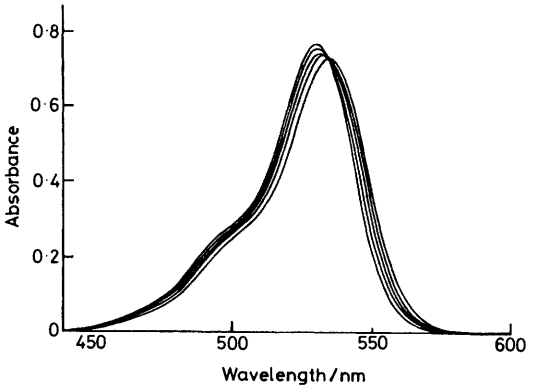

FIGURE 2 Electronic spectra of Rhodamine 6G in the presence of (13): in dimethyl sulphoxide–dioxan–water (10 : 1 : 89 v/v) at 39.4 ± 0.1 °C, pH 10.29 (5.2×10^{-3}M-sodium borate–3.5×10^{-2}M-sodium carbonate), and μ 0.15 (KCl); concentration of Rhodamine 6G, 1.01×10^{-5}M; concentration range of (13), $(0.67$—$6.67) \times 10^{-4}$M. The absorption maximum at 530 nm shifted to longer wavelength upon addition of (13) with decrease in intensity

amyloses with that of (13). Cramer *et al.* have shown that α-cyclodextrin (α-CD) forms a 1 : 1 adduct with nitrophenol at acidic and alkaline pH and that the binding of a series of azo dyes with α-CD is highly stereo-

TABLE 3

Dissociation constants for the inclusion complexes of (13) [a]

Substrate [b]	K_d/ mol l^{-1}	$\lambda_{iso.}$ [c]/ nm	$\lambda_{meas.}$ [d]/ nm	10^4[13]/M
Rhodamine 6G (1.01×10^{-5}M)	2.1×10^{-4}	535	530	0.67—6.67
Quinaldine Red (3.89×10^{-6}M)	2.2×10^{-4}	520	478	0.19—6.30
PAN (6.77×10^{-6}M)	6.78×10^{-4}	494	550	1.65—8.25

[a] All measurements were carried out in dimethyl sulphoxide–ethanol–water (10 : 1 : 89 v/v) at pH 10.29 (5.2×10^{-3}M sodium borate–3.5×10^{-2}M sodium carbonate), μ 0.15 (KCl), and 39.4 ± 0.1 °C. [b] *p*-Nitroaniline (9.88×10^{-6}M), Methyl Orange (1.01×10^{-5}M), and *p*-nitrophenolate (1.00×10^{-5}M) were also used but no spectral changes due to complex formation were detected. [c] Observed isosbestic point. [d] Wavelength used for measurements.

specific.[17] We have investigated in this work the interaction of Rhodamine 6G or PAN with β-cyclodextrin (β-CD) but have failed to observe the formation of the corresponding complexes. Rhodamine 6G seems to be too bulky to be incorporated into the cavity of β-CD (7.5 Å). These results indicate that the electrostatic and hydrophobic interactions play major roles in the binding of (13) with guest molecules, while stereospecificity is exercised in the binding between guest and host molecules for the cycloamylose systems.

Menger and Portnoy have shown that the laurate anion micelle inhibits the hydrolysis of several *p*-nitrophenyl esters.[18] We have observed in this work similar kinetic behaviour with (13) in the alkaline hydrolysis of *p*-nitrophenyl 3,5-dimethylcyclohexylacetate (17), and the resulting saturation-type kinetics are shown in Figure 3. The kinetic data were, therefore, analysed in terms of the Michaelis–Menten treatment based on the reaction pathway given by equation (3): E, S, and P stand for catalyst, substrate, and hydrolysis products, respectively, (ES) is the Michaelis-type inclusion complex; k_{hyd} and k'_{hyd} refer to the rate constants for alkaline

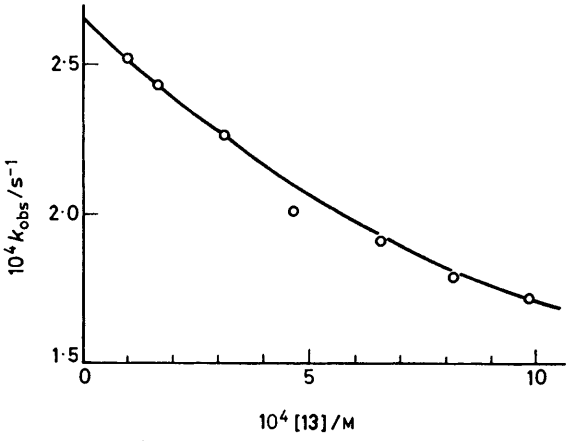

FIGURE 3 Correlation of pseudo-first-order rate constant k_{obs} with concentration of (13) for hydrolysis of *p*-nitrophenyl 3,5-dimethylcyclohexylacetate (17) at 20.5 ± 0.1 °C, pH 10.29 (5.2×10^{-3}M-sodium borate–3.5×10^{-2}M-sodium carbonate), and μ 0.15 (KCl) in dimethyl sulphoxide–dioxan–water (10 : 1 : 89 v/v). Solid line refers to the calculated curve (see text)

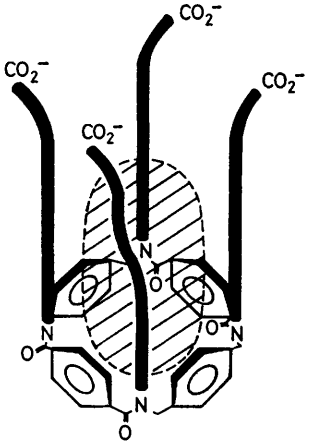

FIGURE 4 Schematic representation of the substrate-binding feature of (13); the shaded portion refers to an incorporated guest molecule

hydrolysis of the free and the bound substrate, respectively; and K_b is the binding constant for the formation of (ES). Equation (4) is consistent with the reaction

$$\begin{array}{ccccc} E + S & \overset{K_b}{\rightleftharpoons} & (ES) & \xrightarrow{k'_{hyd}} & P \\ & \underset{k_{hyd}}{\searrow} & & & \\ & & P & & \end{array} \qquad (3)$$

scheme given by equation (3). The dissociation constant for the complex-forming equilibrium between (13)

$$\frac{1}{k_{obs} - k_{hyd}} = \frac{1}{(k'_{hyd} - k_{hyd})K_b[13]} - \frac{1}{k'_{hyd} - k_{hyd}} \qquad (4)$$

and (17) and the first-order rate constant for the hydrolysis of the bound substrate were evaluated from this relationship: $1/K_b$, 1.79×10^{-3} mol l^{-1}; k'_{hyd}, 1.80×10^{-6} s^{-1}. The rate of hydrolysis of (17) was retarded by 147-fold relative to the spontaneous rate. The active ester carbonyl of the substrate seems to be effectively shielded from the attack of external hydroxide ion by hydrophobic and electrostatic effects. The dissociation constant for the (13)–PAN system is smaller than that for the (13)–3,5-dimethylcyclohexylacetate (17). The cavity size constructed by four benzene rings of aza-[3.3.3.3]paracyclophane and four alkyl branches is suitable for incorporation of a naphthyl moiety. Accordingly, PAN can be more tightly bound to (13) than (17) since the latter does not have such a moiety in the molecule. It should be pointed out that (13) effectively incorporates bulky guest molecules in a concentration range above and below its CMC. This means that the azacyclophane itself forms an effective hydrophobic binding site (Figure 4) and the micelle formation has little effect on the substrate-binding process.

In conclusion, azacyclophanes involving quaternary nitrogens in the cyclic skeleton, (8) and (9), and octopus cyclophane which bears long alkyl chains as branches

of the skeleton (13) incorporate suitably bulky substrates into their macrocyclic cavities by effective hydrophobic interaction. Moreover, (13) undergoes host–guest interaction with bulky cationic substrates and to a lesser extent with neutral ones of similar size. The substrate specificity exercised by (13) is primarily due to the electrostatic and hydrophobic interactions with guest molecules.

EXPERIMENTAL

I.r. spectra were taken with a JASCO DS-403G grating spectrophotometer. ^{1}H N.m.r. spectra were obtained with either a Varian A-60 or a Bruker WH-90 FT spectrometer with tetramethylsilane (in deuteriochloroform or [$^2H_6$]-DMSO) and 3-(trimethylsilyl)propanesulphonic acid (in deuterium oxide) as internal references. Melting points were measured using capillary tubes with a Yamato MP-1 melting point apparatus (oil-bath type). E.s.r. spectra were recorded at room temperature on a JEOL JES-ME-3 X-band spectrometer with the manganese(II) ion, diffused thermally into magnesium oxide, as reference. Electronic spectra were recorded on a Union Giken high sensitivity spectrophotometer SM-401. pH-Measurements were carried out with a Beckman Expandomatic SS-2 pH meter equipped with a Metrohm EA-125 combined electrode, calibrated against standard aqueous buffers. Surface tension measurements were performed at room temperature with a Kyowa DIGI-O-MATIC ESB-IV electro-surface balance assembled by the Wilhelmy principle. The platinum blade was repeatedly washed with distilled water and heated to incandescence in the flame of an alcohol lamp after each measurement. Gel-filtration chromatography was carried out on a column of Sephadex LH-20. Methanol–chloroform (3 : 2 or 1 : 1 v/v) was used as an eluant and components eluted were detected by u.v. absorption at 265 nm.

Terephthalaldehyde (1) was purchased from Nakarai Chemicals and recrystallized from methanol–water (1 : 9 v/v); m.p. 119—120 °C. *NN'*-Dimethyl-*p*-xylylenediamine (2a) was synthesized from (1) and trimethylamine by the method of Sander,[19] b.p. 112—113 °C at 3 mmHg (lit.,[13] 141 °C at 15 mmHg). Terephthaloyl dichloride (3) was purchased from Nakarai Chemicals and distilled before use, b.p. 115 °C at 2 mmHg, m.p. 83 °C. Palladium chloride (Ishizu Pharmaceutical Co.), n-octylamine (Tokyo Kasei Kogyo Co.), 1-aminoundecanoic acid (Nakarai Chemicals), and methyl iodide (Wako Pure Chemical Industries) were obtained from commercial sources. *p*-Nitrophenyl 3,5-dimethylcyclohexylacetate (17) was prepared previously.[4g] 2,2,6,6-*Tetramethyl*-1-*oxyl*-4-*piperidyl cyclohexylacetate* (14) and 2,2,6,6-*tetramethyl*-1-*oxyl*-4-*piperidyl* 2,2-*dimethylpropionate* (15) were prepared from the corresponding acid chlorides and 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl in the presence of dry pyridine in a manner similar to that reported by Waggoner *et al.* for the preparation of 2,2,6,6-tetramethyl-1-oxyl-4-piperidyl octanoate;[20] (14) had m.p. 44—46 °C (Found: C, 69.75; H, 8.8; N, 4.75. C_{17}-$H_{24}O_3N$ requires C, 70.3; H, 8.35; N, 4.8%); (15) had m.p. 97—98 °C (Found: C, 64.45; H, 10.4; N, 5.7. $C_{13}H_{26}NO_3$ requires C, 63.9; H, 10.7; N, 5.75%). Rhodamine 6G (Kishida Chemical Co.), Quinaldine Red (Nakarai Chemicals), 1-(2-pyridylazo)-2-naphthol (Dojin Pharmaceutical Institute), Methyl Orange (Wako Pure Chemical Industries), and *p*-nitroaniline (Ishizu Pharmaceutical Co.) were obtained from commercial sources as extra pure grade and used without further purification.

NN'N''N''' '-*Tetramethyl*-2,11,20,29-*tetra-aza*[3.3.3.3]-*paracyclophane* (6) *and* NN'N''N''' 'N''' ''N''' '' '-*hexamethyl*-2,11,20,29,38,47-*hexa-aza*[3,3,3,3,3,3]*paracyclophane* (7).—A solution of terephthaloyl dichloride (3) (6.1 g) in dry benzene (500 ml) and a solution of *NN'*-dimethyl-*p*-xylylenediamine (2a) (9.8 g) in dry benzene (500 ml) were added dropwise to refluxing dry benzene (1.5 l) at the same rate with vigorous stirring over *ca.* 29 h. Nitrogen gas was passed through the reaction vessel during the reaction. Stirring was continued for another 1 h at reflux temperature. The hot reaction mixture was filtered, and the filtrate was evaporated to afford a white solid (3.1 g, 35% from terephthaloyl dichloride). A mixture of the solid (1.0 g) and lithium aluminium hydride (LAH) (3.0 g) in dry dioxan (300 ml) was refluxed with stirring for 72 h. The reaction mixture was cooled to 0 °C, and 5% aqueous sodium hydroxide (12 ml) was added. The mixture was refluxed for 1 h, filtered, and cooled. The filtrate was evaporated and the crude products (6) and (7) (370 mg) were separated by gel-filtration chromatography on Sephadex LH-20 with methanol–chloroform (3 : 2 v/v) as eluant; (6) (40 mg) had $\nu_{max.}$ (KBr) 2 770 cm^{-1} (NCH_3), δ($CDCl_3$) 2.32 (12 H, s, NCH_3), 3.32 (16 H, s, benzyl), and 7.21 (16 H, s, aromatic); (7) (270 mg) showed $\nu_{max.}$ (KBr) 2 770 cm^{-1} (NCH_3), δ($CDCl_3$) 2.16 (18 H, s, NCH_3), 3.48 (24 H, s, benzyl), and 7.29 (24 H, s, aromatic).

NNN'N'N''N''N''' 'N''' '-*Octamethyl*-2,11,20,29-*tetra-aza*-[3.3.3.3]*paracyclophane Tetraiodide* (8).—A mixture of the azaparacyclophane (6) (50 mg) and methyl iodide (500 mg) in dry dimethylformamide (15 ml) was stirred at 100 °C for 3 h. After the mixture was cooled to room temperature, the precipitates were filtered off and recrystallised from water (47 mg, 45%); $\nu_{max.}$(KBr) 3 420br cm^{-1} (OH); δ(D_2O) 3.14 (24 H, s, NCH_3), 4.67 (16 H, s, benzyl), and 7.59 (16 H, s, aromatic).

NNN'N'N''N''N''' 'N''' 'N''' ''N''' ''N''' '' 'N''' '' '-*Dodeca-methyl*-2,11,20,29,38,47-*hexa-aza*[3.3.3.3.3.3]*paracyclo-phane Hexaiodide* (9).—A mixture of azaparacyclophane (7) (50 mg) and methyl iodide (1.0 g) in dry dioxan (15 ml) was stirred for 1 h under reflux and for another 1 h at room temperature. The precipitates were recovered and washed with dioxan (5 ml), potassium hydroxide–methanol (5%; 10 ml), and methanol (5 ml). The crude material was recrystallised from water, yield 95 mg (91%), $\nu_{max.}$ (KBr) 3 420 cm^{-1} (OH); δ(D_2O) 3.08 (36 H, s, NCH_3), 4.60 (24 H, s, benzyl), and 7.82 (24 H, s, aromatic).

NN'-*Dioctyl*-p-*xylylenediamine* (2b).—A mixture of terephthalaldehyde (10.0 g), n-octylamine (25.0 g), palladium chloride (540 mg), and Norit SX-II (5.4 g) in ethanol (100 ml) was placed in a 300 ml autoclave and the mixture was agitated at room temperature at an initial hydrogen pressure of 20 kg cm^{-2} for 7 h. The catalysts were removed from the mixture by filtration and the filtrate was evaporated *in vacuo*. The residue was dissolved in absolute ethanol (100 ml) and dry hydrogen chloride was bubbled through the solution for 3 h. The precipitates were recovered by filtration and dissolved in aqueous sodium hydroxide (15%; 200 ml). The alkaline solution was extracted with benzene (2 × 100 ml) and the extract dried (Na_2SO_4). Evaporation gave a viscous oil which crystallised on standing at room temperature (14.7 g, 54%), m.p. 39.0—40.0 °C; $\nu_{max.}$ (neat) 2 900 cm^{-1} (NH); δ($CDCl_3$) 0.87 [6 H, br t, $(CH_2)_6CH_3$], 1.25 [24 H, br s, $(CH_2)_6CH_3$]

2.58 [4 H, t, $NHCH_2(CH_2)_6CH_3$], 3.71 (4 H, s, benzyl), and 7.28 (4 H, s, aromatic).

NN'N''N'''-Tetraoctyl-2,11,20,29-tetra-aza[3.3.3.3]*paracyclophane* (11).—Solutions of terephthaloyl dichloride (2.03 g) and diamine (2b) (3.6 g) in dry benzene (each 100 ml) were added dropwise to a refluxing solution (400 ml) of triethylamine (1.01 g) in dry benzene at the same rate with vigorous stirring under nitrogen over 10 h. Stirring was continued for another 1 h under reflux. The hot reaction mixture was filtered and the precipitate washed with dioxan to afford *NN'N''N'''*-tetraoctyl-2,11,20,29-tetra-aza[3.3.3.3]-paracyclophane-3,10,21,28-tetraone (10) (1.4 g, 28.6%), $\nu_{max.}$ (KBr) 1 620 cm^{-1} (C=O). The crude tetraone (1.4 g) and LAH (4.5 g) were placed in dry dioxan (300 ml) and the mixture was refluxed for 73 h with stirring. The mixture was cooled to 0 °C, and aqueous sodium hydroxide (5%; 15 ml) added. The reaction mixture was then refluxed for 1 h with stirring. The hot mixture was filtered and the filtrate was evaporated *in vacuo* to obtain a solid which was washed with ethanol and recrystallised from n-butyl alcohol (438 mg, 33%), ninhydrin negative, $\nu_{max.}$ (KBr) 2 950 (CH), 2 860 (CH), and 2 800 cm^{-1} (CH); $\delta(CDCl_3)$ 0.89 [12 H, br t, $CH_2(CH_2)_6CH_3$], 1.25 [48 H, br s, $CH_2(CH_2)_6CH_3$], 2.43 [8 H, t, $CH_2(CH_2)_6CH_3$], 3.38 (16 H, s, benzyl), and 7.27 (16 H, s, aromatic).

NN'-Bis-(10-*methoxycarbonyldecyl*)-p-*xylylenediamine* (2c).—10-Methoxycarbonyldecylamine hydrochloride was prepared by the esterification of 1-aminoundecanoic acid with dry methanol,[21] m.p. 160—162 °C (lit.,[21] 160 °C). A mixture of terephthalaldehyde (5.0 g), 10-methoxycarbonyldecylamine dihydrochloride (20.0 g), palladium chloride (216 mg), Norit SX-II (2.0 g), and triethylamine (10.0 g) in methanol (180 ml) was placed in a 300 ml autoclave and the mixture agitated at room temperature with an initial hydrogen pressure of 22 kg cm^{-2} for 5 h. The reaction mixture was then warmed to 50 °C and filtered. The filtrate was evaporated *in vacuo* to afford the dihydrochloride of (2c) (9.2 g). The product was dissolved in methanol–water (9 : 11 v/v) (200 ml) containing sodium hydrogencarbonate (10.0 g). The solution was extracted with chloroform (2 × 100 ml) and the extract dried (Na_2SO_4). Evaporation gave an oil which, on standing at room temperature, gave *crystals* (8.0 g, 30%), m.p. 63—63.5 °C, $\nu_{max.}$ (neat) 2900 (NH) and 1740 cm^{-1} (C=O); $\delta(CDCl_3)$ 1.28 [32 H, br s, $CH_2(CH_2)_8CH_2COOCH_3$], 2.30 [4 H, t, $CH_2(CH_2)_8CH_2COOCH_3$], 2.60 [4 H, t, $CH_2(CH_2)_8CH_2COOCH_3$], 3.67 (6 H, s, $COOCH_3$), 3.77 (4 H, s, benzyl), and 7.30 (4 H, s, aromatic) (Found: C, 72.05; H, 10.5; N, 5.25. $C_{32}H_{56}N_2O_4$ requires C, 72.15; H, 10.6; N, 5.25%).

NN'N''N''' -Tetrakis-(10-*methoxycarbonyldecyl*)-2,11,-20,29-*tetra-aza*[3.3.3.3]*paracyclophane*-3,10,21,28-*tetraone* (12).—Solutions of terephthaloyl dichloride (1.52 g) and (2c) (4.0 g) in dry benzene (each 100 ml) were added dropwise to a refluxing solution of triethylamine (21.0 g) in dry benzene (100 ml) at the same rate with vigorous stirring under nitrogen over 11 h and the mixture refluxed with stirring for a further 1 h. The hot mixture was filtered and the filtrate evaporated to give a white solid which was washed thoroughly with methanol and purified on a column of silica gel (Wako gel C-100). The fraction eluted with methylene chloride–ethyl acetate (5 : 2 v/v) was collected and further purified by gel-filtration chromatography (Sephadex LH-20; methanol–chloroform 1 : 1 v/v) (1.09 g, 11%), ninhydrin negative, $\nu_{max.}$ (KBr) 2 900 (CH), 2 840 (CH), 1 740 (C=O), and 1 630 cm^{-1} (C=O); $\delta(CDCl_3)$ 1.27 [64 H, br s, $CH_2(CH_2)_8CH_2COOCH_3$], 2.30 [8 H, t, $CH_2(CH_2)_8CH_2COOCH_3$], 3.50 [8 H, t, $CH_2(CH_2)_8CH_2COOCH_3$], 3.67 [12 H, s, $CH_2(CH_2)_8CH_2COOCH_3$], 4.50 (8 H, br s, benzyl), and 7.27 (16 H, s, aromatic).

NN'N''N''' -Tetrakis-(10-*carboxydecyl*)-2,11,20,29-*tetra-aza*[3.3.3.3]*paracyclophane*-3,10,21,28-*tetraone* (13).—A mixture of (12) (600 mg) and aqueous sodium hydroxide (5%; 6 ml) in methanol (60 ml) was refluxed for 27 h and allowed to stand overnight at room temperature. The mixture was evaporated *in vacuo* to remove methanol, and water (50 ml) was added to the residue. The aqueous solution was cooled to 0 °C and adjusted to pH 3 by adding aqueous citric acid. The precipitate was filtered off, washed with water, and recrystallised from acetone (460 mg, 80%), $\nu_{max.}$ (KBr) 2 900 (CH), 2 840 (CH), 1 720 (C=O), and 1 620 cm^{-1} (C=O); $\delta([^2H_6]DMSO)$ 1.27 [64 H, br s, $CH_2(CH_2)_8CH_2COOH$], 2.20 [8 H, t, $CH_2(CH_2)_8CH_2COOH$], 3.50 [8 H, br t, $CH_2(CH_2)_8CH_2COOH$], 4.50 (8 H, br s, benzyl), and 7.11 (16 H, br s, aromatic).

The authors are grateful to Professor Masaru Yamaguchi and Dr. Tsutomu Katsuki of our University for mass spectral measurements.

[8/1069 *Received*, 8*th June*, 1978]

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