Syntheses of Macrocyclic Enzyme Models. Part 2.[1] Preparation and Substrate-binding Properties of [10.10]Paracyclophanes

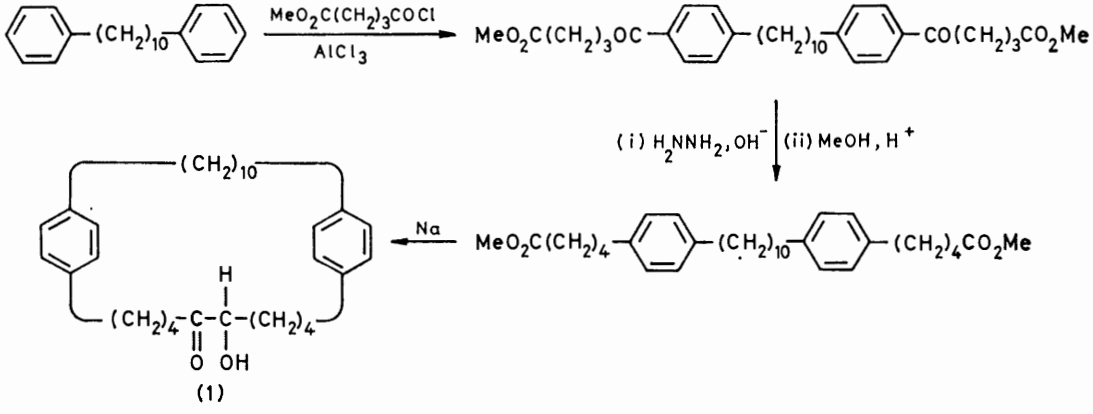
By **Yukito Murakami,* Yasuhiro Aoyama, Masaaki Kida, Akio Nakano, Kazuyuki Dobashi, Chieu Dinh Tran,** and **Yoshihisa Matsuda,** Department of Organic Synthesis, Faculty of Engineering, Kyushu University, Fukuoka 812, Japan

A water-soluble [10.10]paracyclophane (7) was found to form association complexes in 1 : 1 stoicheiometry with a series of *p*-nitrophenyl carboxylates bearing an alicyclic moiety (8)—(12). The hydrolysis of the substrate ester trapped in the paracyclophane cavity was moderately (by 6.5-fold) to significantly (by 99-fold) retarded relative to that in bulk solution. The binding constant for complex formation is relatively large (2.0×10^3—2.8×10^4 l mol^{-1}) and increases as the hydrophobicity of the substrate increases. The geometrical mode for interaction of the paracyclophane with the substrate is most plausibly face-to-face type. Formation of the association complex with (7) was also detected spectroscopically by using 4-cyano-1-dodecylpyridinium iodide (13) or a nitroxide radical bearing a hexadecanoyl moiety (14) as a pseudo-substrate. The CT probe (13) exhibited a new CT-transition band at 330 nm upon complex-formation with (7), which suggests that the micro-environment of the chromophore is less polar than in water but more polar than in methanol. The nitroxide radical (14) was found to lose its rotational mobility significantly upon complex-formation with (7); the rotational correlation time increases from 0.2—0.8 $\times 10^{-10}$ s in ethanol or benzene to 7.7—11.6 $\times 10^{-10}$ s. On the other hand, the isotropic nitrogen hyperfine splitting constant for (14) bound with the paracyclophane is practically the same as that expected in water without complexation with (7). The nature of the substrate-binding site of (7) was characterized on the basis of these spectral data.

HYDROPHOBIC interactions are the most universal driving forces for substrate-binding by many enzymes. Recently we have investigated the catalytic efficiency of various [20]paracyclophanes to explore novel enzyme model systems.[2-10] Functional paracyclophanes show significant rate effects on the deacylation of carboxylic esters due to their hydrophobic interaction with the substrates.[2,3,7-9] Particular cyclophane derivatives with a monofunctional group provide examples of novel hydrophobic [6] and electrostatic catalysis.[4] The most attractive observation is, however, the bifunctional catalysis played by some paracyclophanes, one of nucleophilic (an ionized [5] or unionized oxime group [10]) and the other of acidic character (a quaternary ammonium group [5] or an imidazole group co-ordinated by the cupric ion [10]). These groups are fixed in a definite spatial orientation so that co-operative catalysis can be exercised. Since we have realized that paracyclophane macrocycles are promising organic catalysts which may show various aspects of enzyme-like functions in a condensed sense, it has become necessary to investigate the variation of catalytic features upon modification of the macrocyclic skeleton. We have thus now prepared water-soluble [10.10]paracyclophanes, higher homologues of [20]paracyclophanes studied previously, and have characterized the substrate-binding features from kinetic and spectroscopic viewpoints.

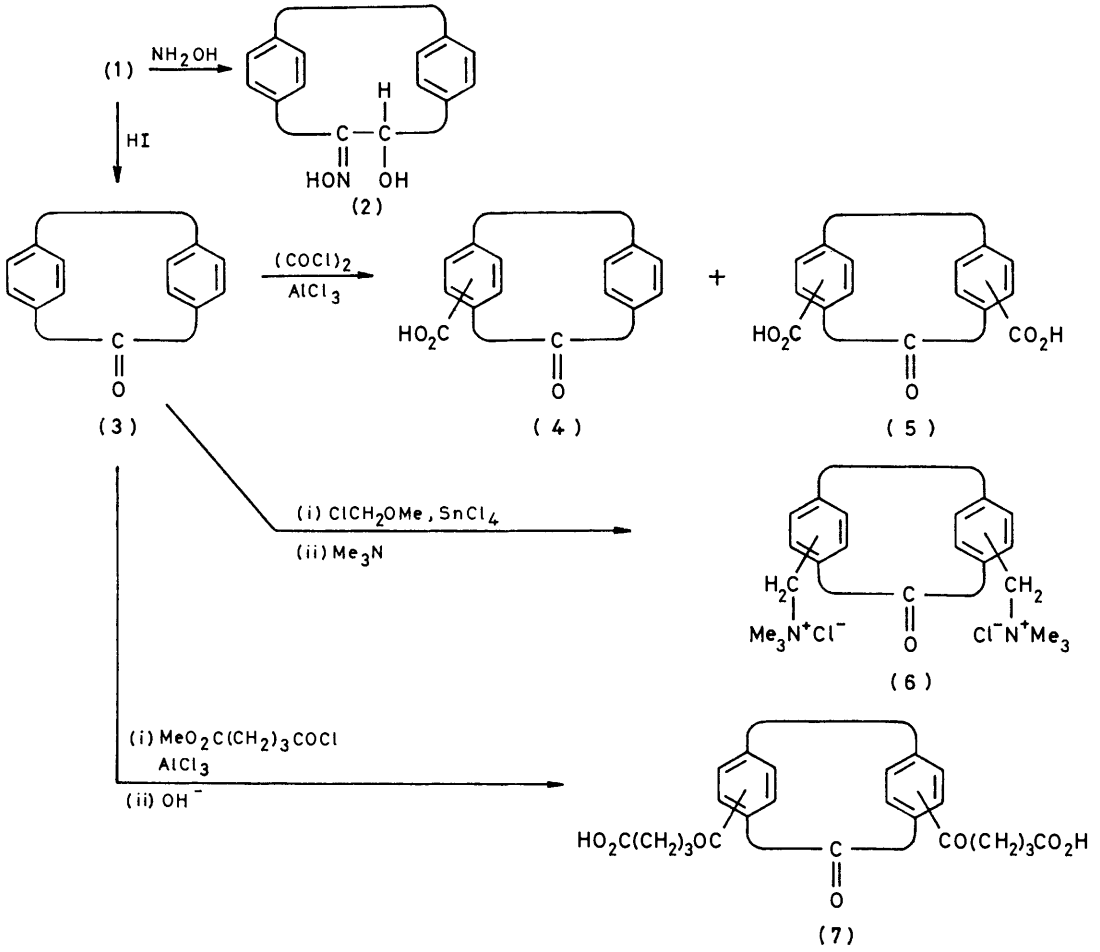
RESULTS AND DISCUSSION

Preparation.—The acyloin form of [10.10]paracyclophane (1) was prepared from 1,10-diphenyldecane *via* a three-step procedure; *i.e.* Friedel–Crafts acylation with 4-methoxycarbonylbutyryl chloride, Wolff–Kishner reduction followed by esterification, and acyloin condensation by high dilution method (Scheme 1). Materials derived from (1) are summarized in Scheme 2. The acyloin was either converted into the hydroxyimino alcohol (2) by treatment with hydroxylamine or reduced to the ketone (3) with hydriodic acid. The solubility of a cyclophane in water was much improved by introduction of a carboxy or a quaternary ammonium group on the skeleton. Such an ionic substituent placed in the vicinity of a hydrophobic site is, however, effective in changing the water structure around it so as to weaken



SCHEME 1

the hydrophobic interaction.[11] Thus, some attempts were made to introduce only one carboxy or quaternary ammonium group into the ketone (3), but these failed to give the desired products. Direct carboxylation with oxalyl chloride gave a mixture of monocarboxy (4) and dicarboxy (5) derivatives. The reaction of (3) with chloromethyl methyl ether, catalyzed by stannic chloride, gave the corresponding chloromethyl derivative, which was converted into the ammonium salt (6) by treatment with triethylamine. The major product of this reaction was the di-substituted derivative (6). We finally prepared a dicarboxy-derivative (7) in which the carboxy group was separated from the benzene ring by four carbon atoms. Friedel–Crafts acylation of (3) with 4-methoxycarbonylbutyryl chloride followed by hydrolysis readily gave (7).



SCHEME 2

Interaction of (7) *with Alicyclic Moieties.*—The interaction of (7) with aliphatic moieties of six- or ten-membered rings in aqueous media was investigated from a kinetic viewpoint. The rates for alkaline hydrolyses of a series of *p*-nitrophenyl carboxylates bearing an alicyclic moiety (8)—(12) were retarded in the presence of an excess of (7). The hydrolyses of (8)—(11) were carried out under the following conditions: 20.0 ± 0.1 °C; in ethanol–dioxan–water (10.9 : 1 : 88.1 v/v) with pH 10.8 ($Na_2B_4O_7$–Na_2CO_3, 0.05 M) and μ 0.15 (KCl); initial substrate concentration 1.0×10^{-5}M for (8)–(10) and 1.0×10^{-6}M for (11). Substrate (12) bearing additional methyl substituents on the nitrophenyl moiety was unreactive under the conditions described above and its hydrolysis required more vigorous conditions: 40.0 ± 0.1 °C; pH 11.8 ($NaOH$–Na_2HPO_4, 0.05M); initial substrate concentration 2.0×10^{-6}M. The observed extent of inhibition is attributed to the formation of an association complex between (7) and a substrate. In this complex the substrate ester is protected from attack by an external hydroxide ion by the steric effect of the cyclophane skeleton and/or the electrostatic effect of the carboxylate groups on the macrocycle which acts to repel an approaching hydroxide ion.* The paracyclophane (7) was shown to exist in a monomeric form under the conditions employed; the surface tension of the aqueous solution of (7) ($<10^{-3}$M) at 32 °C, pH 10.0 ($Na_2B_4O_7$–Na_2CO_3, 0.025M), and μ 0.10 (KCl) steadily decreases with increasing paracyclophane concentration without any

* Inhibition of ester hydrolysis is rather common in anionic micelles, see ref. 12.

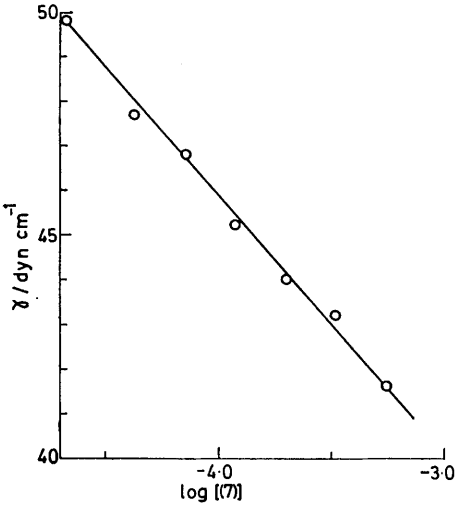


FIGURE 1 Correlation of surface tension γ with concentration for aqueous solutions of (7) at 32 °C, pH 10.0, and μ 0.10 (KCl)

break point corresponding to the critical micelle concentration of a micelle-forming surfactant [13] (Figure 1). The pseudo-first-order rate constants for hydrolyses of (8)—(12) in the presence of various amounts of (7) are shown in Figures 2 and 3. In all cases saturation-type kinetics were observed with respect to cyclophane concentration. All the kinetic data are consistent with the following scheme:

$$\begin{array}{ccccc} S + C & \underset{K_b}{\rightleftharpoons} & (SC) & \xrightarrow[k'_{hyd}]{} & P \\ k_{hyd}\downarrow & & & & \\ P & & & & \end{array}$$

Substrate S and paracyclophane C yield association complex (SC) at a 1 : 1 molar ratio with binding constant K_b, and the free and bound substrates undergo hydrolysis with rate constants k_{hyd} and k'_{hyd}, respectively. The observed rate constant ($k_{obs.}$) is related to the cyclophane concentration for this scheme by equation (1), where $[C]_T$ stands for the total stoicheiometric concentration of the paracyclophane. The double reciprocal plot,

$$\frac{1}{k_{hyd} - k_{obs.}} = \frac{1}{k_{hyd} - k'_{hyd}} + \frac{1}{(k_{hyd} - k'_{hyd})K_b[C]_T} \quad (1)$$

$1/(k_{hyd} - k_{obs.})$ *vs.* $1/[C]_T$, yields a straight line with a correlation coefficient larger than 0.992 as shown typically for (9) in Figure 4, and k'_{hyd} and K_b values were evaluated from the slope and intercept (Table 1). The

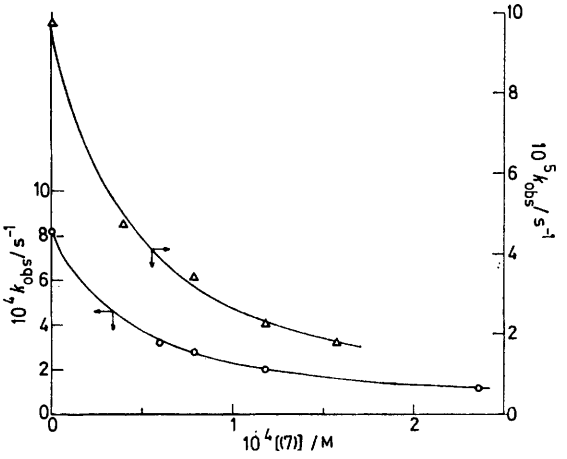


FIGURE 3 Correlations of pseudo-first-order rate constant $k_{obs.}$ with concentration of (7) for hydrolyses of (11) (○) at 20.0 °C and pH 10.8, and of (12) (△) at 40.0 °C and pH 11.8 in ethanol–dioxan–water (10.9 : 1 : 88.1 v/v) (μ 0.15 with KCl). Substrate concentrations: (11), 1.0×10^{-6}M; (12), 2.0×10^{-6}M

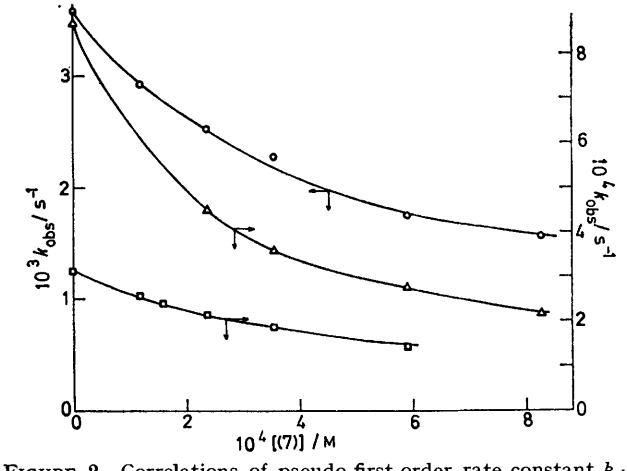


FIGURE 2 Correlations of pseudo-first-order rate constant $k_{obs.}$ with concentration of (7) for hydrolyses of (8) (○), (9) (□), and (10) (△) at 20.0 °C and pH 10.8 in ethanol–dioxan–water (10.9 : 1 : 88.1 v/v) (μ 0.15 with KCl); substrate concentrations 1.0×10^{-5}M

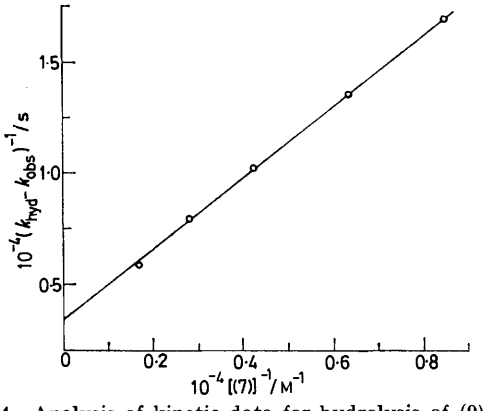


FIGURE 4 Analysis of kinetic data for hydrolysis of (9) in the presence of (7) by means of equation (1): kinetic measurements were carried out at 20.0 °C and pH 10.8 in ethanol–dioxan–water (10.9 : 1 : 88.1 v/v) (μ 0.15 with KCl)

ratio k_{hyd}/k'_{hyd} is a direct measure of the extent of rate inhibition due to complex formation. It is interesting to note a qualitative correlation between k_{hyd} and k_{hyd}/k'_{hyd} values: the less reactive the ester is in a bulk phase (smaller k_{hyd}) the more severely inhibited its hydrolysis is in the association complex (larger k_{hyd}/k'_{hyd}), suggesting that the ester reactivity in the association complex is also controlled primarily by the steric effect.

The binding constants are in the range 10^3—10^4 l mol^{-1} and are larger than those for the complexes formed

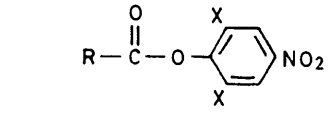
TABLE 1

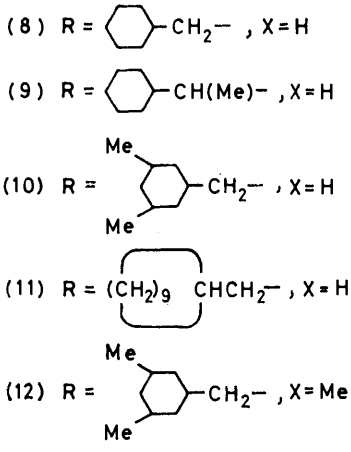
Kinetic parameters for hydrolysis of *p*-nitrophenyl carboxylates in the presence and absence of (7) [a]

Ester [b]	k_{hyd}/s^{-1}	k'_{hyd}/s^{-1}	k_{hyd}/k'_{hyd}	K_b/l mol^{-1}	pH [c]	t/°C
(8)	3.58×10^{-3}	5.5×10^{-4}	6.5	2.3×10^{3}	10.8	20.0
(9)	3.12×10^{-4}	6.0×10^{-6}	52	2.0×10^{3}	10.8	20.0
(10)	8.67×10^{-4}	3.4×10^{-5}	26	4.3×10^{3}	10.8	20.0
(11)	8.23×10^{-4}	1.0×10^{-5}	82	2.6×10^{4}	10.8	20.0
(12)	9.88×10^{-5}	1.0×10^{-6}	99	2.8×10^{4}	11.8	40.0

[a] In ethanol–dioxan–water 10.9 : 1 : 88.1 v/v, μ 0.15 (KCl). [b] Initial concentrations: 1.0×10^{-5}M for (8)—(10), 1.0×10^{-6}M for (11), and 2.0×10^{-6}M for (12). [c] Borax–carbonate buffer (0.05M) for runs at pH 10.8 and sodium hydroxide–phosphate buffer (0.05M) for runs at pH 11.8.

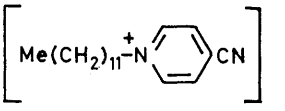
between phenyl esters or azo dyes and cycloamyloses (K_b 10^2—10^3 l mol^{-1}).[14-17] For hydrophobic association of the paracyclophane with the substrates of our present study, the substrate is not necessarily incorporated into the macrocyclic cavity. Upon introduction of two methyl groups into the cyclohexane ring [(10)] or replacement of the cyclohexane ring by the cyclodecane (11), the acyl moiety cannot be incorporated into the paracyclophane cavity as shown by CPK space-filling mole-





cular models. Further substitution of two methyl groups on the nitrophenyl moiety [(12)] makes it impossible to incorporate any part of the substrate molecule into the cavity. In spite of such stereochemical aspects of the substrates, the binding constant increases in the order (8) < (10) < (12). The binding constant for (11) is also significantly large. On the basis of these observations, the following conclusion may be drawn for the geometry of the present host–guest interaction: *p*-nitrophenyl carboxylates bearing an aliphatic moiety of a six- or ten-membered ring interact with the present paracyclophane (7) by a *face-to-face* manner. However, this finding does not exclude the possibility of a strong hydrophobic interaction, between a paracyclophane and a carboxylic ester bearing a long alkyl chain, provided by incorporation of the alkyl chain of the latter into the paracyclophane cavity.[2-6]

Characterization of Substrate-binding Site.—The solvent dependence of an intramolecular charge transfer (CT) energy for a 4-substituted pyridinium iodide is well documented.[18] Thus, a 4-substituted pyridinium iodide is a convenient CT probe for estimation of the polarity of the micro-environment.[19,20] The CT transition energy for 4-cyano-1-dodecylpyridinium iodide (13) decreases as



(13)

the solvent polarity decreases: 362 nm in methanol, 381 nm in ethanol, 425 nm in acetonitrile, and 470 nm in dichloromethane. An aqueous solution of (13) did not exhibit any detectable CT band in a reasonable energy range. A new absorption band appeared at 330 nm (Figure 5) upon addition of a small amount of (7) (3.9×10^{-5}M) to an aqueous solution of (13) at pH 9.0 and μ 0.10 (KCl). The species responsible for the CT band at 330 nm is plausibly due to the association complex between (7) and (13). In reference to the correlation between $\lambda_{max.}$ and solvent polarity, such a transition energy (330 nm) indicates that the micro-environment of the CT

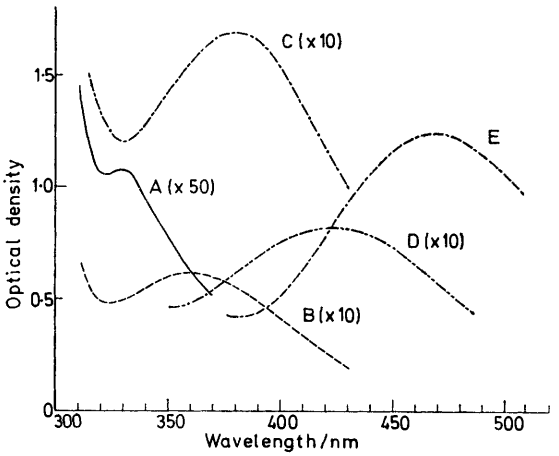


FIGURE 5 Electronic absorption spectra of (13) at 25 °C. A, In water (pH 9.0, μ 0.10 with KCl) containing (13) (3.81×10^{-5}M) and (7) (3.93×10^{-5}M); B, in methanol containing (13) (1.08×10^{-3}M); C, in ethanol containing (13) (1.12×10^{-3}M); D, in acetonitrile containing (13) (0.944×10^{-3}M); E, in dichloromethane containing (13) (0.924×10^{-3}M). Number indicates that the real optical density is obtained by reduction with this factor

probe incorporated into (7) is less polar than in water but significantly more polar than in methanol.*

A carboxylic ester bearing a hexadecanoyl moiety was

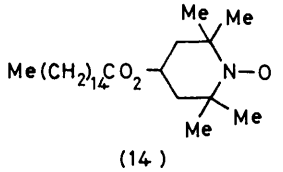
* Mukerjee and Ray [20] have shown that an effective dielectric constant of the micelle surface of dodecylpyridinium iodide is 36.

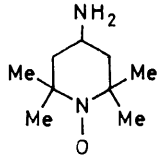
spin-labelled with a nitroxide radical (14) and solubilized in 1% (v/v) aqueous ethanol at pH 10.0 and μ 0.10 (KCl) in the presence of *ca.* 20-fold excess of (7). Under these conditions, there is little doubt of the formation of an association complex between (7) and (14). The *X*-band e.s.r. spectrum shows a highly distorted triplet signal, in marked contrast to the sharp and symmetrical signal for the same probe dissolved in an organic solvent such as ethanol (Figure 6). The e.s.r. spectra provide two kinds of information: the polarity of the micro-environment around the nitroxide moiety, and the rotational mobility of the probe. The isotropic nitrogen splitting constant (A_N) for (14) is 15.98 G in ethanol and 15.53 G in benzene (Table 2). These values are in reasonable agreement with those for a similar nitroxide radical (15) (16.075 G in ethanol and 15.532 G in benzene [21]). Thus, it is reasonable to expect that (14) has an A_N-value in water similar in magnitude to that for (15) (16.990 G) [21] even though such a value was not directly measured for (14) because of its limited solubility. The A_N-value for (14) in water containing (7) is 16.97 G. This is very close to the value estimated for (14) in water without (7), suggesting that the nitroxide moiety of (14) in the association complex is placed essentially in the bulk phase. The rotational mobility of the probe can be quantitatively expressed in terms of a rotational correlation time (τ_c) calculated by a standard method.[22] The τ_c values computed from the e.s.r. line-width and -height are somewhat different from each other possibly because of non-idealised line shape (Table 2). While the τ_c value for (14) in ethanol or benzene is less than 1×10^{-10} s, it is significantly raised upon complex formation with (7). The rotational correlation time is theoretically correlated with the radius of a particle (a) and the viscosity of an environment (η) by equation (2).[23]

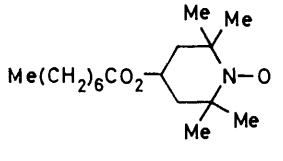
$$\tau_c = \frac{4\pi\eta a^3}{3\kappa T} \qquad (2)$$

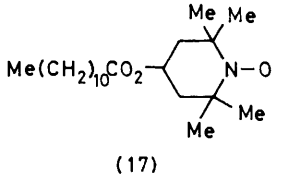
The restricted rotational freedom of the probe is undoubtedly due to its complexation with the large cyclophane molecule. The extent of restricted movement for (14) is much greater than that in liquid paraffin ($\tau_c = 5.1 - 9.2 \times 10^{-10}$ s).

It is interesting to characterize the present paracyclophane systems in the light of similar e.s.r. studies carried out on micellar [24] and cycloamylose systems.[25] The A_N and τ_c values for nitroxide radicals (16) and (17) bound with micellar sodium dodecyl sulphate (SDS) and









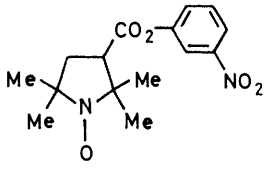


TABLE 2

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

Radical	Medium	A_N/G	$10^{10}\tau_c$/s	Ref.
(14)	Ethanol	15.98	0.7,[a] 0.4 [b]	This work
(14)	Benzene	15.53	0.8,[a] 0.2 [b]	This work
(14)	Liquid paraffin	15.30	5.1,[a] 9.2 [b]	This work
(14)	Water	17.0 [c]		This work
(14–7) [d]	Water [e]	16.97	7.7,[a] 11.6 [b]	This work
(16)	Water	16.80	0.7	25
(16–SDS) [f]	Water	16.40	4	25
(17–SDS) [f]	Water	16.20	3	25
(18)	Water [g]	16.16	0.35	26
(18–CA) [h]	Water [g]	15.75	3.3	26

[a] Calculated from equation (3). [b] Calculated from equation (4). [c] Estimated value, see text. [d] An association complex between (14) (3.0×10^{-5}M) and (7) (5.8×10^{-4}M). [e] Containing 1.0% (v/v) ethanol; pH 10.0 and μ 0.10 (KCl). [f] An association complex between (16) (3.2×10^{-5}M) or (17) (4.3×10^{-5}M) and micellar sodium dodecyl sulphate (5% by weight). [g] Containing 0.5% (v/v) acetonitrile; pH 5.75 (phosphate buffer, 0.07M). [h] An association complex between (18) (1.0×10^{-4}M) and cyclohepta-amylose (4.0×10^{-3}M).

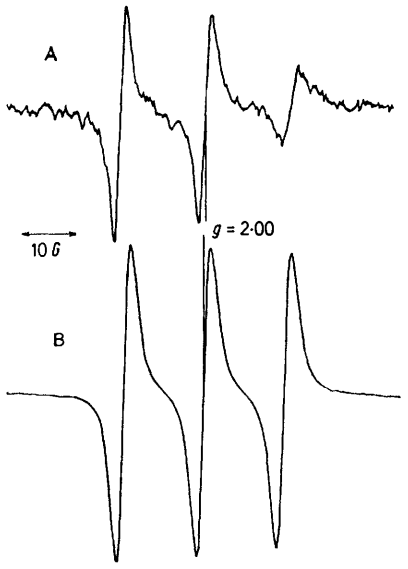


FIGURE 6 *X*-Band e.s.r. spectra of (14) at room temperature. A, In water (pH 10.0, μ 0.10 with KCl) containing (7) (5.8×10^{-4}M) and (14) (3.0×10^{-5}M); B, in ethanol containing (14) (5.0×10^{-5}M)

It is interesting to characterize the present paracyclophane systems in the light of similar e.s.r. studies carried out on micellar [24] and cycloamylose systems.[25] The A_N and τ_c values for nitroxide radicals (16) and (17) bound with micellar sodium dodecyl sulphate (SDS) and

those for nitroxide radical (18) incorporated into the cavity of cyclohepta-amylose are listed in Table 2 along with those observed in the free state. Upon complex formation with a micellar system or cycloamylose, the A_N-value for the probe is reduced relative to that observed in the aqueous phase without any additives. On the other hand, there is little reduction in A_N for the probe bound with paracyclophane. The cyclophane system shows a τ_c value greater than those for micellar and cycloamylose systems, even though it is not justified to compare τ_c values quantitatively for different probes employed in different systems. Even qualitatively, however, this finding is surprising if it is taken into account that the present cyclophane is in a monomeric form. Consequently, the A_N and τ_c values may characterize the paracyclophane system as follows: the apolar interaction between the cyclophane and the alkyl chain of the probe is quite tight, while the cyclophane is not large enough to isolate the nitroxide moiety of the probe from the bulk phase. On the other hand, a surfactant micelle is an aggregate large enough to incorporate the probe and shield it effectively from the bulk phase, while the probe is rotating extremely rapidly for the adsorbed molecule on a hypothetical rigid micelle [25] because of the dynamic nature of a micellar system.

In conclusion, we have successfully developed a paracyclophane macrocycle which is able to incorporate the substrate bearing an alicyclic moiety by hydrophobic interaction of a *face-to-face* mode. Thus, our present results may provide useful information in designing synthetic macrocyclic enzyme models as summarized below. (i) The paracyclophane forms association complexes with a variety of substrates in 1 : 1 stoicheiometry. (ii) The resulting complexes are stable and tight as confirmed by kinetically determined binding constants and τ_c values for the spin-labelled probe. (iii) A part of a substrate molecule bound with the paracyclophane is effectively exposed to the bulk phase as judged by the CT energy of the bound CT probe and the A_N value for the spin-labelled e.s.r. probe.

EXPERIMENTAL

Melting points were measured with a Yamato MP-1 melting point apparatus (oil-bath type). I.r. spectra were recorded on either a JASCO IR-E or a JASCO DS-403G grating spectrophotometer. ^{1}H N.m.r. spectra were obtained with a Varian A-60 spectrometer, with tetramethylsilane as internal reference. Electronic spectra were recorded on a Union Giken SM-401 high sensitivity spectrophotometer. Surface tension measurements were performed at room temperature with a Kyowa DIGI-O-MATIC ESB-IV electrosurface balance. High speed liquid chromatography for preparative purposes was carried out on a Hitachi 635 liquid chromatograph with Hitachi gel 3019. Methanol–dichloromethane (3 : 1 v/v) was used as eluant and the components eluted were detected by u.v. absorption at 254 nm.

E.s.r. spectra were recorded at room temperature on a JEOL JES-ME-3X spectrometer. The isotropic nitrogen hyperfine splitting constant (A_N) was evaluated by dividing a separation between the low- and high-field lines in the spectrum by two. The rotational correlation time (τ_c) was calculated by the standard method [22,24,25] from the linewidth and -height according to equations (3) and (4), respectively.

$$\tau_c = \left(\frac{W_1}{W_0} + \frac{W_{-1}}{W_0} - 2\right) \Big/ \frac{b^2}{4\pi\sqrt{3}W_0} \quad (3)$$

$$\tau_c = \left[\left(\frac{h_0}{h_1}\right)^{1/2} + \left(\frac{h_0}{h_{-1}}\right)^{1/2} - 2\right] \Big/ \frac{b^2}{4\pi\sqrt{3}\ W_0} \quad (4)$$

W_1, W_0, and W_{-1} are peak-to-peak line widths in Hz of the derivatives of the low-, central-, and high-field lines, respectively; h_1, h_0, and h_{-1} are the corresponding peak-to-peak heights; and $b = 3.06 \times 10^8$ s^{-1}.[22] The τ_c values evaluated by equations (3) and (4) are somewhat different from each other because of non-idealized line shape and are listed in Table 2.

The pH and kinetic measurements were carried out as described previously.[4-6,9]

p-*Nitrophenyl Carboxylates*.—*p*-Nitrophenyl esters of cyclohexylacetic acid (8), α-cyclohexylpropionic acid (9), 3,5-dimethylcyclohexylacetic acid (10), and cyclodecylacetic acid (11) were obtained as described previously.[9] Compound (12) was prepared by refluxing a mixture of 3,5-dimethylcyclohexylacetyl chloride [9] (300 mg), 2,6-dimethyl-4-nitrophenol (270 mg), magnesium ribbon (7 mg), and a small amount of iodine in dry benzene (6 ml) for 7 h. The mixture was washed with cold water, saturated aqueous sodium bicarbonate, and again with cold water, and dried (Na_2SO_4). The solvent was removed *in vacuo* and the residue was distilled at 130 °C and 0.2 mmHg to obtain *2,6-dimethyl-4-nitrophenyl 3,5-dimethylcyclohexaneacetate* (12) (160 mg, 32%), m.p. 56—57 °C; δ(CCl_4) 7.92 (s, aromatic), 2.7—2.3 (m, CH_2CO), 2.23 (s, Ph CH_3), 2.1—1.0 (m, cyclohexyl), and 0.96 and 0.87 (each br s, cyclohexyl CH_3) (Found: C, 67.7; H, 7.9; N, 4.45. $C_{18}H_{25}NO_4$ requires C, 67.7; H, 7.9; N, 4.4%).

1,10-*Bis*[4-(4-*methoxycarbonylbutyryl*)*phenyl*]*decane*.— To a mixture of 4-methoxycarbonylbutyryl chloride (45 g) and anhydrous aluminium chloride (60 g) in anhydrous carbon disulphide (400 ml) was added dropwise with vigorous stirring 1,10-diphenyldecane (20 g) at 0 °C over a period of 75 min. Stirring was continued for 3 h at 23 °C and for an additional 1 h at reflux temperature. The mixture was then poured into ice–water (300 g) containing 12N-hydrochloric acid (100 ml) and extracted with benzene (4 × 200 ml). The benzene extract was washed with water, dried (Na_2SO_4), and treated with a small amount of Norit A active charcoal (Wako Pure Chemicals) at reflux temperature for 1 h. Benzene was distilled off *in vacuo* after removal of the charcoal from the extract. Repeated recrystallization of the residue from benzene–light petroleum (1 : 9 v/v) gave the dioxo-diester as yellowish *crystals* (11 g, 31%), m.p. 72.5—74 °C; $\nu_{max.}$ (Nujol) 1 737 (ester C=O), and 1 684 cm^{-1} (ketone C=O) (Found: C, 74.1; H, 8.4. $C_{34}H_{46}O_6$ requires C, 74.2; H, 8.35%).

1,10-*Bis*[4-(4-*carboxybutyl*)*phenyl*]*decane*.—A mixture of the dioxo-diester obtained above (11 g), hydrazine hydrate (15 g), potassium hydroxide (25 g), and diethylene glycol (400 ml) was refluxed for 3 h with stirring. Water and excess of hydrazine were removed by distillation and the residual mixture was refluxed for another 12 h. After

being cooled to room temperature the mixture was acidified with 6N-hydrochloric acid, and the resulting white solid was recovered by filtration, washed with water (6 × 200 ml), and dried *in vacuo*. Recrystallization from methanol afforded the *diacid* (8.5 g, 87%), m.p. 147.5—150 °C; $\nu_{max.}$ (Nujol) 1 695 cm^{-1} (CO_2H) (Found: C, 77.55; H, 9.35. $C_{32}H_{46}O_4$ requires C, 77.75; H, 9.3%).

1,10-*Bis*[4-(4-*methoxycarbonylbutyl*)*phenyl*]*decane*.— The diacid obtained above (8.5 g) was dissolved in methanol (500 ml) containing concentrated sulphuric acid (20 ml) and refluxed for 12 h. Most of the methanol was then distilled off from the mixture. Water (300 ml) was added to the residue and the mixture was extracted with benzene (4 × 200 ml) and recrystallized from methanol to yield the *diester* (7.6 g, 86%), m.p. 48—49.5 °C; $\nu_{max.}$ (Nujol) 1 737 cm^{-1} (C=O) (Found: C, 78.15; H, 9.7. $C_{34}H_{50}O_4$ requires C, 78.15; H, 9.6%).

6-*Hydroxy*[10.10]*paracyclophan*-5-*one* (1).—Under purified nitrogen sodium metal (4.0 g) was added with vigorous stirring to refluxing dry xylene (2 l). A solution of the above diester (7.65 g) in dry xylene (400 ml) was added dropwise into this mixture over 24 h, and refluxing was continued for another hour. The mixture was cooled to room temperature under nitrogen, and acetic acid (60 ml) and dry xylene (60 ml) were added dropwise. The xylene solution was washed with water (4 × 500 ml), dried ($CaSO_4$), and evaporated *in vacuo* under nitrogen to give a yellowish viscous oil which was recrystallized from ether–hexane (1 : 1 v/v) to afford the *acyloin* (1) (4.2 g, 62%), m.p. 67.5—70.5 °C; $\nu_{max.}$ (KBr) 3 430 (OH) and 1 700 cm^{-1} (C=O); $\delta(CCl_4)$ 6.93 (s, aromatic), 3.92 (br s, C*H*OH), 2.51 (t, benzyl), 2.23 (m, CH_2C=O), and 1.9—1.0 (m, other CH_2 and OH); *m/e* 462 (M^+) (Found: C, 82.8; H, 10.05. $C_{32}H_{46}O_2$ requires C, 83.06; H, 10.0%).

6-*Hydroxyimino*[10.10]*paracyclophan*-5-*ol* (2).—A mixture of the acyloin (1) (262 mg), hydroxylamine hydrochloride (180 mg), and powdered KOH (230 mg) in ethanol (50 ml) was refluxed with stirring for 30 min. The mixture was neutralised with 4N-hydrochloric acid and extracted with ether (2 × 70 ml). The combined extracts were washed with water, dried (Na_2SO_4), and evaporated to dryness. The residue was recrystallized from light petroleum–benzene (5 : 1 v/v) to give the *oxime* (2) (190 mg, 70%), m.p. 122—123 °C; $\nu_{max.}$ (KBr disc) 3 240 cm^{-1} (OH); $\delta(CDCl_3)$ 7.03 (s, aromatic), 4.5—3.0 (br m, C*H*OH and OH), 2.57 (t, benzyl), 2.35 (m, CH_2C=NOH), and 2.0—1.1 (m, other CH_2) (Found: C, 80.35; H, 9.85; N, 2.85. $C_{32}H_{47}NO_2$ requires C, 80.45; H, 9.95; N, 2.95%).

[10.10]*Paracyclophan*-5-*one* (3).—A solution of the acyloin (1) (2.5 g) and 57% hydriodic acid (5.0 g) in acetic acid (80 ml) was refluxed for 4 h. Water (100 ml) was added to the cooled solution and the mixture was poured into water (200 ml) containing sodium hydroxide (50 g) and sodium hydrogensulphite (6 g). The organic material was extracted with benzene (4 × 100 ml) and worked up as usual to give a yellowish oil which was then chromatographed on silica gel (Wako gel C-100; benzene as eluant) to afford the ketone (3) as an *oil* (2.05 g, 85%); $\nu_{max.}$ (neat) 1 714 cm^{-1} (C=O); $\delta(CCl_4)$ 6.93 (s, aromatic), 2.51 (t, benzyl), 2.20 (m, CH_2 C=O), and 1.9—1.0 (m, other CH_2) (Found: C, 85.8; H, 10.4. $C_{32}H_{46}O$ requires C, 86.05; H, 10.4%).

5-*Oxo*[10.10]*paracyclophane*-12(13)-*carboxylic Acid* (4) *and* 5-*Oxo*[10.10]*paracyclophane*-12(13),28(29)-*dicarboxylic Acid* (5).—Oxalyl chloride (450 mg) in carbon disulphide (50 ml) was added to a mixture of the ketone (3) (1.0 g) and aluminium chloride (1.2 g) in carbon disulphide (50 ml) at 0 °C over a period of 40 min. The mixture was stirred at 15 °C for 2.5 h, poured into ice–water (200 g) containing concentrated hydrochloric acid (50 ml), and extracted with benzene (5 × 100 ml). The benzene extract was evaporated to give an oil, which was subsequently stirred in 5% aqueous sodium hydroxide (800 ml) containing methanol (400 ml) for 20 h. The mixture was evaporated *in vacuo* to remove methanol and extracted with ether (100 ml). The aqueous layer was acidified with 12N-hydrochloric acid and extracted with benzene (3 × 200 ml). After the benzene was evaporated off, the residual oil was chromatographed on silica gel [Wako gel C-100; benzene (300 ml) as eluant] to recover the unchanged ketone (100 mg). Further elution with ether (200 ml) afforded an oily mixture (400 mg) of the monocarboxy (4) and dicarboxy (5) derivatives in the ratio 1 : 1.4 (n.m.r.); $\nu_{max.}$ (neat) 1 700 cm^{-1} (CO_2H); $\delta(CCl_4)$ 11.26 (s, CO_2H), 7.76 (s, *ortho*-ArH), 7.09 (m, unsubst. ArH and *meta*- and *para*-ArH), 2.90 (m, *ortho*-benzyl), 2.55 (m, unsubst. benzyl and *meta* benzyl), 2.25 (m, CH_2C=O), and 1.9—0.8 (m, other CH_2). High speed liquid chromatography failed to separate (4) and (5).

12(13), 28(29)-*Bis*(*trimethylammoniomethyl*)[10.10]*paracyclophan*-5-*one Dichloride* (6).—A solution of chloromethyl methyl ether (320 mg) in dichloromethane (30 ml) was added to a mixture of (3) (450 mg) and anhydrous stannic chloride (1.0 g) in dichloromethane (100 ml) at 0 °C with stirring over 100 min. The mixture was stirred at 0 °C for another 5.5 h and poured into ice–water (100 g) containing concentrated hydrochloric acid (30 ml). The organic layer was separated and the aqueous layer extracted with dichloromethane (4 × 100 ml). The combined extracts were washed with water, dried ($MgSO_4$), and evaporated to give a dark brown oil. The oil was dissolved in ether (5 ml) and the solution was filtered to separate the inorganic materials. The usual work-up gave the chloromethyl derivative of (3) as an oil (375 mg, 75%); $\delta(CCl_4)$ 7.02 and 6.95 (m, aromatic), 4.47 (s, CH_2Cl), 3.2—1.85 (m, benzyl and CH_2C=O), and 1.85—0.6 (m, other CH_2). Gaseous trimethylamine generated by addition of aqueous trimethylamine (30%, 100 g) to solid sodium hydroxide (60 g) was dried over soda-lime and introduced into a solution of the chloromethyl derivative of (3) (365 mg) in methanol–benzene (1 : 1 v/v; 50 ml) at room temperature over a period of 80 min. The mixture was stirred for another 4 h at room temperature, and the solvent and excess of trimethylamine were removed *in vacuo*. The residue (331 mg) was purified by repeated high speed liquid chromatography to give the *dichloride* (6) (30 mg); $\delta(CD_3OD)$ 6.93 (s, aromatic), 4.27 (s, $CH_2N^+Me_3$), 2.75 (s, Me_3N^+), 2.7—1.8 (m, benzyl and CH_2C=O), and 1.6—0.7 (m, other CH_2) (Found: C, 69.85; H, 9.85; N, 4.0. $C_{46}H_{66}N_2Cl_2O{\cdot}H_2O$ requires C, 70.65; H, 10.1; N, 4.1%).

12(13),28(29)-*Bis*(4-*carboxybutyryl*)[10.10]*paracyclophan*-5-*one* (7).—4-Carboxybutyryl chloride (1.0 g) in carbon disulphide (20 ml) was added with stirring to a mixture of the ketone (3) (250 mg), anhydrous aluminium chloride (1.3 g), and carbon disulphide (50 ml) at 0 °C over 30 min. The mixture was stirred for 1 h at 0 °C, for 1 h at room temperature, and for another 6 h at reflux temperature. The mixture was then poured into ice–water (50 g) containing concentrated hydrochloric acid (30 ml), and extracted with benzene (4 × 100 ml). Evaporation of benzene from the extract gave a yellow oil; $\delta(CCl_4)$ 3.60 (CO_2CH_3). The oil was hydrolysed in 5% aqueous sodium hydroxide

(800 ml) containing methanol (300 ml), evaporated to remove methanol, and acidified with 6N-hydrochloric acid. The mixture was extracted with benzene (3 × 150 ml), and the extract was evaporated to give a yellow oil, which was chromatographed on silica gel (Wako gel C-100). A small amount of the unchanged ketone was eluted using benzene (300 ml) and dichloromethane (200 ml). The crude (7) (180 mg), eluted using methanol, was dissolved in ether (100 ml) and extracted with 5% aqueous sodium hydroxide (200 ml). The organic layer was washed with water (6 × 500 ml). The aqueous extract and the washings were combined, acidified with 12N-hydrochloric acid, and extracted with benzene (4 × 200 ml). Usual work-up of the benzene extract gave an oil (140 mg), which was further purified by means of high speed liquid chromatography with a recycling device to afford pure (7) as an *oil* (80 mg, 21%); $\nu_{max.}$ (neat) 1 700 cm^{-1} (CO_2H); $\delta(CCl_4)$ 10.75 (br s, CO_2H), 7.26 (s, *ortho*-ArH), 7.00 (s, *meta*- and *para*-ArH), 3.1—1.8 (m, benzyl, $CH_2C{=}O$, and CH_2CO_2H), and 1.8—0.6 (m, other CH_2) (Found: C, 74.1; H, 8.7. $C_{42}H_{58}O_7$ requires C, 74.75; H, 8.65%).

4-Cyano-1-dodecylpyridinium Iodide (13).—Stoicheiometric amounts of dodecyl iodide (2.85 g) and 4-cyanopyridine (1.0 g) were heated together at 80 °C for 20 h, and cooled to room temperature. Ether (10 ml) was added to the mixture and the yellow precipitates (0.78 g, 20%) were recovered by filtration. Repeated recrystallization of the precipitates from absolute ethanol–ether (1 : 6 v/v) gave yellow *needles*, m.p. 142—143.5 °C (Found: C, 53.75; H, 7.35; N, 7.0. $C_{18}H_{29}N_2I$ requires C, 54.0; H, 7.3; N, 7.0%).

2,2,6,6-Tetramethyl-1-oxyl-4-piperidyl Palmitate (14).—This compound was prepared by a slight modification of the procedure for preparation of the octanoate and decanoate analogues.[24] Freshly distilled palmitoyl chloride (301 mg) in ether (1 ml) was added dropwise to a mixture of 2,2,6,6-tetramethyl-4-piperidinol-1-oxyl [26] (172 mg) and pyridine (79 mg) in ether (3 ml) at 0 °C with stirring. After a few minutes the solution was allowed to warm to room temperature and was left for 2 h. The solution was then washed several times with 5% aqueous sodium bicarbonate, dried ($MgSO_4$), and evaporated *in vacuo*. The resulting red oil was chromatographed on active alumina (5% deactivated, Ishizu Pharmaceutical Co.). Elution with ethyl acetate–benzene (1 : 1 v/v) and storage of the product for 2 days in a desiccator afforded *crystals*, m.p. 32—32.5 °C (Found: C, 73.1; H, 11.75; N, 3.3. $C_{25}H_{48}NO_3$ requires C, 73.1; H, 11.8; N, 3.4%).

This work was supported in part by a Scientific Research Grant from the Ministry of Education, Japan.

[8/950 *Received*, 22*nd May*, 1978]

REFERENCES

[1] Part 1, Y. Murakami, Y. Aoyama, K. Ohno, K. Dobashi, T. Nakagawa, and J. Sunamoto, *J.C.S. Perkin I*, 1976, 1320.
[2] Y. Murakami, J. Sunamoto, and K. Kano, *Bull. Chem. Soc. Japan*, 1974, **47**, 1238.
[3] Y. Murakami, J. Sunamoto, H. Okamoto, and K. Kawanami, *Bull. Chem. Soc. Japan*, 1975, **48**, 1537.
[4] Y. Murakami, Y. Aoyama, K. Dobashi, and M. Kida, *Bull. Chem. Soc. Japan*, 1976, **49**, 3633.
[5] Y. Murakami, Y. Aoyama, and K. Dobashi, *J.C.S. Perkin II*, 1977, 24.
[6] Y. Murakami, Y. Aoyama, and K. Dobashi, *J.C.S. Perkin II*, 1977, 32.
[7] Y. Murakami, J. Sunamoto, H. Kondo, and H. Okamoto, *Bull. Chem. Soc. Japan*, 1977, **50**, 2420.
[8] J. Sunamoto, H. Kondo, H. Okamoto, and K. Taira, *Bioorganic Chemistry*, 1977, **6**, 95.
[9] Y. Murakami, Y. Aoyama, M. Kida, and A. Nakano, *Bull. Chem. Soc. Japan*, 1977, **50**, 3365.
[10] Y. Murakami, Y. Aoyama, M. Kida, and J. Kikuchi, *J.C.S. Chem. Comm.*, 1978, 494.
[11] W. B. Danklicker and V. A. de Saussure, in ' The Chemistry of Biosurfaces,' ed. M. L. Hair, Marcel Dekker, New York, 1971, vol. 1, ch. 1.
[12] (*a*) J. H. Fendler and E. J. Fendler, ' Catalysis in Micellar and Macromolecular Systems,' Academic Press, New York, 1975; (*b*) F. M. Menger and M. J. McCreery, *J. Amer. Chem. Soc.*, 1974, **96**, 121.
[13] J. P. Kratohvil and H. T. DelliColli, *Canad. J. Biochem.*, 1968, **46**, 945.
[14] F. Cramer, W. Saenger, and H.-Ch. Spatz, *J. Amer. Chem. Soc.*, 1967, **89**, 14.
[15] R. L. VanEtten, J. F. Sebastian, G. A. Clowes, and M. L. Bender, *J. Amer. Chem. Soc.*, 1967, **89**, 3242.
[16] R. E. VanEtten, G. A. Clowes, J. F. Sebastian, and M. L. Bender, *J. Amer. Chem. Soc.*, 1967, **89**, 3253.
[17] D. L. VanderJagt, F. L. Killian, and M. L. Bender, *J. Amer. Chem. Soc.*, 1970, **92**, 1016.
[18] E. M. Kosower, 'An Introduction to Physical Organic Chemistry,' Wiley, New York, 1968, ch. 2.
[19] J. H. Fendler and L.-J. Liu, *J. Amer. Chem. Soc.*, 1975, **97**, 999.
[20] P. Mukerjee and A. Ray, *J. Phys. Chem.*, 1966, **70**, 2144.
[21] B. R. Knauer and J. J. Napier, *J. Amer. Chem. Soc.*, 1976, **98**, 4395.
[22] A. S. Waggoner, O. H. Griffith, and C. R. Christensen, *Proc. Nat. Acad. Sci. U.S.A.*, 1967, **57**, 1198.
[23] N. Bloembergen, E. M. Purcell, and R. V. Pound, *Phys. Rev.*, 1948, **73**, 679.
[24] A. S. Waggoner, A. D. Keith, and O. H. Griffith, *J. Phys. Chem.*, 1968, **72**, 4129.
[25] K. Flohr, R. M. Paton, and E. T. Kaiser, *J. Amer. Chem. Soc.*, 1975, **97**, 1209.
[26] R. Briere, H. Lemaire, and A. Rassat, *Tetrahedron Letters*, 1964, 1775.