Hydrophobic Vitamin B_{12}. Part 6.† Carbon-skeleton Rearrangement *via* Formation of Host–Guest Complexes derived from an 'Octopus' Azaparacyclophane and Hydrophobic Vitamin B_{12} Derivatives: a Novel Holoenzyme Model System

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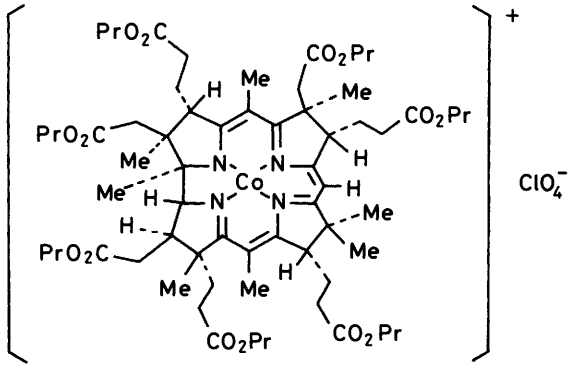
The alkylation reactions of a hydrophobic vitamin B_{12} derivative with alkyl bromides in an 'octopus' azaparacyclophane having eight hydrocarbon chains have been investigated. Molecular discrimination has been shown to originate from electrostatic interaction between the octopus cyclophane and the alkyl bromides. Alkylation was enhanced by desolvation and proximity effects operating on the reacting species *via* formation of a ternary complex composed of the octopus cyclophane, the hydrophobic vitamin B_{12} derivative, and an alkyl halide. Carbon-skeleton rearrangement reactions of alkyl ligands bound to the hydrophobic vitamin B_{12} were found to be markedly favoured in the hydrophobic cavity provided by the octopus cyclophane, relative to the reactions in methanol and benzene, under anaerobic photolysis conditions at ordinary temperatures. The same reactions took place readily in solid benzene below 4 °C under similar conditions. The central cobalt atom of the hydrophobic vitamin B_{12} participates in the rearrangement reaction *via* formation of a tight pair with an alkyl radical species. Non-enzymic rearrangement reactions have been shown here to proceed quite efficiently by employing a relevant apoenzyme model.

Vitamin-B_{12}-dependent enzymes catalyse various isomerization reactions for which clear explanations in terms of organic and organometallic chemistry are not yet available. Clarification of the reaction mechanisms involved by utilizing relevant model reaction systems is a current research target in bioinorganic chemistry.[1] Various cobalt complexes have been synthesized as model complexes,[2] and mechanistic studies of various enzyme-mimic reactions have been carried out to ascertain whether the isomerization reactions proceed *via* radical or anionic mechanisms, and whether or not the cobalt of the vitamin B_{12} participates in the rearrangement process.[3] In order to simulate various functions of vitamin B_{12} as exerted in the hydrophobic active sites of the enzymes concerned, we have been dealing with hydrophobic vitamin B_{12} derivatives which have ester groups in place of the peripheral amide moieties of the naturally occurring vitamin.[4–14] We have reported previously that the carbon-skeleton rearrangement reactions of alkyl halides bearing various functional groups are catalysed by a hydrophobic vitamin B_{12} derivative under electrochemical conditions.[15] Even though the rearrangement reaction is generally considered to proceed *via* radical mechanisms *in vivo*, the anionic intermediates generated by electrochemical reduction readily afford the corresponding rearrangement products. In connection with the biological reaction, a free radical rearrangement reaction involving 1,2-migration of a thioester group was investigated in organic solvents as a model for the methylmalonyl-CoA mutase reaction; the rearrangement product was obtained in relatively small yield (1—9%).[16] Since radical species formed during the enzymic reactions must be different in nature from those generated in homogeneous solutions, the need for appropriate apoenzyme models, into which vitamin B_{12} models can be incorporated, must be emphasized.

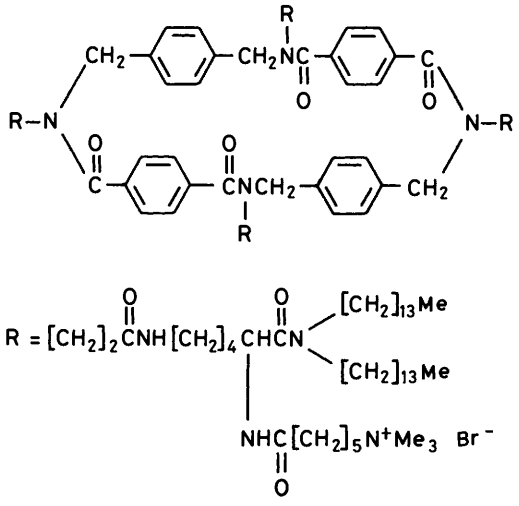
The naturally occurring apoproteins, which provide relevant reaction sites for vitamin B_{12}, are considered to play crucial roles in the isomerization reactions.[17] However, studies of non-enzymic reactions have been exclusively related to clarification of the catalytic functions of the coenzyme, vitamin B_{12}, and relevant apoprotein models have received little treatment. We have been interested in the catalytic activity of vitamin B_{12} in hydrophobic environments, to simulate the catalytic functions of the holoenzymes concerned, and have previously investigated various reactions of hydrophobic vitamin B_{12} derivatives in single-compartment vesicles of peptide amphiphiles.[6,14]

Various cyclophanes have been studied extensively in view of their potential for functional simulation of enzymes.[18] However, the hydrophobic cavities of conventional cyclophanes are generally small and shallow, so that relatively bulky molecules cannot be incorporated. Recently, we have examined macrocyclic compounds which provide sizeable intramolecular cavities, from the viewpoint of host–guest chemistry.[19] An 'octopus' azaparacyclophane having eight hydrocarbon chains, $APC[C_2Lys(C_5N^+)2C_{14}]_4$, behaves as an effective cationic host over a wide pH range in aqueous media,[20] and incorporates a hydrophobic vitamin B_{12} derivative in 1:1 molar ratio.[13] The 'octopus' azacyclophane is superior to the bilayer membrane as a host for a hydrophobic vitamin B_{12}, since the former provides the guest-binding site in a simpler stoicheiometric relationship. In view of model systems for methylmalonyl-CoA mutase-like reactions, an alkyl halide must react initially with a hydrophobic vitamin B_{12} in the Co^I state to afford the corresponding alkylated complex before the alkyl ligand is activated for carbon-skeleton rearrangement. With this in mind, a hydrophobic vitamin B_{12} derivative, $[Cob(II)7C_3ester]ClO_4$, was reduced to the Co^I state, and the alkylation of the Co^I species with alkyl bromides in the hydrophobic cavity of $APC[C_2Lys(C_5N^+)2C_{14}]_4$ has been investigated. Carbon-skeleton rearrangement reactions of alkyl ligands bearing various electron-withdrawing groups, co-ordinated to the hydrophobic vitamin B_{12}, were then studied under anaerobic photolysis conditions. The results showed that the isomerization reaction proceeds efficiently in the hydrophobic site of the 'octopus' azacyclophane, on the basis of product analyses. The fate of radical species generated by cobalt–carbon bond cleavage under anaerobic photolysis conditions is discussed in the light of related microenvironmental effects.

† Part 5, ref. 15.

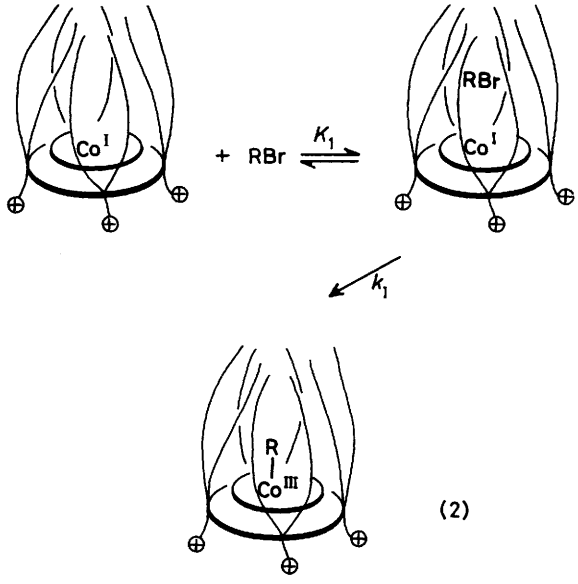


[Cob(II)7C$_3$ester]ClO$_4$



APC[C$_2$Lys(C$_5$N$^+$)2C$_{14}$]$_4$

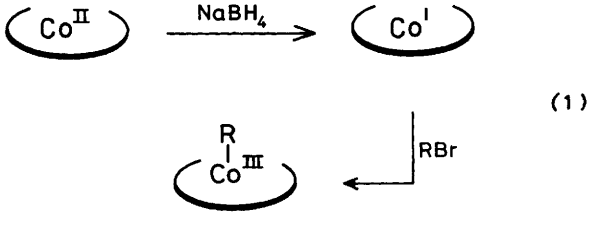
reduced to the Co^I state with NaBH$_4$; then an alkyl halide (RX) was added to the resulting assembly. Non-ionic, cationic, and anionic alkyl bromides were employed, and the reactions were followed by electronic spectroscopy. The rate constants were evaluated from the absorbance decay of the band at 392 nm, due to the Co^I species, according to the method described previously.[6] The alkylation proceeded *via* a two-step process shown in equation (2); K_1 and k_1 are the binding constant for incorporation of RX into the cyclophane and the rate constant for reaction of Cob(I)7C$_3$ester with RX, respectively. RX is first incorporated into the cyclophane, and then subjected to nucleophilic attack by the Cob(I)7C$_3$ester in the cyclophane to afford an alkylated complex. The kinetic parameters calculated on the basis of equation (2) are listed in Table 1.† Cob(I)7C$_3$ester is



(2)

Results and Discussion

Alkylation of Hydrophobic Vitamin B$_{12}$ in 'Octopus' Azaparacyclophane.—Conventional cyclophanes generally provide small and shallow hydrophobic cavities, in which a bimolecular reaction cannot take place. APC[C$_2$Lys(C$_5$N$^+$)2C$_{14}$]$_4$ provides a hydrophobic cavity of considerable depth and is able to incorporate a hydrophobic vitamin B$_{12}$ derivative through the induced-fit mechanism in aqueous media.[13] Thus we were able to study the alkylation reactions of the hydrophobic vitamin B$_{12}$ derivative, incorporated into APC[C$_2$Lys(C$_5$N$^+$)2C$_{14}$]$_4$, with various alkyl halides in aqueous media [equation (1)].



(1)

The alkylation reactions were carried out as follows. First, [Cob(II)7C$_3$ester]ClO$_4$ was incorporated into APC[C$_2$Lys(C$_5$N$^+$)2C$_{14}$]$_4$ in 1:1 molar ratio in an aqueous buffer, because the hydrophobic vitamin B$_{12}$ derivative is insoluble in aqueous media;* the central cobalt atom of the former was quite hydrophobic and is incorporated completely into the cyclophane cavity; the microenvironmental polarity experienced by the hydrophobic vitamin B$_{12}$ is equivalent to that provided by dichloromethane.[13] The rate constants for all the alkyl bromides employed here are similar to each other in dichloromethane. However, in the cyclophane the non-ionic and anionic substrates reacted much more readily with Cob(I)7C$_3$ester to afford the corresponding alkylated complexes, while the cationic substrate did not react at all with the Co^I complex. Since APC[C$_2$Lys(C$_5$N$^+$)2C$_{14}$]$_4$ acts as a cationic host in aqueous media, the host is able to bind the neutral and anionic guest molecules strongly but fails to bind the cationic one, as a result of electrostatic repulsion.[20] The rate constants for the non-ionic and anionic alkyl bromides are much larger than those for the reactions in dichloromethane. This apparently indicates that Cob(I)7C$_3$ester and RX are effectively desolvated and allowed to approach more closely (a proximity effect) in the hydrophobic cavity of APC[C$_2$Lys(C$_5$N$^+$)2C$_{14}$]$_4$. Consequently, the octopus azaparacyclophane clearly demonstrates molecular discrimination based on an electrostatic effect, and the alkylation enhancement is brought about by desolvation and proximity effects.

Carbon-skeleton Rearrangement in 'Octopus' Azaparacyclophane.—Isomerization reactions catalysed by vitamin-B$_{12}$-dependent enzymes have attracted much attention in recent

* The hydrophobic vitamin B$_{12}$ must be located inside the cyclophane cavity, as confirmed by fluorescence spectroscopy (see later).

† The binding constant (K_2) for incorporation of two molecules of RX into the cyclophane was found to be negligible; see Experimental section.

Table 1. Kinetic parameters for reactions of Cob(I)7C_3ester with alkyl bromides in APC[C_2Lys(C_5N^+)2C_{14}]$_4$ at 20.0 ± 0.1 °C[a]

Alkyl bromide[b]	APC[C_2Lys(C_5N^+)2C_{14}]$_4$[c] K_1/dm^3 mol^{-1}	10^3 k_1/s^{-1}	K_1k_1/dm^3 mol^{-1} s^{-1}	CH_2Cl_2 k_2/dm^3 mol^{-1} s^{-1}
Br[CH_2]$_5CH_3$	1 000	9.9	9.9	0.11
Br[CH_2]$_5CO_2^-$	900	9.1	8.2	0.10
Br[CH_2]$_5N^+Me_3$		No reaction		0.09

[a] Cob(I)7C_3ester, 2.0 × 10^{-5} mol dm^{-3}. [b] Initial concentration 9.0 × 10^{-4}—6.5 × 10^{-3} mol dm^{-3}. [c] APC[C_2Lys(C_5N^+)2C_{14}]$_4$, 2.0 × 10^{-5} mol dm^{-3}; in phosphate–borate buffer (0.05 mol dm^{-3}; pH 9.2).

Table 2. Product analyses for photolysis of Ac(CO_2Et)MeCCH_2Cob(III)-7C_3ester in various media at 20.0 ± 0.1 °C[a]

Medium	Yield (%) (A)	(B)	(C)
Methanol	70	Trace[c]	8.0
Benzene	65	Trace[d]	10
APC[C_2Lys(C_5N^+)2C_{14}]$_4$[b]	23	Trace[d]	65

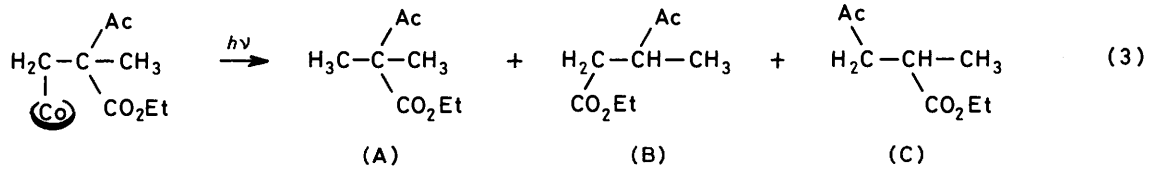
[a] A solution containing Ac(CO_2Et)MeCCH_2Cob(III)7C_3ester (5.0 × 10^{-5} mol dm^{-3}) was irradiated with a 500 W tungsten lamp for 1 h at a distance of 30 cm. Products were analysed by g.l.c. [b] A dichloromethane solution containing APC[C_2Lys(C_5N^+)2C_{14}]$_4$ and Ac(CO_2Et)MeCCH_2Cob(III)7C_3ester was evaporated *in vacuo* to remove the solvent completely in the dark, and an aqueous phosphate–borate buffer (0.05 mol dm^{-3}; pH 9.2) was added to the residue; initial concentration of both cyclophane and cobalt complex, 5.0 × 10^{-5} mol dm^{-3}. [c] Yield less than 0.1%. [d] Yield less than 1%.

years in view of their controversial reaction mechanisms. In particular, carbon-skeleton rearrangement reactions as typically performed by methylmalonyl-CoA mutase have been investigated by adopting relevant model systems without apoprotein models. These enzymic reactions are known to be initiated by homolytic cleavage of the cobalt–carbon bond to form a 5′-deoxyadenosyl radical, which abstracts a hydrogen atom from a substrate to generate a substrate free radical.[21] Inevitably the important roles of the apoproteins concerned have been emphasized. We have adopted here a holoenzyme model composed of APC[C_2Lys(C_5N^+)2C_{14}]$_4$ and the hydrophobic vitamin B_{12}.

Hydrophobic vitamin B_{12} derivatives bearing various alkyl ligands at one axial site of the nuclear cobalt were incorporated into the hydrophobic cavity of APC[C_2Lys(C_5N^+)2C_{14}]$_4$ in aqueous media and then irradiated with visible light under anaerobic conditions. Product analyses for the photolysis of the alkylated complex, Ac(CO_2Et)MeCCH_2Cob(III)7C_3ester, in the octopus cyclophane, methanol, and benzene are summarized in Table 2 [see equation (3)]. The yield of acetyl-migration product (C) was significantly increased in the octopus cyclophane as compared with those in methanol and benzene. A bivalent copper salt, $CuCl_2$, is known to serve as a selective scavenger toward radicals that enter the bulk aqueous phase.[22] For this reason, $CuCl_2$ was added to the reaction solution containing the octopus cyclophane and the alkylated complex in 1:1 molar ratio in order to confirm that all the reaction took place in the cyclophane cavity. Regardless of the addition of $CuCl_2$, the yields of all the products given in Table 2 remained unchanged. This clearly indicates that the rearrangement reaction proceeded in the cyclophane, not in the bulk aqueous phase.

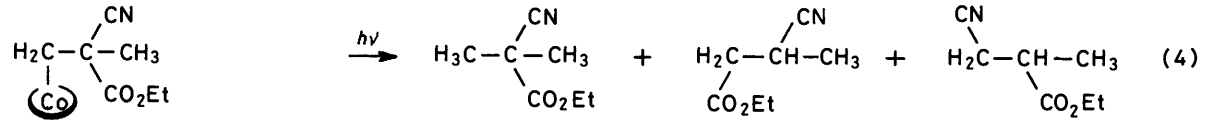
Similar reaction behaviour with respect to the medium effect was observed for the 1,2-migration of electron-withdrawing groups such as carboxylic ester, thioester, acetyl, and cyano [see equations (4)—(7)]. It is of interest to compare the apparent migratory aptitude of these electron-withdrawing groups with that previously observed under electrochemical conditions.[15] The carboxylic ester group migrates more readily than the cyano group under electrochemical reduction conditions that allow the reaction to proceed *via* formation of anionic intermediates, whereas the groups migrate with comparable aptitude under the present conditions.[23]

Correlations of the concentration of APC[C_2Lys(C_5N^+)-2C_{14}]$_4$ with product yields are shown in Figure 1 for the photolysis of Ac(CO_2Et)MeCCH_2Co^{III}7C_3ester. When the cyclophane concentration decreased below the concentration of the alkylated hydrophobic vitamin B_{12}, the yield of the acetyl migration product was progressively reduced. In the concentration range above 5.0 × 10^{-5} mol dm^{-3}, while the concentration of the alkylated complex remained constant at 5.0 × 10^{-5} mol dm^{-3}, the product molar ratio reached a constant level (reduction product *vs.* rearrangement product 1:3). Similar behaviour was observed for the reaction of (CN)(Co_2Et)MeCCH_2Cob(III)7C_3ester (5.0 × 10^{-5} mol dm^{-3}); the molar ratio of reduction product *vs.* rearrangement products became constant (1:1) in a concentration range of the octopus cyclophane above 5.0 × 10^{-5} mol dm^{-3}. When $CuCl_2$ was added to a reaction solution in which the cyclophane concentration had been adjusted below the concentration of the alkylated complex, the product ratio (1:3 molar ratio) was identical with that observed in a sufficiently high concentration of octopus cyclophane even though the total yield was reduced. As a result, it became clear that the reduction and rearrangement reactions proceed at a kinetic ratio of 1:3 in the octopus cyclophane under the present conditions.



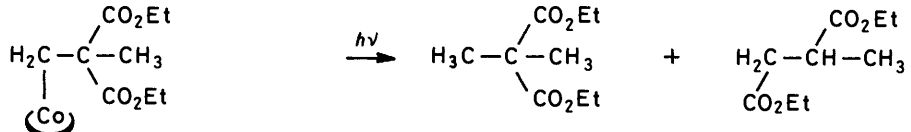
(3)

Mode of Cobalt–Carbon Bond Cleavage.—The photolysis reaction was followed by spectroscopic methods in order to clarify the mechanism for 1,2-migration of the functional groups in APC[C_2Lys(C_5N^+)2C_{14}]$_4$. The electronic spectral change observed in the photolysis of (CN)(CO_2Et)MeCCH_2Cob(III)-7C_3ester, which was incorporated into the octopus cyclophane in an aqueous buffer, is shown in Figure 2. The alkylated



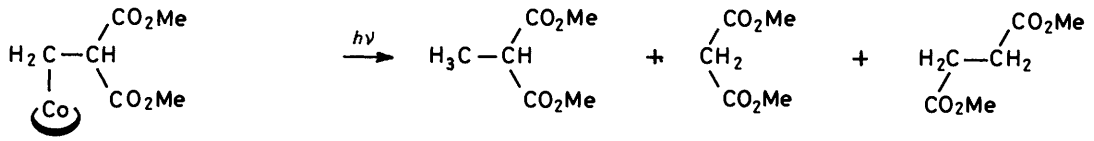
methanol (20 °C)	87%	0%	0%
benzene (20 °C)	78%	trace	trace
(0 °C)	46%	19%	15%
APC[C_2Lys(C_5N^+)2C_{14}]$_4$ (20 °C)	40%	21%	19%

(4)



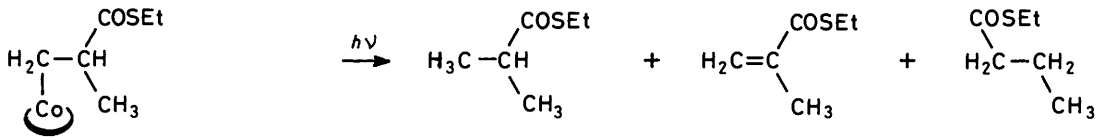
methanol (20 °C)	88%	0%	
benzene (20 °C)	82%	1·3%	
(0 °C)	71%	15%	
APC[C_2Lys(C_5N^+)2C_{14}]$_4$ (20 °C)	75%	13%	

(5)



methanol (20 °C)	86%	1·7%	0%
benzene (20 °C)	86%	1·5%	1·1%
APC[C_2Lys(C_5N^+)2C_{14}]$_4$ (20 °C)	60%	12%	6·0%

(6)



methanol (20 °C)	83%	0%	0%
benzene (20 °C)	80%	trace	trace
(0 °C)	65%	5%	12%
APC[C_2Lys(C_5N^+)2C_{14}]$_4$ (20 °C)	60%	4%	15%

(7)

complex was transformed into the bivalent cobalt species upon irradiation with visible light under anaerobic conditions.[10] Identical behaviour was observed for photolysis in methanol or benzene.

The cleavage of the cobalt–carbon bond in $(CO_2Et)_2$-$MeCCH_2$Cob(III)7C_3ester was examined under irradiation conditions by the spin-trapping technique with α-phenyl-*N*-(t-butyl)nitrone (PBN). E.s.r. signals attributable to the PBN spin adduct (A_N = 13.5 G, A_H = 2.0 G; 10^4 G = 1 T) were clearly observed in methanol and in benzene (Figure 3). This apparently indicates that the radical species is generated by photolytic cobalt–carbon cleavage. Although such e.s.r. signals were not detected at room temperature in the presence of the octopus cyclophane, typical signals attributable to the bivalent cobalt species were observed during photolysis by e.s.r. measurements at 77 K.[4] Since a very limited amount of PBN can be incorporated into the cyclophane in aqueous media, spin adduct formation is difficult to detect by e.s.r. In the light of the foregoing spectroscopic results, which were obtained from the alkylated complex under anaerobic irradiation conditions, the alkylated hydrophobic vitamin B_{12} must undergo homolysis of the cobalt–carbon bond in all the reaction media studied to afford the bivalent cobalt complex and the corresponding alkyl radical species under photolysis conditions.

The alkyl radical generated by photolysis of $(CN)(CO_2Et)$-$MeCCH_2$Cob(III)7C_3ester and the identical free radical produced by the reaction of the corresponding alkyl bromide with the tin radical species (Scheme 1) [24,25] were detected by e.s.r.

$$R'OOR' \xrightarrow{h\nu} 2R'O^{\bullet}$$

$$R'O^{\bullet} + Bu^n_3SnH \longrightarrow R'OH + Bu^n_3Sn^{\bullet}$$

$$Bu^n_3Sn^{\bullet} + RBr \longrightarrow Bu^n_3SnBr + R^{\bullet}$$

Scheme 1.

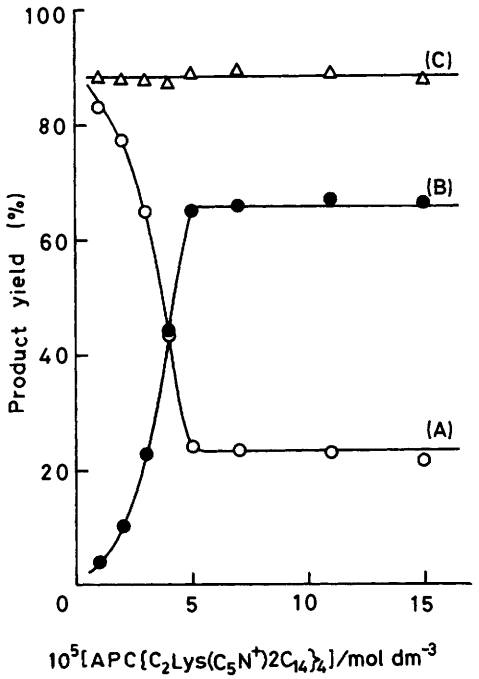


Figure 1. Correlations between concentration of $APC[C_2Lys(C_5N^+)2C_{14}]_4$ and product yield for photolysis of $Ac(CO_2Et)MeCCH_2Cob(III)7C_3ester$ (5.0×10^{-5} mol dm^{-3}) in phosphate–borate buffer (0.05 mol dm^{-3}; pH 9.2) at 20.0 ± 0.1 °C: (A) ethyl 2,2-dimethyl-3-oxobutyrate; (B) ethyl 2-methyl-4-oxopentanoate; (C) total yield

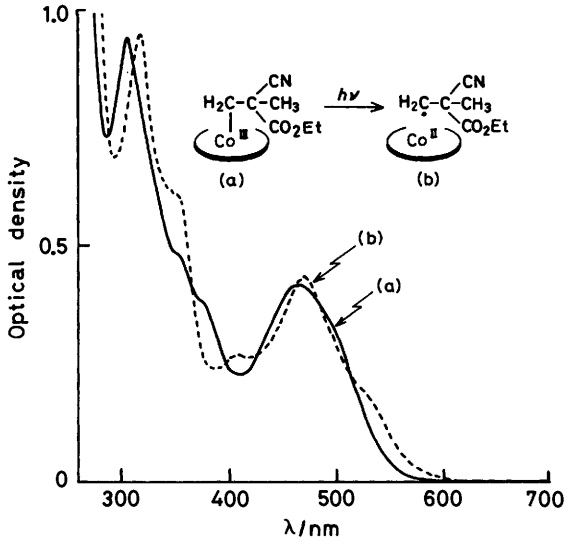


Figure 2. Electronic spectra of cobalt species incorporated into $APC[C_2Lys(C_5N^+)2C_{14}]_4$ (3.6×10^{-5} mol dm^{-3}) in phosphate–borate buffer (0.05 mol dm^{-3}; pH 9.2) at 20.0 ± 0.1 °C: (a) $(CN)(CO_2Et)MeCCH_2Cob(III)7C_3ester$ (3.6×10^{-5} mol dm^{-3}) incorporated into $APC[C_2Lys(C_5N^+)2C_{14}]_4$ before photolysis; (b) (a) irradiated with a 500 W tungsten lamp at a distance of 30 cm for 10 min [formation of $Cob(II)7C_3ester$]

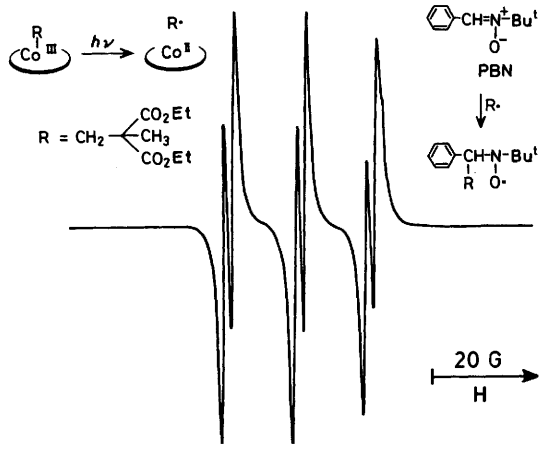


Figure 3. E.s.r. spectrum of a benzene solution containing $(CO_2Et)_2MeCCH_2Cob(III)7C_3ester$ (2.0×10^{-3} mol dm^{-3}) and PBN (0.10 mol dm^{-3}) at room temperature upon irradiation with a high-pressure mercury lamp at a distance of 30 cm

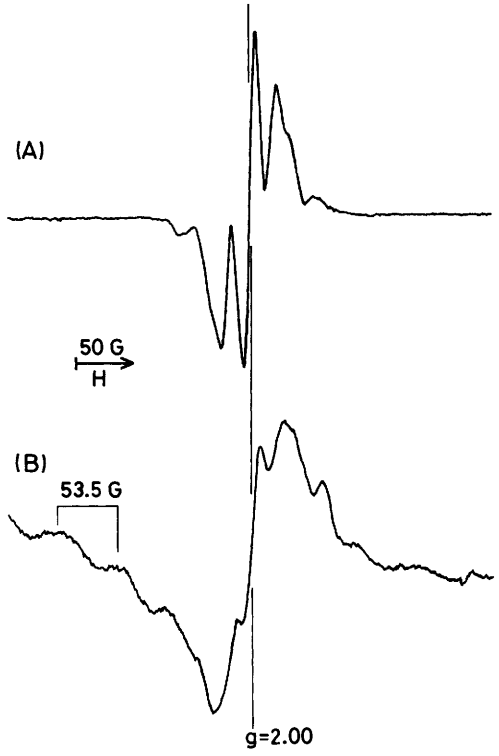
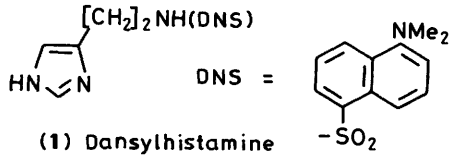


Figure 4. E.s.r. spectra observed during anaerobic photolysis with a 500 W xenon lamp at a distance of 30 cm at 77 K: (A) ethyl 3-bromo-2-cyano-2-methylpropanoate (5.0×10^{-2} mol dm^{-3}), Bu^n_3SnH (5.0×10^{-2} mol dm^{-3}), and benzoyl peroxide (5.0×10^{-2} mol dm^{-3}) incorporated into $APC[C_2Lys(C_5N^+)2C_{14}]_4$ (5.0×10^{-2} mol dm^{-3}) in phosphate–borate buffer (0.05 mol dm^{-3}; pH 9.2); (B) $(CN)(CO_2Et)MeCCH_2Cob(III)7C_3ester$ (5.0×10^{-2} mol dm^{-3}) incorporated into $APC[C_2Lys(C_5N^+)2C_{14}]_4$ (5.0×10^{-2} mol dm^{-3}) in the identical phosphate–borate buffer

spectroscopy. The alkyl radical produced by the reaction of the alkyl bromide with Bu^n_3SnH and benzoyl peroxide is shown in Figure 4(A), and that generated from the alkylated hydrophobic vitamin B_{12} by photolysis in Figure 4(B). The spectral features observed at around $g = 2.00$ (±70 G) are similar, though the signals in Figure 4(B) are rather broad. Signals with a hyperfine splitting constant of 53.5 G, presumably due to an interaction of the radical species with the cobalt nucleus, are observed in a low magnetic-field range as shown in Figure 4(B). A detailed explanation of this feature is not available at present.

Microenvironment around Hydrophobic Vitamin B_{12} in 'Octopus' Azaparacyclophane.—$[(CN)Cob(III)7C_3ester]ClO_4$



(1) Dansylhistamine

co-ordinated at the residual axial site by dansylhistamine [2-(imidazol-4-yl)-*N*-[5-(dimethylamino)-1-naphthylsulphonyl]-ethylamine (**1**)] as a fluorescent probe was employed to evaluate the microenvironment around the hydrophobic vitamin B_{12} derivative in $APC[C_2Lys(C_5N^+)2C_{14}]_4$.* The microscopic polarity experienced by the dansyl moiety bound to the hydrophobic vitamin B_{12} is reflected in its fluorescence maximum.[26] In order to obtain the reference data, fluorescence maxima of dansylhistamine co-ordinated to $[(CN)Cob(III)7C_3$-ester$]ClO_4$ were measured in various mixtures of water and dioxane as shown in Figure 5; the fluorescence maximum is shifted to lower wavelength as the solvent polarity decreases. It is clear that the octopus cyclophane provides a microenvironment for the dansyl moiety that is equivalent to a medium polarity between those of methanol and ethanol. Since the hydrophobic vitamin B_{12} itself in the octopus cyclophane is in a microenvironment equivalent in medium polarity to dichloromethane,[13] the dansyl moiety seems to be situated at a site in the cyclophane cavity closer to the bulk aqueous phase. This result provides us with the valuable information that not only the hydrophobic vitamin B_{12} itself but also an alkyl fragment bound to the complex is markedly desolvated in the cyclophane cavity.

Large fluorescence polarization (*P*) values were obtained at various temperatures for the dansylhistamine-co-ordinated hydrophobic vitamin B_{12} when the complex was incorporated into the octopus cyclophane: 0.2—0.3 in the temperature range 40—45 °C (Figure 6). This apparently indicates that the molecular motion of the guest in the host molecule is markedly repressed, since *P* values in methanol and benzene are 0.005—0.006 and 0.02—0.04, respectively, in the same temperature range. Thus, the microenvironmental effect provided by the cyclophane is quite different from those produced by simple organic solvents which solubilize the complex homogeneously.

To confirm that the repression of motion of the alkylated hydrophobic vitamin B_{12} results in enhancement of the isomerization reaction, the photolysis reactions of the alkylated complexes were examined in benzene at various temperatures. Product analyses for the photolysis of $Ac(CO_2Et)MeCCH_2$-$Cob(III)7C_3$ester are summarized in Table 3. The yield of acetyl migration product (C) was much greater below the m.p. of benzene than above it. A similar temperature effect was observed for photolyses of other alkylated complexes, shown in equations (4)—(7). Thus the hydrophobic cage effect of the octopus cyclophane acts to repress the molecular motion of the alkylated hydrophobic vitamin B_{12} and to desolvate it to a significant extent, so that the intermediate radical pair generated by photolysis can remain closely associated in the cage for a sufficient period of time for 1,2-migration to occur (see Scheme 2).

Role of Vitamin B_{12} in the Isomerization Process.—In order to clarify the role of the hydrophobic vitamin B_{12} in the 1,2-migration process, we examined the reactivity of the 2-acetyl-2-ethoxycarbonylpropyl radical produced by the reaction of the corresponding bromide with the tin radical species ($Bu^n{}_3Sn^\cdot$) derived from $Bu^n{}_3SnH$ and benzoyl peroxide under

* The axial ligation constant (*K*) of $[(CN)Cob(III)7C_3ester]ClO_4$ with the dansylhistamine was determined by electronic spectroscopy: log $K = 5.3$ in an aqueous buffer containing $APC[C_2Lys(C_5N^+)2C_{14}]_4$.

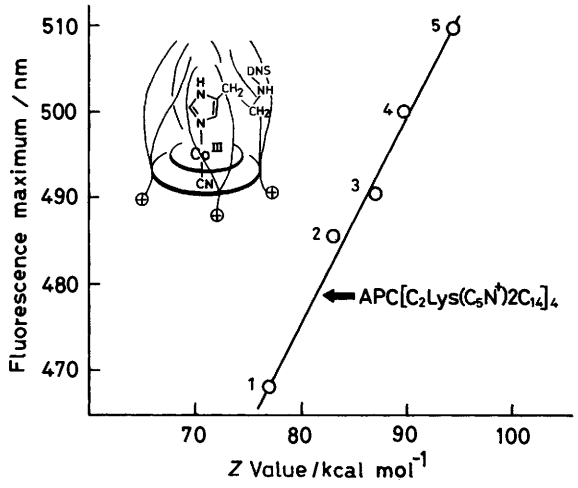


Figure 5. Solvent effect on fluorescence of dansylhistamine (1.0×10^{-5} mol dm^{-3}) co-ordinated to $[(CN)Cob(III)7C_3ester]ClO_4$ (1.0×10^{-5} mol dm^{-3}) incorporated into $APC[C_2Lys(C_5N^+)2C_{14}]_4$ (1.0×10^{-5} mol dm^{-3}) in phosphate–borate buffer (0.05 mol dm^{-3}; pH 9.2) at 20.0 ± 0.1 °C. Reference data obtained in water–dioxane at the following ratios (v/v): 1, 1:9; 2, 3:7; 3, 1:1; 4, 7:3; 5, 1:0

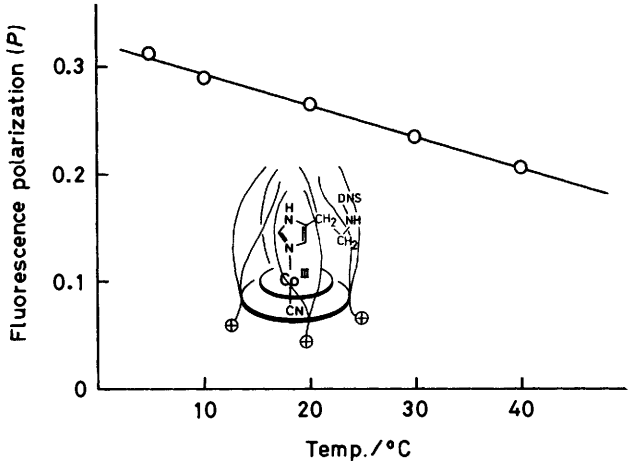
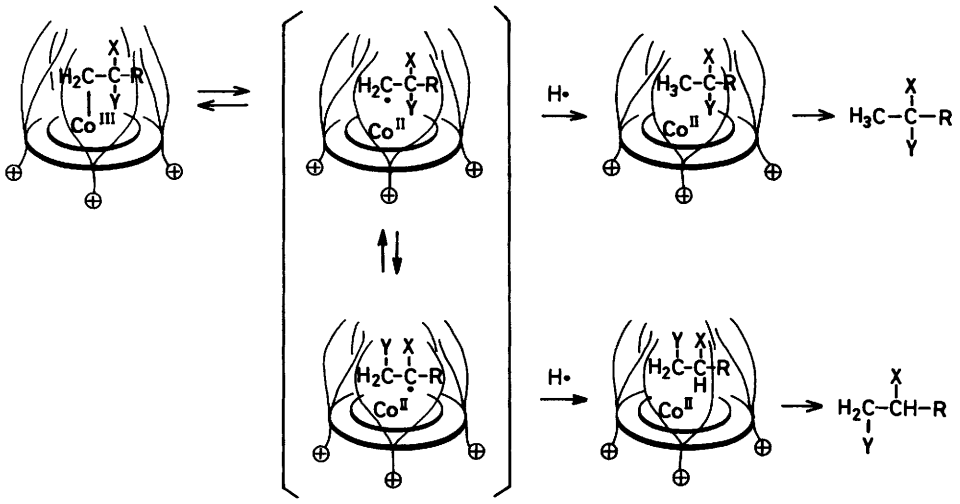


Figure 6. Temperature effect on fluorescence polarization of dansylhistamine (1.0×10^{-5} mol dm^{-3}) co-ordinated to $[(CN)Cob(III)7C_3$-ester$]ClO_4$ (1.0×10^{-5} mol dm^{-3}) incorporated into $APC[C_2Lys(C_5N^+)2C_{14}]_4$ (1.0×10^{-5} mol dm^{-3}) in phosphate–borate buffer (0.05 mol dm^{-3}; pH 9.2)

Table 3. Product analyses for photolysis of $Ac(CO_2Et)MeCCH_2$-$Cob(III)7C_3$ester in benzene[a]

	Yield (%)		
Temp. (°C)[b]	(A)	(B)	(C)
0.0	32	Trace[c]	55
4.0	30	Trace[c]	53
7.0	62	Trace[c]	13
10.0	63	Trace[c]	12
20.0	65	Trace[c]	10
30.0	64	Trace[c]	11
40.0	66	Trace[c]	13

[a] A benzene solution containing $Ac(CO_2Et)MeCCH_2Cob(III)7C_3ester$ (5.0×10^{-5} mol dm^{-3}) was irradiated with a 500 W tungsten lamp at a distance of 30 cm for 1 h at temperatures 7 °C and above and for 30 h at temperatures 4 °C and below. Products were analysed by g.l.c. [b] Accuracy, ±0.1 °C. [c] Yield less than 1%.



Scheme 2.

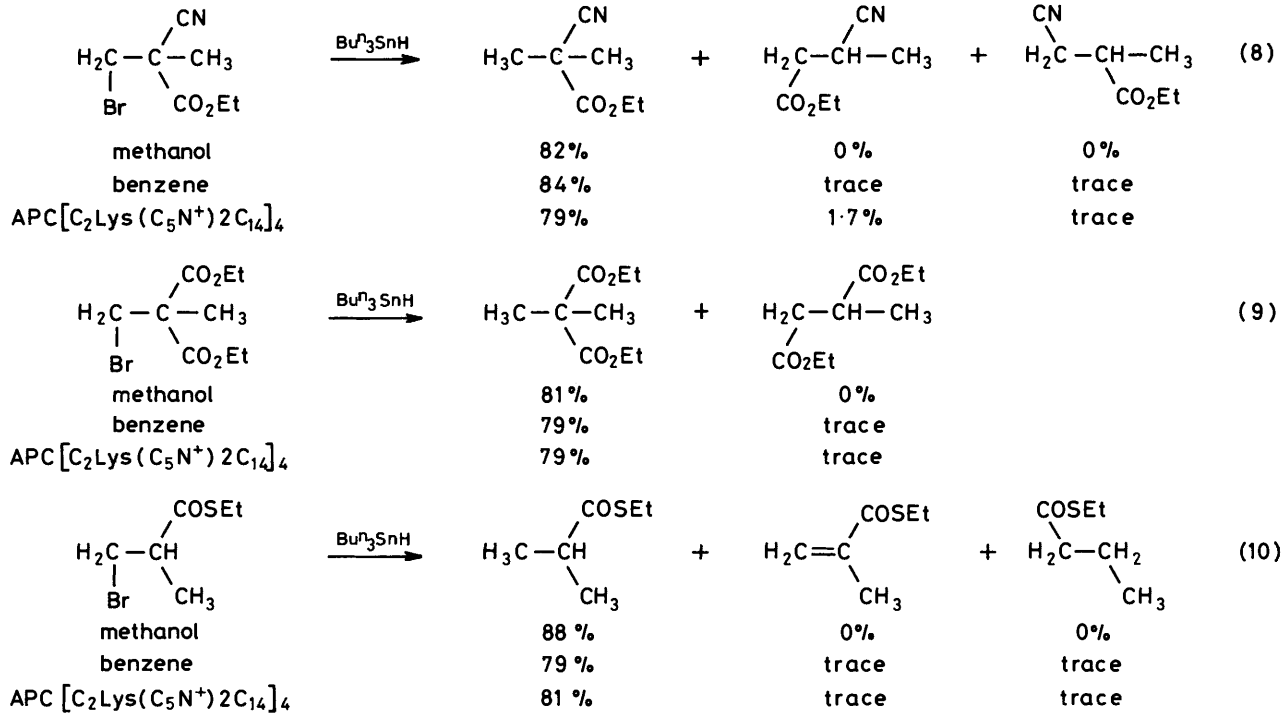


Table 4. Product analyses for reaction of radical species generated from ethyl 2-bromomethyl-2-methyl-3-oxobutyrate in various media[a]

Medium	Temp. (°C)[b]	Yield (%) (A)	(B)	(C)
Methanol	10.0	84	0	Trace[c]
	30.0	85	0	Trace[c]
Benzene	10.0	86	0	Trace[c]
	30.0	88	0	Trace[c]
APC[C_2Lys(C_5N^+)2C_{14}]$_4$[d]	10.0	78	0	2.9
	30.0	80	0	2.8

[a] A solution containing the ester (5.0×10^{-5} mol dm^{-3}), Bu$^n_3$SnH (5.1×10^{-5} mol dm^{-3}), and benzoyl peroxide (5.2×10^{-5} mol dm^{-3}) was irradiated with a 500 W tungsten lamp for 1 h at a distance of 30 cm. Products were analysed by g.l.c. [b] Accuracy, ±0.1 °C. [c] Yield less than 0.1%. [d] A dichloromethane solution containing APC[C_2Lys(C_5N^+)2C_{14}]$_4$, the ester, and benzoyl peroxide was evaporated *in vacuo* to remove the solvent completely in the dark, and then an aqueous phosphate–borate buffer (0.05 mol dm^{-3}; pH 9.2) was added to the residue: APC[C_2Lys-(C_5N^+)2C_{14}]$_4$, 5.0×10^{-5} mol dm^{-3}; concentrations of other species were identical with those given above.

photolysis conditions. The rearrangement products (B) and (C) were not detected in the homogeneous solutions, and the product (C) was obtained in quite small yield even in the presence of the octopus cyclophane (Table 4).* Similar results were obtained for other alkyl halides at 10 °C as given in equations (8)—(10). Consequently, the hydrophobic vitamin B_{12} is an essential factor for efficient promotion of the rearrangement reaction. In other words, the alkyl radical species alone cannot readily undergo the 1,2-migration since the radical species rapidly abstracts a hydrogen atom from some source under ordinary conditions. Participation of the bivalent cobalt species is required for the rearrangement reaction, so as to suppress abstraction of hydrogen *via* formation of a tight pair with the radical species.

Concluding Remarks.—In the light of the present study, the following became apparent.

(i) APC[C_2Lys(C_5N^+)2C_{14}]$_4$ has a sizeable hydrophobic cavity, such that the bimolecular alkylation reactions of Cob(I)7C_3ester with various alkyl halides (RX) can take place in the host molecule. In these reactions, molecular recognition based on an electrostatic effect becomes operative, and the rate enhancement originates from desolvation of the reacting species through formation of a ternary complex composed of APC[C_2Lys(C_5N^+)2C_{14}]$_4$, Cob(I)7C_3ester, and RX.

(ii) The octopus azaparacyclophane is effective as an apoenzyme model for functional simulation of vitamin-B_{12}-dependent enzymes. The 1,2-migration of the electron-withdrawing groups arises from both repression of motion and desolvation effects operating on the alkylated cobalt complexes situated in the cyclophane.

(iii) The nuclear bivalent cobalt of the hydrophobic vitamin B_{12} promotes the isomerization reaction *via* formation of a tight pair with the radical intermediate; free radicals formed in homogeneous solutions do not afford the corresponding rearrangement products under the present mild conditions. This study shows novel examples of vitamin B_{12}-dependent holoenzyme models that allow effective isomerization accompanied by carbon-skeleton rearrangement, and provides a useful guide for designing relevant apoenzyme models.

Experimental

General Analyses and Measurements.—Elemental analyses were performed at the Microanalysis Centre of Kyushu University. A Beckman Φ71 pH meter equipped with a Beckman 39505 combined electrode was used for pH measurements after calibration with a combination of appropriate standard aqueous buffers. Electronic absorption spectra were recorded with a Hitachi 340 or a Hitachi 220A spectrophotometer; fluorescence spectra were obtained with a Hitachi 650-60 spectrofluorometer. ^{1}H N.m.r. spectra were taken with a Hitachi R-24B spectrometer, and e.s.r. spectra with a JEOL JES-FE1G *X*-band spectrometer equipped with Advantest TR-5213 microwave counter and Echo Electronics EFM-2000 n.m.r. field meter. Fluorescence polarization measurements were made with a Union Giken FS-501A fluorescence polarization spectrophotometer equipped with a Sord microcomputer M200 Mark II; emission at 480 nm was monitored upon excitation at 325 nm with a slit width of 3.5 nm for both excitation and emission sides. Fluorescence polarization (P) was calculated according to a method used previously.[19,27] G.l.c. analyses were carried out with a Shimadzu GC-9A apparatus equipped with a Shimadzu C-R3A-FFC Chromatopac.

Materials.—Heptapropylcobyrinate perchlorate, [Cob(II)-7C_3ester]ClO_4, and various alkylated hydrophobic vitamin B_{12} derivatives, R-Cob(III)7C_3ester, were prepared with reference to a method reported previously.[9,14] The preparation of the octopus azaparacyclophane, APC[C_2Lys(C_5N^+)2C_{14}]$_4$, is described in detail elsewhere.[20,†] Preparative procedures for various alkyl halides used as substrates and corresponding authentic samples for product analyses have been reported previously.[15] 1-Bromohexane and 1-bromohexanoic acid were purchased from Wako Pure Chemical Industries, Osaka, Japan and distilled just before use. Methanol and benzene were purified and dried just before use according to standard procedures.[28]

(6-Bromohexyl)trimethylammonium Bromide, $Br[CH_2]_6N^+Me_3Br^-$.—6-Hydroxyhexyltrimethylammonium bromide (6.0 g, 0.025 mol), prepared from 6-bromohexan-1-ol[29] and trimethylamine, was mixed with phosphorus tribromide (150 g, 0.55 mol), and the mixture was stirred for 24 h at room temperature and then carefully poured into crushed ice (200 g). A combined diethyl ether extract (70 ml × 3) was evaporated to dryness and the residue was purified by gel-filtration chromatography on a column of Sephadex LH-20 with methanol as eluant: yield 4.1 g (53%), m.p. 75.4—76.1 °C (Found: C, 35.7; H, 7.05; N, 4.45. $C_9H_{21}Br_2N$ requires C, 35.65; H, 7.0; N, 4.6%); δ_H(60 MHz; $CDCl_3$) 1.7 (8 H, m, CH_2), 3.2 [11 H, m, $(CH_3)_3N^+CH_2$], and 3.6 (2 H, t, CH_2Br).

2-(Imidazol-4-yl)-N-[5-(dimethylamino)-1-naphthylsulphonyl]ethylamine (**1**).—A solution (200 ml) of 5-(dimethylamino)naphthalene-1-sulphonyl chloride (dansyl chloride; 1.0 g, 3.7×10^{-3} mol) in acetone was added to histamine (122 mg, 1.1×10^{-3} mol) dissolved in aqueous 0.1 mol dm^{-3} triethylamine (50 ml) on an ice-bath. The mixture was stirred in an ice-bath for 8 h and kept in a refrigerator for 10 h. A benzene extract was evaporated to dryness to give an oily material (820 mg, 77%); δ_H(60 MHz; $CDCl_3$) 2.74 [18 H, s, $N(CH_3)_2$], 2.80 (2 H, t, Im-CH_2), 3.00 (2 H, m, CH_2N), 6.75 (1 H, s, NH), and 7.3—8.3 (20 H, m, aromatic). This product, N,N^{Im},N^{Im}-tridansylhistamine (800 mg, 9.9×10^{-4} mol), was placed in 30% (w/w) formic acid (100 ml) and stirred for 4 h at room temperature. The mixture was evaporated to dryness, and the residue was purified by gel-filtration chromatography on columns of Sephadex LH-20 and Toyopearl HW-40 Fine with methanol to give the *dansylhistamine* (220 mg, 65%), m.p. 45.6—46.1 °C (Found: C, 58.95; H, 5.9; N, 16.1. $C_{17}H_{20}N_4O_2S$ requires C, 59.3; H, 5.85; N, 16.25%); δ_H(60 MHz; $CDCl_3$) 2.74 [6 H, s, $N(CH_3)_2$], 2.80 (2 H, t, Im-CH_2), 3.00 (2 H, m, CH_2N), 6.40 (1 H, s, NH), and 7.3—8.2 (8 H, m, aromatic).

Kinetic Measurements.—The pseudo-first-order rate constants ($k_{obs.}$) for the alkylation reactions of Cob(I)7C_3ester (2.0×10^{-5} mol dm^{-3}) with alkyl bromides (9.0×10^{-4}—6.5×10^{-3} mol dm^{-3}) in aqueous phosphate–borate buffer (0.05 mol dm^{-3}; pH 9.2) containing APC[C_2Lys(C_5N^+)2C_{14}]$_4$ (2.0×10^{-5} mol dm^{-3}) were obtained as described previously.[6] Correlations between the initial concentration of an alkyl bromide and the pseudo-first-order rate constant are shown in

* Recently, Dowd *et al.* reported that a combination of Bu^n_3SnH and 2,2′-azobisisobutyronitrile promoted rearrangement of the same alkyl bromide in refluxing benzene: P. Dowd and S.-C. Chang, *J. Am. Chem. Soc.*, 1987, **109**, 3493. We repeated their reaction under identical conditions: acetyl migration was observed (*ca.* 10% yield based on the initial amount of substrate). However, other functional groups such as cyano and ethoxycarbonyl did not undergo migration.

† Found for APC[C_2Lys(C_5N^+)2C_{14}]$_4$: C, 67.65; H, 10.35; N, 6.95. Calc. for $C_{216}H_{392}Br_4N_{20}O_{16}$: C, 67.45; H, 10.3; N, 7.3%.

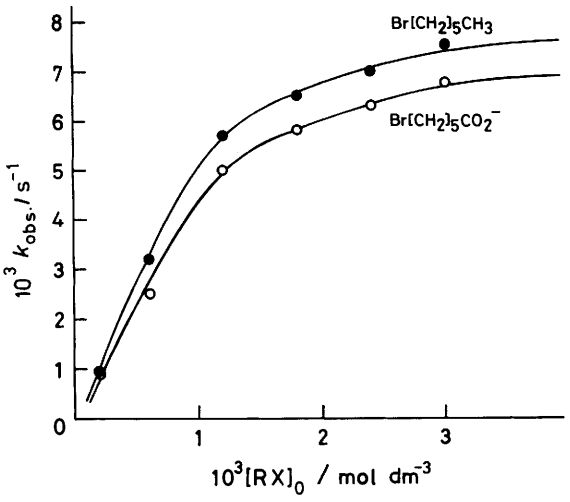


Figure 7. Correlations between initial concentration of an alkyl halide and pseudo-first-order rate constant for the alkylation reaction of Cob(I)7C_3ester (2.0×10^{-5} mol dm^{-3}) incorporated into APC[C_2Lys-(C_5N^+)2C_{14}]$_4$ (2.0×10^{-5} mol dm^{-3}) in phosphate–borate buffer (0.05 mol dm^{-3}; pH 9.2) at 20.0 ± 0.1 °C

Figure 7. Kinetic parameters were evaluated with the aid of equation (13), derived on the basis of equations (11) and (12), where B_{12}·APC and RX·B_{12}·APC refer to the binary complex composed of Cob(I)7C_3ester and APC[C_2Lys(C_5N^+)2C_{14}]$_4$ and the ternary complex composed of an alkyl bromide (RX), Cob(I)7C_3ester, and APC[C_2Lys(C_5N^+)2C_{14}]$_4$, respectively; K_1 and K_2 are the formation constants for the complexes involving one and two molecules of RX, respectively, while k_I and k_{II} are the respective alkylation rate constants and $[RX]_0$ is the initial concentration of an alkyl bromide. The values of K_1, K_2, k_I, and

$$B_{12}\text{·APC} + RX \overset{K_1}{\rightleftharpoons} RX\text{·}B_{12}\text{·APC} \xrightarrow{k_I} R\text{-}B_{12}\text{·APC} \quad (11)$$

$$B_{12}\text{·APC} + 2RX \overset{K_2}{\rightleftharpoons} 2RX\text{·}B_{12}\text{·APC} \xrightarrow{k_{II}} R\text{-}B_{12}\text{·APC·}RX \quad (12)$$

$$k_{obs.} = [RX]_0(k_1K_1 + k_{II}K_2[RX]_0)/(1 + K_1[RX]_0 + K_2[RX]_0^2) \quad (13)$$

k_{II} were obtained by regression analysis, which minimizes the sum of squares of errors (U), applied to all the experimental data [equation (14)]

$$U = \Sigma\{k_{obs.} - [RX]_0(k_1K_1 + k_{II}K_2[RX]_0)/(1 + K_1[RX]_0 + K_2[RX]_0^2)\}^2 \quad (14)$$

It turned out that only the kinetic process given by equation (11) is valid under the present kinetic conditions.

Photolysis and Product Analyses.—A solution containing equimolar quantities of APC[C_2Lys(C_5N^+)2C_{14}]$_4$ and the alkylated complex, R-Cob(III)7C_3ester in dichloromethane was evaporated *in vacuo* to remove the solvent completely in the dark, and then an aqueous phosphate–borate buffer (0.05 mol dm^{-3}; pH 9.2) was added to the residue to obtain the reaction sample with concentrations of the components at 5.0×10^{-5} mol dm^{-3}. The solution was deoxygenated with nitrogen, then irradiated with a 500 W tungsten lamp for 1 h at a distance of 30 cm and at the desired temperature. Complete decomposition of the alkylated complex was confirmed by electronic spectroscopy, and then the product was extracted with dichloromethane and analysed by g.l.c. The products were identified by means of g.l.c., with coinjection of authentic samples as described previously in detail.[15] As for reaction in methanol or benzene, a reaction mixture was evaporated to dryness *in vacuo* before extraction with hexane. Total yields listed in this paper are less than 100% owing to losses during extraction. However, we confirmed that no other by-products were obtained.

Acknowledgements

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References

1 For example Y. Murakami, 'Vitamin B_{12} Models with Macrocyclic Ligands,' in 'Biomimetic Chemistry,' ACS Advances in Chemistry Series No. 191, eds. D. Dolphin, C. McKenna, Y. Murakami, and I. Tabushi, American Chemical Society, Washington D.C., 1980, pp. 179—199.
2 J. Halpern, 'Chemistry and Significance of Vitamin B_{12} Model Systems,' in 'B_{12},' ed. D. Dolphin, Wiley, New York, 1982, vol. 1, pp. 501—542.
3 With reference to model reactions for methylmalonyl-CoA mutase, see for example: (*a*) H. Flohr, W. Pannhorst, and J. Rétey, *Helv. Chim. Acta*, 1978, **61**, 1565; (*b*) A. I. Scott, J. B. Hansen, and S. K. Chung, *J. Chem. Soc., Chem. Commun.*, 1980, 388; (*c*) J. H. Grate, J. W. Grate, and G. N. Schrauzer, *J. Am. Chem. Soc.*, 1982, **104**, 1588; (*d*) P. Dowd and B. K. Trivedi, *J. Org. Chem.*, 1985, **50**, 206; (*e*) W. M. Best, A. P. F. Cook, J. J. Russell, and D. A. Widdowson, *J. Chem. Soc., Perkin Trans. 1*, 1986, 1139. With reference to model reactions for α-methyleneglutarate mutase, see for example: (*f*) J. W. Grate and G. N. Schrauzer, *Z. Naturforsch. Teil B*, 1984, **39**, 821; (*g*) P. Dowd, B. K. Trivedi, M. Shapiro, and L. K. Marwaha, *J. Chem. Soc., Perkin Trans. 2*, 1985, 413. With reference to model reactions for diol dehydrase, see for example: (*h*) B. T. Golding, C. S. Sell, and P. J. Sellers, *J. Chem. Soc., Perkin Trans. 2*, 1980, 961; (*i*) P. Muller and J. Rétey, *J. Chem. Soc., Chem. Commun.*, 1983, 1342; (*j*) R. G. Finke, D. A. Schiraldi, and B. J. Meyer, *Coord. Chem. Rev.*, 1984, **54**, 1.
4 Y. Murakami, Y. Hisaeda, and A. Kajihara, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 3642.
5 Y. Murakami, Y. Hisaeda, A. Kajihara, and T. Ohno, *Bull. Chem. Soc. Jpn.*, 1984, **57**, 405.
6 Y. Murakami, Y. Hisaeda, and T. Ohno, *Bull. Chem. Soc. Jpn.*, 1984, **57**, 2091.
7 Y. Murakami, Y. Hisaeda, and T. Ozaki, *Chem. Lett.*, 1985, 473.
8 Y. Murakami, Y. Hisaeda, T. Ohno, and T. Ozaki, *Chem. Lett.*, 1985, 477.
9 Y. Murakami and Y. Hisaeda, *Bull. Chem. Soc. Jpn.*, 1985, **58**, 2652.
10 Y. Murakami, Y. Hisaeda, T. Ozaki, and T. Ohno, *Chem. Lett.*, 1985, 1711.
11 Y. Murakami, Y. Hisaeda, T. Tashiro, and Y. Matsuda, *Chem. Lett.*, 1985, 1813.
12 Y. Murakami, Y. Hisaeda, T. Tashiro, and Y. Matsuda, *Chem. Lett.*, 1986, 555.
13 Y. Murakami, Y. Hisaeda, J. Kikuchi, T. Ohno, M. Suzuki, and Y. Matsuda, *Chem. Lett.*, 1986, 727.
14 Y. Murakami, Y. Hisaeda, T. Ohno, and Y. Matsuda, *Chem. Lett.*, 1986, 731.
15 Y. Murakami, Y. Hisaeda, T. Ozaki, T. Tashiro, T. Ohno, Y. Tani, and Y. Matsuda, *Bull. Chem. Soc. Jpn.*, 1987, **60**, 311.
16 S. Wollowitz and J. Halpern, *J. Am. Chem. Soc.*, 1984, **106**, 8319.
17 J. M. Pratt, 'Coordination Chemistry of the B_{12} Dependent Isomerase Reactions,' in 'B_{12},' ed. D. Dolphin, Wiley, New York, 1982, vol. 1, pp. 325—392.
18 Y. Murakami, 'Functionalized Cyclophanes as Catalysts and Enzyme Models,' in 'Cyclophanes II,' Topics in Current Chemistry No. 115, ed. F. Vögtle, Springer-Verlag, Berlin, 1983, pp. 107—155.
19 Y. Murakami, J. Kikuchi, M. Suzuki, and T. Takaki, *Chem. Lett.*, 1984, 2139.
20 Y. Murakami, J. Kikuchi, M. Suzuki, and T. Matsuura, *J. Chem. Soc., Perkin Trans. 1*, in the press.

21 B. T. Golding, 'Mechanisms of Action of the B_{12} Coenzyme: Theory and Models,' in 'B_{12},' ed. D. Dolphin, Wiley, New York, 1982, vol. 1, pp. 543—582.
22 N. J. Turro and J. Mattay, *J. Am. Chem. Soc.*, 1981, **103**, 4200.
23 J. Rétey, 'Vitamin B_{12},' ed. B. Zagalak and W. Friedrich, de Gruyter, Berlin, 1979, pp. 439—460.
24 C. Chatgilialoglu, K. U. Ingold, and J. C. Scaiano, *J. Am. Chem. Soc.*, 1981, **103**, 7739.
25 U. Aeberhard, R. Keese, E. Stamm, U. Vögeli, W. Lau, and J. K. Kochi, *Helv. Chim. Acta*, 1983, **66**, 2740.
26 C. J. Seliskar and L. Brand, *J. Am. Chem. Soc.*, 1971, **93**, 5414.
27 Y. Murakami, J. Kikuchi, K. Nishida, and A. Nakano, *Bull. Chem. Soc. Jpn.*, 1984, **57**, 1371.
28 J. A. Riddick and W. B. Bunger, 'Organic Solvents,' Wiley-Interscience, New York, 1970.
29 Y. Murakami, A. Nakano, and H. Ikeda, *J. Org. Chem.*, 1982, **47**, 2137.

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