Carbon-skeleton Rearrangement of an Amino Acid Derivative as Mediated by Hydrophobic Vitamin B_{12} Covalently Bound to a Lipid Species in a Bilayer Membrane

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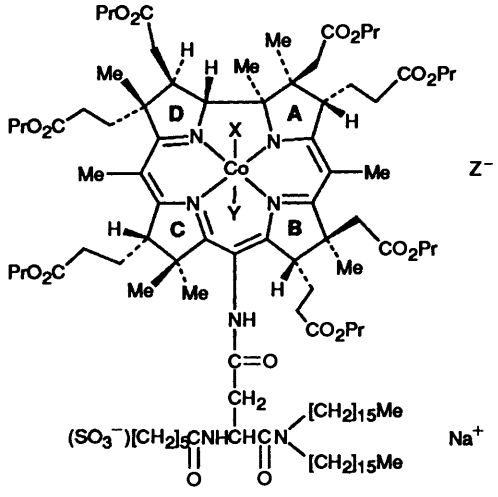
Diethyl 2-acetylamino-2-methylpropanedioate co-ordinated to a hydrophobic vitamin B_{12} covalently bound to a peptide lipid underwent a carbon-skeleton rearrangement to afford diethyl 2-acetylaminobutanedioate in the single-walled vesicle of *N,N*-bis(hexadecyl)-N^{α}-(6-sulfohexanoyl)-L-alaninamide under photolysis conditions.

We have been interested in the catalytic activity of vitamin B_{12} placed in hydrophobic microenvironments so as to simulate catalytic functions of the holoenzymes concerned. In this context, we have prepared an artificial holoenzyme composed of a synthetic bilayer membrane and a hydrophobic vitamin B_{12}. The artificial holoenzyme was found to catalyse isomerization reactions characteristic of the natural holoenzymes, as effected by both motional repression and desolvation effects provided by synthetic bilayer membranes.[1] In order to enhance the catalytic efficiency in the isomerization reactions, we have prepared novel hydrophobic vitamin B_{12} derivatives covalently bound to a lipid species[2] and performed model reactions in simulation of catalysis by methylmalonyl-CoA mutase and α-methyleneglutarate mutase, as mediated by the novel vitamin B_{12} lipid in a bilayer membrane formed with (SO_3^-)-C_5Ala2C_{16}.[3] For the purpose of exploring the catalytic efficiency of the vitamin B_{12} lipid (**1**) in an isomerization reaction of a non-natural substrate, the reaction of diethyl 2-acetylamino-2-methylpropanedioate bound to the hydrophobic vitamin B_{12} was investigated in the $(SO_3^-)C_5Ala2C_{16}$ vesicle under photolysis conditions as described below.

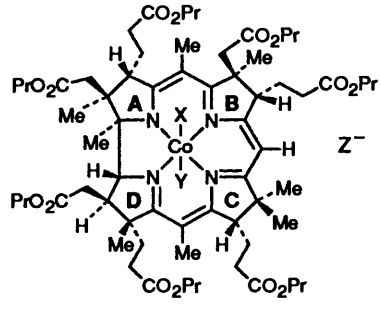
Diethyl 2-acetylamino-2-bromomethylpropanedioate, a brominated non-natural substrate, was prepared after a method reported previously[4] as shown by reaction (1). This brominated substrate was identified by ^1H NMR and IR measurements as well as by elemental analysis. Alkylated complex **2** was prepared by the reaction of **1** with diethyl 2-acetylamino-2-bromomethylpropanedioate and sodium tetrahydroborate in a manner as reported previously[1] [reaction (2)] to give a dark brown solid.† Complex **4** was derived from **3** by following a similar method to give a dark brown solid.‡ The alkylated complexes (**2** and **4**) showed electronic spectra characteristic of tervalent cobalt complexes with a Co–C bond and were converted into the corresponding tervalent cobalt complexes without an alkyl ligand upon cleavage of the Co–C bond by aerobic photolysis (Fig. 1).

Peptide lipid $(SO_3^-)C_5Ala2C_{16}$ was prepared previously.[5] Diethyl 2-acetylamino-2-methylpropanedioate (**A**) and diethyl 2-acetylaminobutanedioate (**B**) were prepared as authentic samples for the corresponding reaction products after procedures reported previously[6] and confirmed to be sufficiently pure by ^1H NMR measurements as well as by GLC and elemental analyses [refer to reaction (3)].

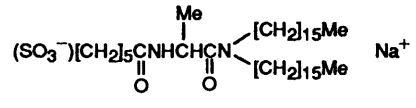
In homogeneous solutions in methanol or benzene, the reaction of the alkylated hydrophobic vitamin B_{12} (**4**) was



1 X = Y = none, Z = ClO_4
2 X = $CH_2C(NHCOMe)(CO_2Et)_2$, Y = H_2O, Z = ClO_4



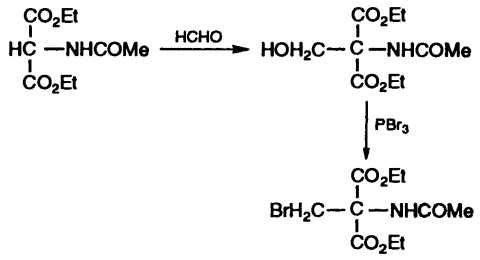
3 X = Y = none, Z = ClO_4
4 X = $CH_2C(NHCOMe)(CO_2Et)_2$, Y = H_2O, Z = ClO_4



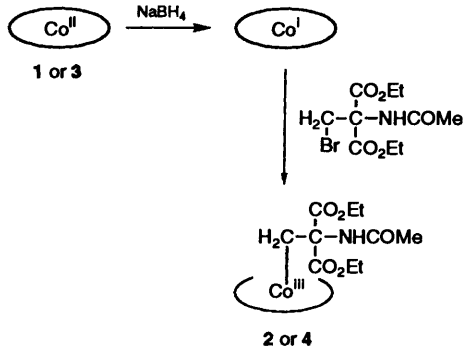
$(SO_3^-)C_5Ala2C_{16}$

† Yield 63%; $\lambda_{max}(CH_2Cl_2)$/nm 266 (ε 1.6 × 10^3 dm^3 mol^{-1} cm^{-1}), 305 (1.9 × 10^3), 317 (1.9 × 10^3), 421 (7.3 × 10^2) and 466 (8.5 × 10^2) (Found: C, 61.6; H, 8.6; N, 4.8. $C_{118}H_{197}ClCoN_8NaO_{29}S$ requires: C, 60.53; H, 8.48; N, 4.79%).

‡ Yield 74%; $\lambda_{max}(CH_2Cl_2)$/nm 264 (ε 1.8 × 10^3 dm^3 mol^{-1} cm^{-1}), 293 (1.6 × 10^3), 313 (1.5 × 10^3), 416 (7.0 × 10^2) and 454 (6.6 × 10^2) (Found: C, 57.9; H, 7.4; N, 4.5. $C_{76}H_{119}ClCoN_5O_{24}$ requires: C, 57.73; H, 7.59; N, 4.43%).



(1)



(2)

Table 1 Product analyses for photolysis of hydrophobic vitamin B_{12} derivatives in various media at 20.0 °C[a]

Medium[b]	Complex	Yield[c] (%) A	B	C
CH_3OH	4	55–57	0–2	19–25
C_6H_6	4	2–6	0–3	78–82
$(SO_3^-)C_5Ala2C_{16}$ vesicle	4	29–33	22–29	22–26
$(SO_3^-)C_5Ala2C_{16}$ vesicle	2	25–26	29–36	10–12

[a] A solution containing a complex (1.0×10^{-4} mol dm^{-3}) was irradiated with a 500 W tungsten lamp at a distance of 30 cm under argon atmosphere for 1 h. [b] $(SO_3^-)C_5Ala2C_{16}$ (5.0×10^{-3} mol dm^{-3}) in aqueous phosphate buffer (20 cm^3; 1.0×10^{-3} mol dm^{-3}; μ 0.01 with KCl; pH 7.0). [c] Products were analysed by GLC. Total yields are less than 100% due to losses during extraction and evaporation treatments.

carried out under anaerobic irradiation with visible light in a manner as described previously.[1] On the basis of GLC analysis, an unexpected product was obtained in addition to the simple reduction product (**A**) and the rearranged one (**B**) [refer to reaction (3)]. The unexpected product was found to be a major component of the whole products in benzene, *ca.* 80%. Therefore, the reaction mixture was concentrated and hexane was added to the concentrate to precipitate the hydrophobic vitamin B_{12}. The filtrate was evaporated to dryness and the residue was dissolved in a small amount of dichloromethane. The resulting solution was applied on a preparative GLC column packed with Silicon SE-30 to give a white powder.§ The unknown product was confirmed to be diethyl 2-acetylaminopropanedioate (**C**) [refer to reaction (3)] by referring to NMR and GLC data obtained for the authentic sample obtained from Tokyo Kasei Co., Tokyo, Japan as a guaranteed reagent.

The reactions of alkylated complexes **2** and **4** were carried out in the single-compartment vesicle as follows. An aqueous phosphate buffer (20 cm^3; 1.0×10^{-3} mol dm^{-3}; μ 0.01 with KCl; pH 7.0) containing $(SO_3^-)C_5Ala2C_{16}$ (1.0×10^{-4} mol)

§ δ_H(500 MHz; $CDCl_3$, Me_4Si) 1.30 (6 H, t), 2.08 (3 H, s), 4.24–4.31 (4 H, m), 5.16 (1 H, d) and 6.52 (1 H, d).

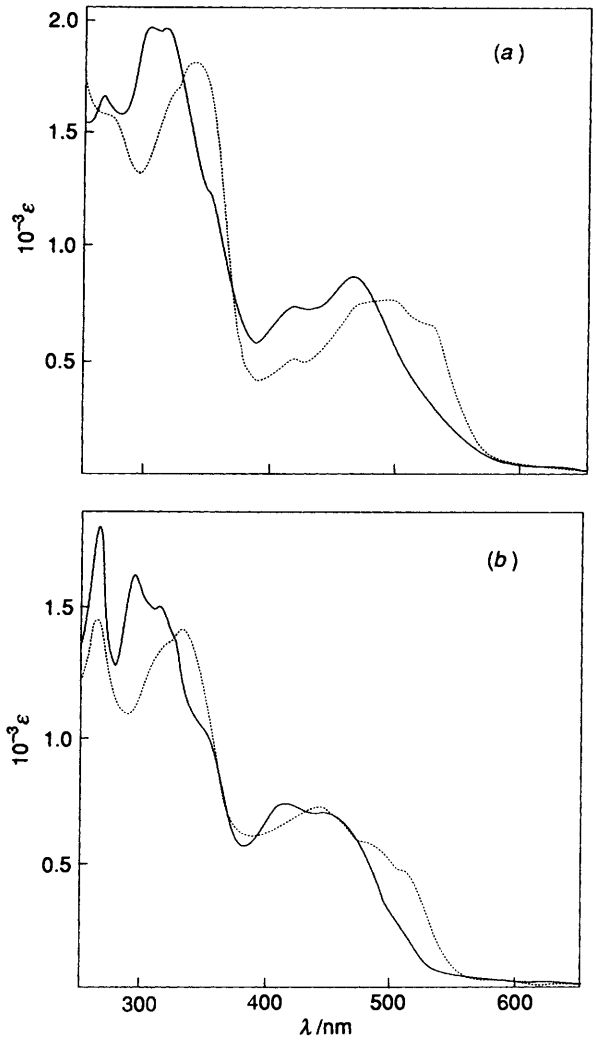
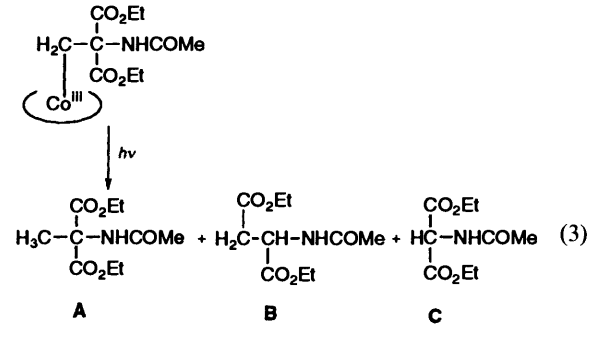


Fig. 1 Electronic spectra of hydrophobic vitamin B_{12} derivatives before photolysis (solid lines) and after irradiation with a 500 W tungsten lamp at a distance of 40 cm for 1 min (dotted lines) in dichloromethane at 20.0 ± 0.1 °C: (*a*), **2** (3.7×10^{-4}); (*b*), **4** (4.8×10^{-5} mol dm^{-3})



(3)

was sonicated for 15 min with a probe-type sonicator at 30 W to give a clear solution. After the solution was deoxygenated with argon gas, *ca.* 0.2 cm^3 of a methanol solution containing the alkylated complex (**2** or **4**) (2.0×10^{-6} mol) was added to it. The alkylated complex was allowed to undergo photolysis with visible light under argon atmosphere at 20.0 ± 0.1 °C. After the alkylated complex was completely photolysed as confirmed by electronic spectroscopy, the resulting mixture was lyophilized. The residue was dissolved in dichloromethane and the filtrate was analysed by GLC (Table 1).

The product analyses for the reaction in various media are summarized in Table 1. The simple reduction product (**A**) was largely obtained in methanol because the solvent acts as an efficient hydrogen donor, while the methyl-eliminated product (**C**) was a major one in benzene due to lack of the hydrogen-donating ability of the medium. As a reference experiment in benzene, the 2-acetylamino-2,2-bis(ethoxycarbonyl)ethyl radical was produced by the reaction of the corresponding bromide with the $Bu_3Sn^{\bullet}$ radical derived from Bu_3SnH and benzoyl peroxide under photolysis conditions. Under such conditions without the hydrophobic vitamin B_{12}, only the reduction product (**A**) was generated. This result indicates that the methyl-eliminated product (**C**) is not obtained without participation of the hydrophobic vitamin B_{12}. On the other hand, relatively large amounts of the rearrangement product (**B**) were obtained in the $(SO_3^-)C_5Ala2C_{16}$ vesicle which provides a hydrophobic microenvironment for **2** and **4** in aqueous media, as compared with the reactions in methanol and benzene. In particular, the substrate species co-ordinated to the vitamin B_{12} lipid (**1**) underwent photolysis to afford the rearrangement product as the largest component in the whole products. As we have clarified previously,[3] the hydrophobic vitamin B_{12} derivative covalently bound to the lipid species is subjected to marked motional repression in the bilayer membrane, as compared with the simple hydrophobic vitamin B_{12} without lipid linkage, to enhance the rearrangement reaction.

In conclusion, the motional repression and desolvation effects operated on the substrate-bound hydrophobic vitamin B_{12} tend to enhance the rearrangement of the substrate radical formed by photolysis. This fact also suggests that participation of the hydrophobic vitamin B_{12} in the transition state is crucial for the rearrangement reaction. Such microenvironmental effects provided by the bilayer membrane becomes more pronounced when the hydrophobic vitamin B_{12} is covalently bound to the lipid species. It must also be noted that the ethoxycarbonyl group seems to migrate much more readily than the acetylamino group under the present experimental conditions.

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