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Hydrophobic Vitamin B_{12}. I. Preparation and Axial Ligation Behavior of Hydrophobic Vitamin B_{12r}[†]

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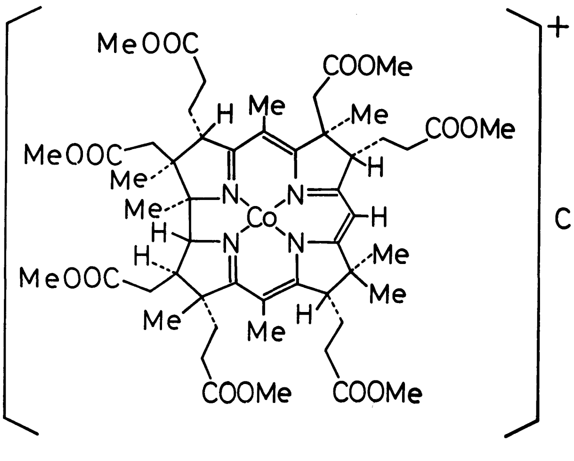
Heptamethyl cobyrinate perchlorate, [Cob(II)ester]ClO_4, was prepared in a good yield from cyanocobalamin. This hydrophobic complex is structurally comparable to the base-off form of vitamin B_{12r}. Thermodynamic parameters (ΔG, ΔH, and ΔS) for axial ligation of [Cob(II)ester]ClO_4 with various amines in dichloromethane and toluene were determined by electronic spectroscopy. The isoequilibrium temperatures obtained from plots of ΔH vs. ΔS were 194±20 and 317±20 K in dichloromethane and toluene, respectively. The equilibrium is primarily controlled by entropy at ordinary temperatures and the log K_1 value varies sensitively along with the change in pK_a of amine in dichloromethane, while the equilibrium values were almost invariant to the choice of amine in toluene because the measurements were carried out near the isoequilibrium temperature. The log K_1 value for [Cob(II)ester]ClO_4, different from the case of cobalt(II)–porphyrin complexes, depends on the nature of solvent used and undergoes wide variations. The possible functions of equatorial ligands involved in these two different complexes in the axial ligation behavior were discussed.

We have prepared previously the cobalt complex of 1,19-dimethyl-AD-didehydrocorrin (Hbdhc), having additional double bonds at peripheral positions and an additional angular methyl group compared with the parent corrinoid, and investigated its structural properties from the viewpoint of vitamin B_{12} chemistry.[1)] The bdhc complex is quite analogous to the corrinoid as far as the electronic properties are concerned on the basis of its spectroscopic, electrochemical, and axial coordination behaviors. On the other hand, the angular methyl groups in the complex exert an unusually large steric effect in the bimolecular reactions of Co^I(bdhc) with alkyl donors which result in the formation of an alkyl-cobalt bond. Even though the bdhc complex is considered to be a good B_{12} model, it does not catalyze the 1–2 migration as observed with vitamin B_{12}. This may indeed be due to the steric repulsion of the angular methyl groups which prevents the initially formed alkyl fragments upon C–Co cleavage from staying close to the cobalt. In order to improve these situations, it is necessitated to develop another good vitamin B_{12} model.

The model studies of coenzyme B_{12}-dependent enzymatic reactions have been carried out in aqueous media[2)] since vitamin B_{12} is soluble in water but hardly in apolar solvents. In order to simulate the functions of vitamin B_{12} as exerted in the hydrophobic active sites of enzymes concerned, however, vitamin B_{12} needs to be modified so that the model studies can be carried out in ordinary apolar organic solvents. Therefore, we synthesized in this work heptamethyl cobyrinate by converting amide groups of vitamin B_{12} into ester ones. Heptamethyl cobyrinate involving cobalt(II) has been prepared by Werthemann *et al.* and isolated as the iodo-adduct.[3)] In the present work, we prepared heptamethyl cobyrinate perchlorate, [Cob(II)ester]ClO_4, in a good yield according to some modified method. This complex is structurally comparable to vitamin B_{12r} in the base-off form, but much more stable in solution and solid states and much less readily oxidized in the air than vitamin B_{12r} itself.

Physicochemical data for vitamin B_{12r} are lacking because B_{12r} is readily oxidized to B_{12a} in the air,[4)] even though such information is important for characterizing its catalytic functions. Therefore, axial ligation constants and thermodynamic parameters for the reaction of [Cob(II)ester]ClO_4 with some pyridines, imidazoles, and aliphatic amines were evaluated in organic solvents under nitrogen atmosphere, and compared with the corresponding data for (5,10,15,20-tetraphenylporphinato)cobalt(II), Co^{II}(tpp).



[Cob(II)ester] ClO_4

Experimental

Materials. Cyanocobalamin was purchased from Nakarai Chemicals and used without further purification. Dicyanocobyrinic acid heptamethyl ester, $(CN)_2$Cob(III)ester, was prepared from cyanocobalamin according to the method of Werthemann *et al.*,[3)] purified by thin-layer chromatography on silica gel (Kiesel gel 60H) with chloroform–methanol (9 : 1 v/v), and reprecipitated from benzene upon addition of hexane. The product was identified by electronic, IR, ^{1}H-NMR, and ^{13}C-NMR spectroscopy[5,6)] as well as by elemental analysis. (5,10,15,20-Tetraphenylporphinato)cobalt(II), Co^{II}(tpp), was prepared after the method of Alder *et al.*,[7)] purified by liquid chromatography on a column of silica gel (Wakogel C-100) with chloroform as an eluant followed by recrystallization from

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chloroform, and identified by electronic and IR spectroscopy as well as by elemental analysis.

Heptamethyl Cobyrinate Perchlorate, [Cob(II)ester]ClO_4. $(CN)_2$Cob(III)ester (50 mg, 4.6×10^{-5} mol) was dissolved in 40 ml of dichloromethane, and the resulting purple solution was treated with 25 ml of 30% aqueous perchloric acid. The orange dichloromethane layer was separated from the acidic aqueous layer and washed with distilled water. After being dried over sodium sulfate, the organic layer was evaporated to dryness at room temperature to obtain monocyanocobyrinic acid heptamethyl ester, [(CN)(H_2O)Cob(III)ester]ClO_4, in a nearly quantitative yield. The monocyano-complex, [(CN)(H_2O)Cob(III)ester]ClO_4, was dissolved in 50 ml of methanol, and 50 ml of distilled water was added to this solution. The solution was deoxygenated by bubbling nitrogen gas through it for 30 min, and treated with 1.0 g of sodium tetrahydroborate and 15 ml of distilled water with vigorous shaking and frequent venting under nitrogen atmosphere. At the instance that the color of solution changed to dark green, 3 ml of 60% aqueous perchloric acid was added carefully to decompose excess sodium tetrahydroborate and to convert Cob(I)ester into Cob(II)-ester. The resulting cobalt complex was extracted with dichloromethane and washed with distilled water. After being dried over sodium sulfate, the dichloromethane solution was evaporated to dryness at room temperature. The residue was reprecipitated four times from benzene upon addition of hexane to afford fine brown crystals: yield 45 mg (87%); λ_{max} (CH_2Cl_2) 266 (ε 1.90×10^4), 316 (2.35×10^4), 403 (0.63×10^4), and 468 nm (1.21×10^4); IR (KBr) 1730 (ester C=O), and 1100 and 620 cm^{-1} (ClO_4^-).

Found: C, 54.68; H, 6.41; N, 5.00%. Calcd for $C_{52}H_{73}ClCoN_4O_{18}$: C, 54.95; H, 6.47; N, 4.93%.

Spectroscopic Measurements. Electronic absorption spectra were recorded on a Hitachi 340 or a Hitachi 220A spectrophotometer. ESR spectra were obtained with a JEOL JES-ME-3 X-band spectrometer equipped with a 100-kHz field modulation unit; a standard MgO/Mn^{II} sample calibrated with a NMR magnetometer was employed for calibration of the magnetic field.

Measurements of Equilibrium Constants and Thermodynamic Calculations. Liquid amines were distilled and solid amines were sublimed at 60 °C/1.33 Pa before use. Dichloromethane and toluene purified and dried according to the standard procedures[8] were redistilled and degassed by bubbling nitrogen gas through them before use. An equilibrium constant for coordination of an amine base to [Cob(II)ester]ClO_4 in dichloromethane was determined by measuring spectral change of a series of solutions containing the cobalt complex (*ca.* 8×10^{-5} mol dm^{-3}) and varying amounts of an amine base under nitrogen atmosphere. Upon addition of each of pyridines, imidazoles, and aliphatic amines, the visible peak at 468 nm decreased in intensity along with slight shift to longer wavelength, and clear isosbestic points were observed. Equilibrium constants were calculated by Eq. 1.

$$\log[(A_0-A)/(A-A_\infty)] = n\log[\mathrm{B}] + \log K_n \qquad (1)$$

Here, A designates an absorbance at a given concentration of an amine base ([B]), A_0 is an absorbance at [B]=0, and A_∞ stands for a limiting absorbance in the presence of a sufficient amount of a base. The values of log K_n, measured at three or four different temperatures over the 10—30 °C range, were used to evaluate ΔH and ΔS values. As for [Cob(II)ester]ClO_4 in toluene, and Co^{II}(tpp) in dichloromethane and toluene, the equilibrium constants were also calculated according to the same method on the basis of absorbance changes at 470 nm for the former complex and 527.5 nm for the latter.

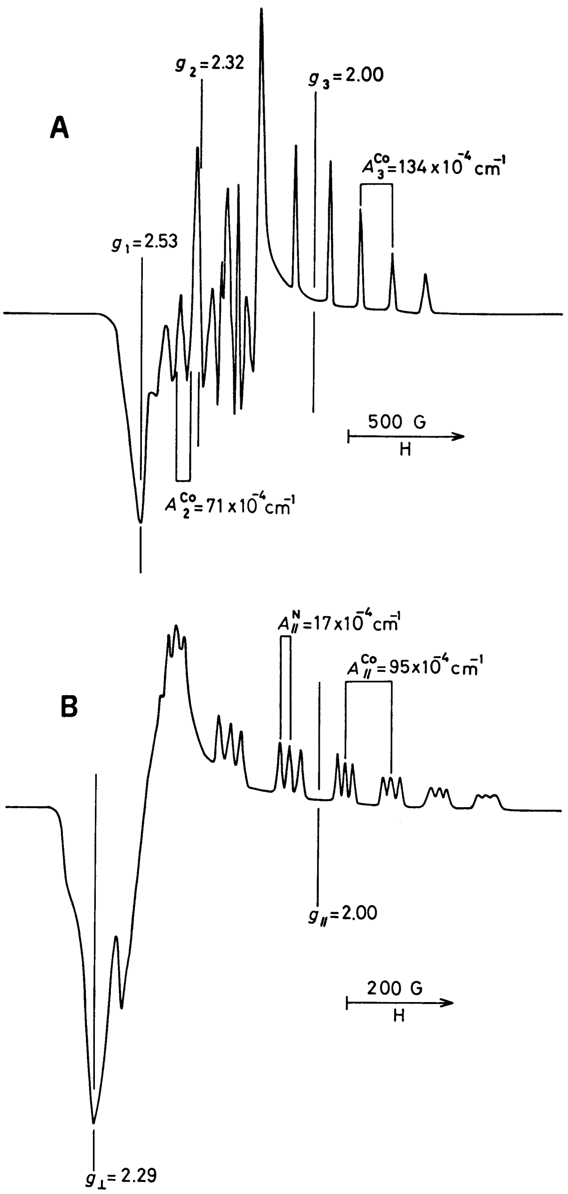

Fig. 1. ESR spectra of [Cob(II)ester]ClO_4 in chloroform–benzene (2 : 1 v/v) at 77 K: A, 9×10^{-3} mol dm^{-3} [Cob(II)ester]ClO_4; B, 9×10^{-3} mol dm^{-3} [Cob(II)-ester]ClO_4 and 4.5×10^{-2} mol dm^{-3} pyridine.

Results and Discussion

ESR spectra of [Cob(II)ester]ClO_4 in base-off and base-on forms are shown in Fig. 1. The spectra and the corresponding spin Hamiltonian parameters are comparable to those of aqueous vitamin B_{12r}.[9,10] Electronic spectra of the present complex also show similar features as observed for aqueous vitamin B_{12r}.[11]

The spectral change upon addition of a base to [Cob(II)ester]ClO_4 is typically shown in Fig. 2. The corresponding straight line plots on the basis of Eq. 1 are given in Fig. 3; n=1.00±0.05. This indicates that only one molecule of the base is coordinated to one molecule of the complex under the present conditions, being consistent with the ESR data (Fig. 1). The equilibrium constants and thermodynamic parameters are summarized in Table 1 for the reaction of [Cob(II)ester]-

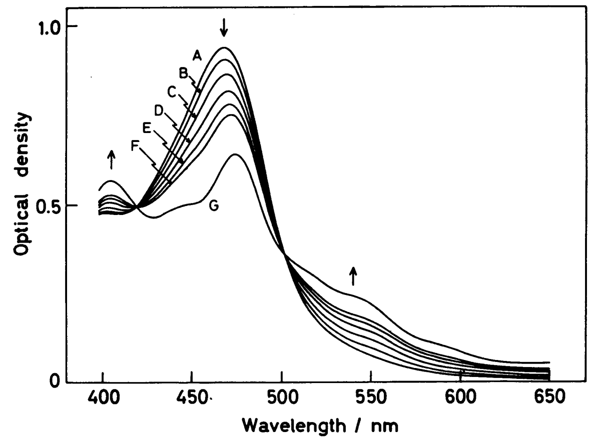

Fig. 2. Spectral change at 18.6 °C for the reaction of [Cob(II)ester]ClO$_4$ in dichloromethane with morpholine at various concentrations: A, 0; B, 7.62×10^{-5}; C, 2.28×10^{-4}; D, 4.56×10^{-4}; E, 7.59×10^{-4}; F, 1.19×10^{-3}; G, 1.32×10^{-1} mol dm^{-3}. Trends of spectral change with increase in base concentration are shown by arrows.

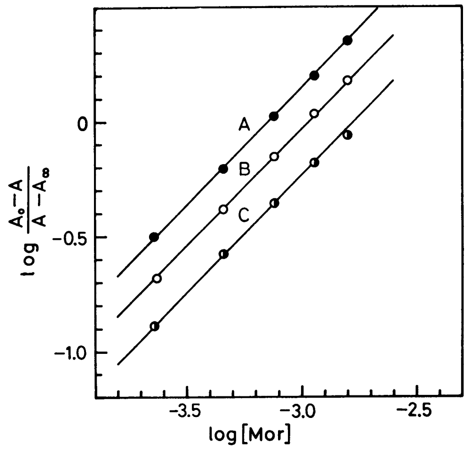

Fig. 3. Analysis of spectral data for the reaction of [Cob(II)ester]ClO$_4$ with morpholine in dichloromethane at temperatures: A, 28.0; B, 23.7; C, 18.6 °C (refer to Eq. 1). Morpholine concentrations are given in mol dm^{-3}.

ClO$_4$ with various bases in dichloromethane and toluene.

The linear correlations are observed between log K_1 and pK_a[12] values for the axial ligation of [Cob(II)ester]ClO$_4$ with various bases as shown in Fig. 4 specifically in dichloromethane: a single straight line correlation for each of the three different types of amine bases; imidazoles, pyridines, and aliphatic amines. The log K_1 value for the reaction with 2-methylpyridine deviates largely from the corresponding correlation line apparently due to a steric hindrance caused by the 2-methyl group. The stability order with respect to the type of amine base is in an increasing sequence: aliphatic amines, pyridines, and imidazoles. However, this order cannot be directly correlated with the nature and strength of cobalt–nitrogen bond even though such explanation is a tempting one,[13] since the entropy effect must be taken into account. An increment in $-\Delta G$ (from 21.1 to 25.6 kJ mol^{-1}) is accompanied by a decrement in $-\Delta H$ (from 44 to 36 kJ mol^{-1}) for the reaction of the complex with imidazole derivatives in dichloromethane (Table 1). Such trends are also noted for the reaction with aliphatic amines and some pyridine bases in the same solvent (Table 1). The thermodynamic parameters for ligand coordination in the liquid state seem to reflect the interaction modes of a medium with the metal complex and a coordinating ligand. Such modes must be intensively correlated with the extent of charge delocalization within the metal complex as well as with the extent of steric interaction between a base and the complex. Thus, the enthalpy and entropy parameters were treated on the basis of isoequilibrium relationship.[14,15]

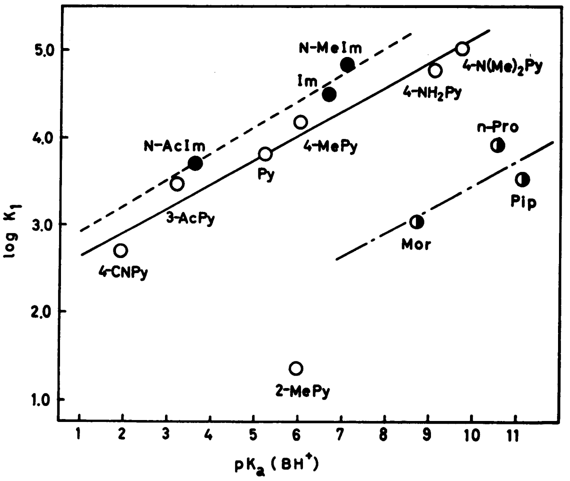

Fig. 4. Correlations between log K_1 and amine basicity for the reaction of [Cob(II)ester]ClO$_4$ with various bases in dichloromethane at 23.5 °C; K_1 in mol^{-1} dm^3.

The enthalpy and entropy changes are linearly correlated by Eq. 2.

$$\Delta H = \Delta H_0 + \beta\Delta S \tag{2}$$

Here, ΔH_0 is the ΔH value corresponding to $\Delta S=0$, and β denotes the isoequilibrium temperature. Combination of Eq. 2 with the correlation, $\Delta G=\Delta H-T\,\Delta S$, gives the following equations.

$$\Delta G = (T/\beta)\Delta H_0 + (1 - T/\beta)\Delta H \tag{3}$$

$$\Delta G = \Delta H_0 + (\beta - T)\Delta S \tag{4}$$

Consequently, the variation of the free energy change ($\Delta\Delta G$) along with the axial coordination of amines is given by either Eq. 5 or 6, and $\Delta\Delta G$ is concerned with a temperature range under measurements with respect to β.

$$\Delta\Delta G = (1 - T/\beta)\Delta\Delta H \tag{5}$$

$$\Delta\Delta G = (\beta - T)\Delta\Delta S \tag{6}$$

Plots of ΔH *vs.* ΔS are shown in Fig. 5 (refer to Table 1). As regards the data obtained in dichloromethane, all points appear to lie along the same line irrespective of the nature of base except for the sterically hindered one (2-MePy): correlation coefficient, 0.99; β, 194$\pm$20 K. It is not surprising, therefore, that increments in ΔG are accompanied by decrements in ΔH at the temperature range of present study which is about 100 K above the isoequilibrium temperature. The stability order for the axial ligation of [Cob(II)ester]ClO$_4$ (see Fig. 4) decreases in the order: imidazoles, pyridines,

Table 1. Thermodynamic parameters for axial ligation of [Cob(II)ester]ClO_4 with various amines

Solvent	Amine[a]	$pK_a(BH^+)$	$\log K_1$[b,c]	$-\Delta G$[b] / kJ mol^{-1}	$-\Delta H$ / kJ mol^{-1}	$-\Delta S$[d] / J K^{-1} mol^{-1}
Dichloromethane	2-MePy	5.97	1.36	7.7	32 ± 4	92
	4-CNPy	1.90	2.70	15.3	37 ± 4	71
	3-AcPy	3.18	3.48	19.8	39 ± 4	63
	Py	5.22	3.83	21.8	38 ± 4	54
	4-MePy	5.98	4.20	23.9	36 ± 8	42
	4-NH_2Py	9.11	4.79	27.2		
	4-N$(Me)_2$Py	9.70	5.02	28.5		
	N-Aclm	3.60	3.72	21.1	44 ± 7	79
	lm	6.65	4.51	25.6	36 ± 5	38
	N-Melm	7.06	4.85	27.5		
	Mor	8.70	3.05	17.3	48 ± 6	105
	Pip	11.12	3.53	20.0		
	n-Pro	10.53	3.93	22.3	44 ± 4	79
Toluene	3-AcPy	3.18	1.98	11.2	38 ± 4	92
	Py	5.22	1.81	10.3	38 ± 4	92
	4-MePy	5.98	2.16	12.3	46 ± 4	117
	Mor	8.70	2.06	11.7	44 ± 7	109
	Pip	11.12	2.14	12.2	36 ± 5	84

a) Abbreviations: 2-MePy, 2-methylpyridine; 4-CNPy, 4-cyanopyridine; 3-AcPy, 3-acetylpyridine; Py, pyridine; 4-MePy, 4-methylpyridine; 4-NH_2Py, 4-aminopyridine; 4-N$(Me)_2$Py, 4-dimethylaminopyridine; *N*-Aclm, *N*-acetylimidazole; lm, imidazole; *N*-Melm, *N*-methylimidazole; Mor, morpholine; Pip, piperidine; *n*-Pro, propylamine. b) Evaluated at 296.6 K. c) Accuracy within ±0.1; K_1 in mol^{-1} dm^3. d) Accuracy within ±15.

Table 2. Thermodynamic parameters for axial ligation of Co^{II} (tpp) with various amines

Solvent	Amine	$pK_a(BH^+)$	$\log K_1$[a,b]	$-\Delta G$[a] / kJ mol^{-1}	$-\Delta H$ / kJ mol^{-1}	$-\Delta S$[c] / J K^{-1} mol^{-1}
Dichloromethane	Py	5.22	2.91	16.5	41 ± 4	79
	N-Aclm	3.60	2.50	14.2	42 ± 5	88
	N-Melm	7.06	3.05	17.3	44 ± 5	92
	n-Pro	10.53	3.08	17.5	56 ± 4	130
	Mor	8.70	3.35	19.0	52 ± 4	113
	Pip	11.12	3.55	20.2	46 ± 4	88
Toluene	Py	5.22	2.86	16.2	35 ± 6	63
	N-Aclm	3.60	2.89	16.4	28 ± 4	42
	N-Melm	7.06	3.22	18.3	34 ± 4	54
	n-Pro	10.53	3.13	17.8	38 ± 4	71
	Mor	8.70	3.36	19.1	36 ± 4	54
	Pip	11.12	3.43	19.5	38 ± 4	63

a) Evaluated at 296.6 K. b) Accuracy within ±0.1; K_1 in mol^{-1} dm^3. c) Accuracy within ±15.

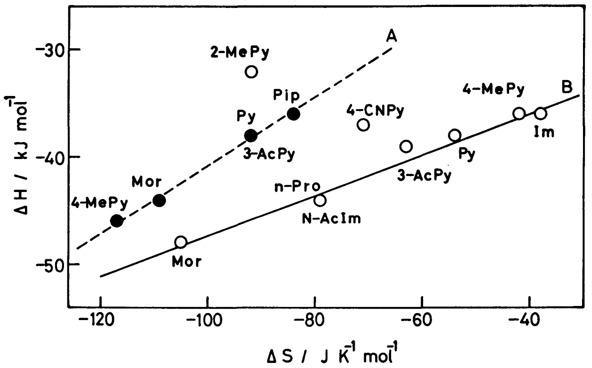

Fig. 5. Plots of ΔH *vs.* ΔS for the reaction of [Cob(II)-ester]ClO_4 with amines in toluene (A) and dichloromethane (B).

and aliphatic amines. Dichloromethane is considered to be inert for metal-coordination and interaction with the corrin skeleton. Thus, such stability sequence seems to be concerned with the extent of steric interaction between the cobalt complex and amine bases. Further, space-filling molecular models indicate that serious van der Waals repulsion can be generated between the hydrogen atoms bound to the carbons *α* to the nitrogen atom of the aliphatic amines and the angular hydrogen atom bound to the carbon atom at 19 position of [Cob(II)ester]ClO_4. The molecular models show that the restriction of rotation about the carbon-nitrogen bond decreases in the order: cyclic aliphatic amines, pyridine derivatives, and imidazole derivatives. This is attributed to the interactions between an amine base and the side chains of [Cob(II)ester]ClO_4 and

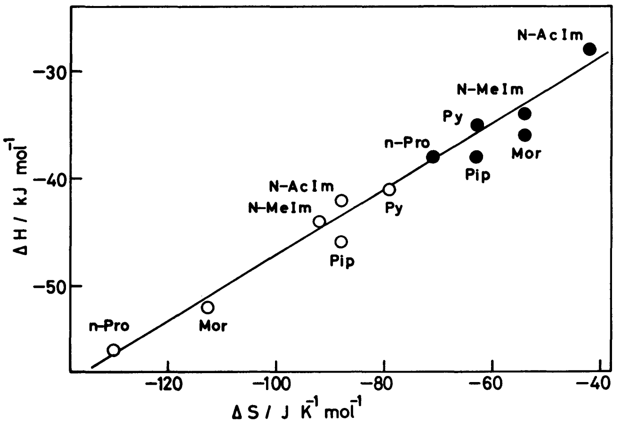

Fig. 6. Plots of ΔH vs. ΔS for the reaction of Co$^{\mathrm{II}}$(tpp) with amines in dichloromethane (○) and toluene (●). The solid line is based on the least squares calculation applied to all points.

between the hydrogen atoms bound to α-carbon atoms of amines and the angular hydrogen of the cobalt complex.

In toluene, the enthalpy-entropy relationship with a correlation coefficient of 0.99 (Fig. 5) gives $\beta = 317 \pm 20$ K. The isoequilibrium temperature is very close to the temperature range for present measurements. As a consequence, the values of log K_1 are nearly invariant to the choice of amine in contrast to those observed in dichloromethane.

Thermodynamic parameters for the reaction of Co$^{\mathrm{II}}$(tpp) with various amines in dichloromethane and toluene are tabulated in Table 2. The values of log K_1 are nearly invariant to the choice of amine or solvent. Plots of ΔH vs. ΔS for determination of the isoequilibrium temperatures in both solvents are shown in Fig. 6. All points appear to lie along the same line: correlation coefficient, 0.98; β, 300 ± 20 K. Calculations for each individual solvent system result in isoequilibrium temperatures of 301 ± 30 and 327 ± 50 K for dichloromethane and toluene, respectively. Because the isoequilibrium temperatures are obviously close to room temperature, the log K_1 value for axial ligation of Co$^{\mathrm{II}}$(tpp) in dichloromethane and toluene is insensitive to the change in pK_a of amine. The results obtained for Co$^{\mathrm{II}}$(tpp) are comparable to those for [5,10,15,20-tetrakis(*p*-methoxyphenyl)porphinato]cobalt(II), Co$^{\mathrm{II}}$(t(*p*-CH$_3$O)pp).[16] Axial ligation constants for the reaction of Co$^{\mathrm{II}}$(t(*p*-CH$_3$O)pp) with pyridine, piperidine, and *N*-methylimidazole in several solvents have been reported to be nearly constant at room temperature. This was attributed to the fact that the reaction was carried out near the isoequilibrium temperature (320 ± 24 K). Being consistent with the previous and present results, plots of ΔH vs. ΔS result in a single line correlation for the axial ligation of a cobalt(II)-porphyrin complex, irrespective of the nature of amines and solvents. On the other hand, the correlation of ΔH vs. ΔS for the axial ligation of [Cob(II)ester]ClO$_4$ apparently depends on the nature of solvent employed (Fig. 5). The present complex, [Cob(II)ester]ClO$_4$, involves the monoanionic corrin moiety as its equatorial ligand while Co$^{\mathrm{II}}$(tpp) has the dianionic porphyrin ligand. The positive charge in the former complex is supposed to be delocalized throughout the whole molecule and the π-π interaction of charge-transfer type between the equatorial ligand and toluene is expected along with the reduction of axial ligation ability as a consequence. Such interaction is apparently not expected with dichloromethane.

In conclusion, the isoequilibrium relationship applies to axial ligation of [Cob(II)ester]ClO$_4$ in a manner as demonstrated for cobalt(II)- and iron(II)-porphyrin complexes.[13,16,17] In dichloromethane which has no tendency to interact with [Cob(II)ester]ClO$_4$, the equilibrium constant is primarily controlled by entropy at ordinary temperatures and the log K_1 value varies sensitively along with the change in pK_a of amine. The log K_1 value for the present hydrophobic vitamin B$_{12r}$, in contrast to that for cobalt(II)-porphyrin complexes, depends on the nature of medium. This suggests that the axial ligation equilibrium of vitamin B$_{12}$ changes widely with the microenvironmental property in the enzyme active sites.

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