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PREPARATION AND CHARACTERIZATION OF HYDROPHOBIC VITAMIN B_{12} COMPLEXES HAVING A PROXIMAL BASE

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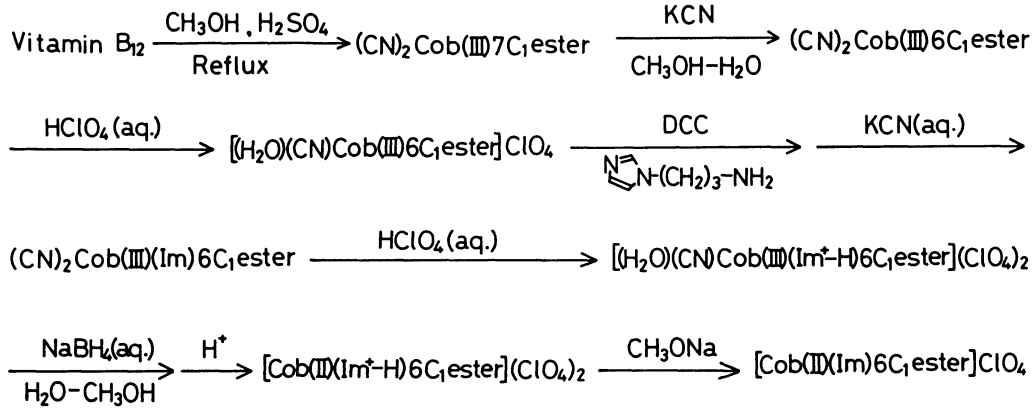
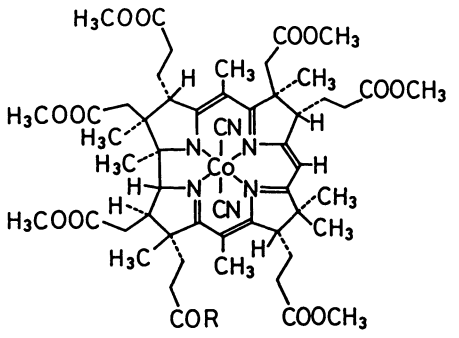
Hexamethyl cobyrinate complexes having an imidazolyl segment as a proximal base, with and without the axial cyano ligands, were prepared and characterized by means of electronic, CD, ^{1}H-NMR, and ESR spectroscopy. The redox behavior of the complex without the axial cyano ligands was examined by cyclic voltammetry.

We have prepared hydrophobic vitamin B_{12} derivatives in order to simulate various functions of vitamin B_{12} as exerted in the hydrophobic reaction sites of enzymes concerned, and investigated their axial coordination and redox behavior in non-aqueous media as well as their alkylation reactions in various molecular aggregates.[1-3] The hydrophobic vitamin B_{12} derivatives so far synthesized by us do not have any proximal base capable of coordinating to the nuclear cobalt at its axial sites within each molecule. Even though the catalytic role of the axial base in vitamin B_{12} has not been understood clearly, it has been considered that the axial base has some electronic and steric effects on the cobalt-carbon bond cleavage in enzymic systems.[4,5] In this regard, we prepared hydrophobic vitamin B_{12} complexes having an imidazolyl segment, branched from the equatorial skeleton, as an axial base according to the following procedure based on modification of cyanocobalamin (vitamin B_{12}).



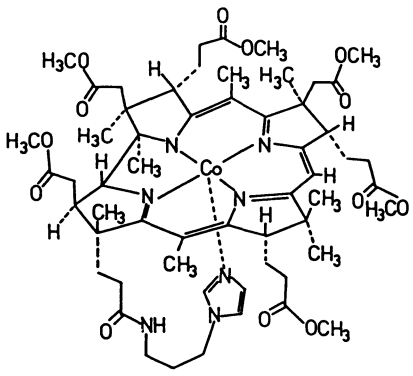
$(CN)_2$Cob(III)7C_1ester[1] (2.54 g, 2.33 mmol) was hydrolyzed in a medium composed of methanol containing 1%(w/w) potassium cyanide (25 mL) and water (1 mL) for 1 h at 30 °C. After the mixture was evaporated in vacuo, dichloromethane was added to



R=OCH$_3$: (CN)$_2$Cob(III)7C$_1$ester

=O$^-$K$^+$: (CN)$_2$Cob(III)6C$_1$ester

=N-(CH$_2$)$_3$-N : (CN)$_2$Cob(III)(Im)6C$_1$ester



[Cob(III)(Im)6C$_1$ester]ClO$_4$

the residue and the undissolved material (potassium cyanide) was removed by filtration. The filtrate was evaporated to dryness in vacuo, and the product was purified by gel-filtration chromatography on a column of Sephadex LH-20 with methanol as an eluant. The second fraction among five components[6] was collected and evaporated to dryness to afford a dark purple solid, (CN)$_2$Cob(III)6C$_1$ester: yield 729 mg (28%); UV_{max} (0.01%(w/w) KCN in CH_3OH) 278 (ε 9.01 x 10^3), 315 (8.17 x 10^3), 369 (2.29 x 10^4), 420 (2.13 x 10^3), 545 (6.86 x 10^3), and 584 nm (8.24 x 10^3); ^{13}C-NMR (CD_3OD, TMS) δ=172.3, 173.1, 173.6, 174.2, 174.9, and 175.2 (ester CO), and 180.5 (carboxylate CO). Found: C, 55.61; H, 6.41; N, 7.34%. Calcd for $C_{53}H_{70}CoKN_6O_{14}\cdot(3/2)H_2O$: C, 55.83; H, 6.45; N, 7.37%.

(CN)$_2$Cob(III)6C$_1$ester (521 mg, 0.468 mmol) was dissolved in dichloromethane (100 mL), and the purple solution was treated with 30%(w/w) 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 orange layer was evaporated to dryness. The residue was reprecipitated from chloroform upon addition of hexane to afford fine red crystals, [(H_2O)(CN)Cob(III)6C$_1$ester]-ClO_4: yield 476 mg (87%); UV_{max} (CH_3OH) 275, 358, and 532 nm; IR (KBr) 1730 (ester C=O), and 1120 and 635 cm^{-1} (ClO_4^-).

A dry dichloromethane solution (10 mL) of the above product (1.55 g, 1.34 mmol) and N,N'-dicyclohexylcarbodiimide (DCC; 276 mg, 1.34 mmol) was stirred for 0.5 h at 0 °C, and 3-(1-imidazolyl)propylamine (156 mg, 1.34 mmol) was added to the solution which was subsequently stirred for 3 h at 0 °C and for 24 h at room temperature. The precipitates (N,N'-dicyclohexylurea) were removed by filtration, and the filtrate was evaporated in vacuo. The residue was subjected to gel-filtration chromatography on a column of Sephadex LH-20 with methanol as an eluant. The major red fraction was evaporated to dryness, and the residue was dissolved in dichloromethane and treated with 0.5%(w/w) aqueous potassium cyanide. The unreacted portion was eliminated as (CN)$_2$Cob(III)6C$_1$ester by gel-filtration chromatography on a column of Sephadex LH-20 with methanol. The purple fraction eluted first was evaporated to dryness, and the residue was dissolved in methanol containing hydrogen chloride (ca. 1%(w/w)) and evaporated to dryness. Dichloromethane was added to the

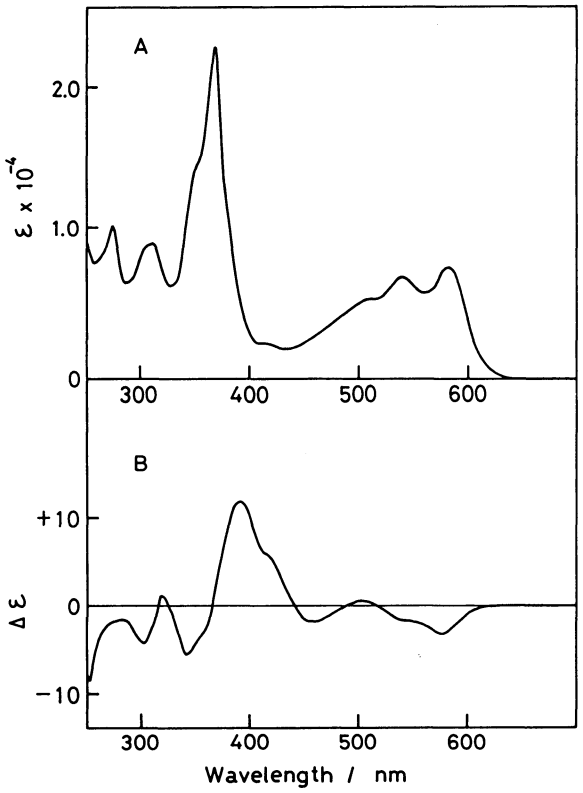


Fig. 1. Electronic (A) and CD (B) spectra of $(CN)_2$Cob(III)(Im)6C_1ester in CH_3OH containing 0.01%(w/w) KCN at 20.0 °C: UV_{max} 279 (ε 9.26 x 10^3), 316 (9.71 x 10^3), 369 (2.17 x 10^4), 423 (2.78 x 10^3), 548 (6.92 x 10^3), and 586 nm (7.76 x 10^3); CD 251 (Δε -8.69), 304 (-4.69), 343 (-5.92), 396 (+11.9), 423 (+5.23), and 582 nm (-3.54).

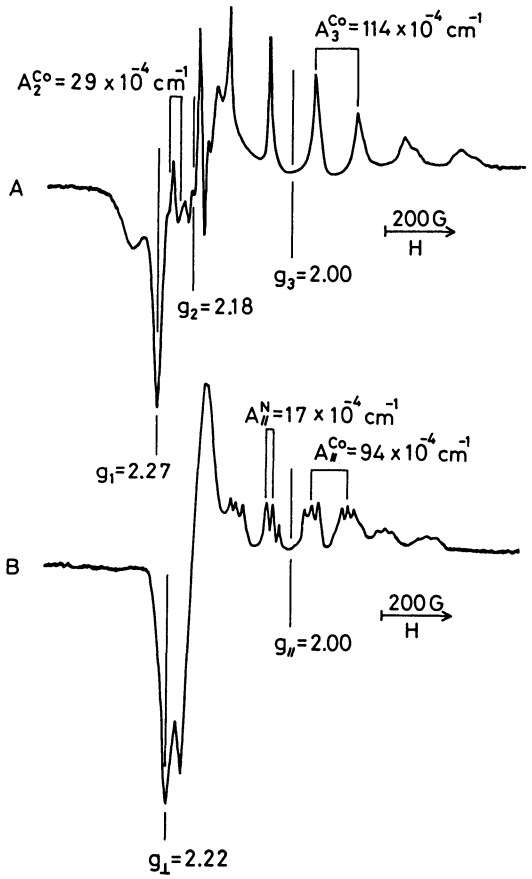


Fig. 2. ESR spectra of hydrophobic vitamin B_{12}'s in ether—methanol (1:1 v/v) at 77 K: A, [Cob(II)(Im^+-H)6C_1ester]$(ClO_4)_2$ (5.0 x 10^{-3} mol dm^{-3}); B, [Cob-(II)(Im)6C_1ester]ClO_4 (5.0 x 10^{-3} mol dm^{-3}).

residue, and the undissolved material was removed by filtration. The filtrate was treated with aqueous potassium cyanide, and purified by gel-filtration chromatography in a manner as mentioned above. The major purple fraction was evaporated to dryness, and the residue was reprecipitated from chloroform upon addition of hexane to afford fine dark purple crystals, $(CN)_2$Cob(III)(Im)6C_1ester: yield 756 mg (49%). Electronic and CD spectra are shown in Fig. 1 and comparable to those of dicyanocobalamin. IR (KBr) 1730 (ester C=O) and 1655 cm^{-1} (amide C=O); ^{1}H-NMR (CD_3OD, TMS) δ=1.67 (CH_2N), 2.08 (CH_2), 3.60 (18H, m, CO_2CH_3), 3.84 [2H, t, $C\underline{H}_2$N(Im)], 5.76 (1H, s, H at C-10), 6.76 (1H, s, Im-4H), 6.92 (1H, s, Im-5H), and 7.47 (1H, s, Im-2H). Found: C, 59.15; H, 6.90; N, 10.69; Co, 4.82%. Calcd for $C_{59}H_{80}CoN_9O_{13}\cdot H_2O$: C, 59.04; H, 6.89; N, 10.50; Co, 4.96%.

[Cob(II)(Im^+-H)6C_1ester]$(ClO_4)_2$ was prepared from $(CN)_2$Cob(III)(Im)6C_1ester (55 mg, 4.8 x 10^{-5} mol) after the method adopted for the preparation of heptamethyl

cobyrinate perchlorate:[1] yield 30 mg (48%); UV_{max} (CH_3OH) 267, 316, 409, and 470 nm; IR (KBr) 1730 (ester C=O), 1655 (amide C=O), and 1120 and 635 cm^{-1} (ClO_4^-). [Cob(II)(Im)6C_1ester]ClO_4 was prepared by adding an equimolar amount of sodium methoxide to [Cob(II)(Im^+-H)6C_1ester](ClO_4)$_2$ in methanol. The solution was evaporated to dryness, and the residue was extracted with dichloromethane to afford [Cob(II)(Im)6C_1ester]ClO_4; UV_{max} (CH_3OH) 267, 315, 350sh, 472, and 540sh nm. The final complex without the axial cyano ligands is readily oxidized to the Co^{III} species under aerobic conditions.

ESR spectra of [Cob(II)(Im^+-H)6C_1ester](ClO_4)$_2$ and [Cob(II)(Im)6C_1ester]ClO_4 are shown in Fig. 2. The spectral feature and spin Hamiltonian parameters for the former complex are comparable to those for the base-off form of vitamin B_{12r} and the protonated imidazolyl moiety is free from metal-coordination, while the spectral pattern and spin Hamiltonian parameters for the latter complex are comparable to those for the base-on form of vitamin B_{12r} and indicate that the imidazolyl moiety is completely coordinated to the nuclear cobalt.[7]

The Co(II)/Co(I) redox couple for [Cob(II)(Im)6C_1ester]ClO_4 was observed at -0.69 V vs. SCE in dimethyl sulfoxide (DMSO) by means of cyclic voltammetry, while that for heptamethyl cobyrinate perchlorate having no axial base has been observed at -0.64 V vs. SCE.[2] Therefore, the intramolecular coordination of the imidazolyl group to the nuclear cobalt shifts the redox potential to the cathodic side by 50 mV. When a large excess amount (50 times as much) of N-methylimidazole was added to the latter complex, the redox couple appeared at -0.69 V vs. SCE in DMSO. The similar behavior with respect to the Co(II)/Co(I) redox potential has been observed for cobalamin in an aqueous buffer; -0.74 V vs. SCE for the base-off form, -0.85 V vs. SCE for the base-on form.[8]

In the light of the above observations, it became apparent that the physicochemical properties of the present hydrophobic vitamin B_{12} derivatives are quite comparable to those of the naturally occurring vitamin B_{12}.

References

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6) Five components are different from each other as regards the extent of ester hydrolysis as confirmed by ^{1}H-NMR spectroscopy, and eluted in the following sequence; heptamethyl dicyanocobyrinate, hexamethyl ester of dicyanocobyrinic acid, and pentamethyl ester of dicyanocobyrinic acid. The organic solvents, which can dissolve these components, are as follows in apolar end: the heptamethyl ester, carbon tetrachloride [E_T(30) 32.5]; the hexamethyl ester, benzene [E_T(30) 34.5]; the pentamethyl ester, chloroform [E_T(30) 39.1].
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