the photochemical modification of single tyrosine residue has some relevance to the decrease in the hormones-binding ability of NP-I.

Since NP-I and NP-II both possess a very similar amino acid sequence[4] and essentially identical affinity constants for either oxytocin or lysine-vasopressin,[6] it may be considered that their hormone binding sites would consist of similar amino acid residues. This is supported by the finding that the histidine residue in NP-I as well as the methionine residue in NP-II is nonessential for the hormone binding process.

A certain movement of the tyrosine-49 from a hydrophobic environment to a hydrophilic environment upon complex formation between NP and hormones has been reported both in NP-I[7] and in NP-II.[3a,b,d,8] These may strongly suggest an important role of the tyrosine-49 for hormone binding. Our previous findings have suggested that the movement of tyrosin-49 residue in NP-II on the hormone binding would provide a more sterically acceptable binding site for hormones.[5] The decrease in the hormones-binding ability of NP-I by the photodegradation of the tyrosine-49 residue, which was found in the present work may also be explained as the same conception as described for NP-II.

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[6] E. Breslow and R. Walter, *Mol. Pharmacol.*, **8**, 75 (1972).
[7] J.H. Griffin, R. Alazard, and P. Cohen, *J. Biol. Chem.*, **248**, 7975 (1973).
[8] P. Balaram, A.A. Bothner-By, and E. Breslow, *Biochemistry*, **12**, 4695 (1973); R. Alazard, P. Cohen, J.S. Cohen, and J.H. Griffin, *J. Biol. Chem.*, **249**, 6895 (1974).

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The Cleavage of Cobalt-Carbon Bond in Methyl-Vitamin B_{12} by Cupric Ions

The cupric ions were found to promote the demethylation of methylcobalamin in solutions only when coordinated with chloride anions.

The cleavage of Co-C bond in methyl-vitamin B_{12} (Me-Co) is a subject of current interest, in which the methyl group is shown to be transferred from Co to the other metal ions such as Hg(II),[1,2,3] Tl(III),[4] Pd(II),[5] Cr(II),[6] Pt(II)·(IV),[4] Au(I)·(III).[4] In this communication

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[1] J. Halpern and J.P. Maher, *J. Am. Chem. Soc.*, **86**, 2311 (1964).
[2] R.E. Desimone, M.W. Penley, L. Charbonneau, S.G. Smith, J.M. Wood, H.A.O. Hill, J.M. Pratt, S. Ridsdale, and R.J.P. Williams, *Biochimica et Biophysica Acta*, **304**, 851 (1973). Earlier papaers were cited in 2) and 3).
[3] H. Yamamoto, T. Yokoyama, C.L. Chen, and T. Kwan, *Bull. Chem. Soc. Japan*, **48**, 844 (1975).
[4] G. Agnes, S. Bendle, H.A.O. Hill, F.R. Williams, and R.J.P. Williams, *Chem. Commun.*, **1971**, 850.
[5] W.M. Scovell, *J. Am. Chem. Soc.*, **96**, 3451 (1974).
[6] J.H. Espenson and T.D. Sellers, *J. Am. Chem. Soc.*, **96**, 94 (1974).

we describe an additional example of this sort as effected by copper (II) chloride in solutions.

The methyl displacement was investigated spectrophotometrically by adding a suitable amount of $CuCl_2$ to an aqueous solution of Me-Co kept in a spectrophotometer cell. When the solution containing $CuCl_2$ (10 mM) and Me-Co (50 μM) was kept standing at room temperature (23—28°), no change was observed in the spectrum over 10 days, suggesting that $CuCl_2$ was inert. However, because of the remarkable effect of liganding anions on the methyl transfer to Hg(II),[3] we were conducted to close investigation of the reaction under various anion environments.

As the result, the methyl displacement reaction was found to proceed, for example, by 30% for 5 hr in the coexistence of 1.5M LiCl. The reaction took place almost completely for 24 hr as judged from the spectral change from methyl-vitamin B_{12} to aquovitamin B_{12} (characteristic increase of optical absorbance at 355 nm). The greater the concentration of LiCl the faster was the reaction rate. The rates of absorbance change (355 nm) caused by the methyl displacement reaction in the presence of 4—9M LiCl are illustrated in Fig. 1(a). This kind of accelerating effect was also observed in the presence of NaCl or KCl, but not with $NaClO_4$, and led us to suggest an active Cu(II) species coordinated with Cl^- to a certain extent.

A complex of the form $Cu^{2+}(H_2O)_4(Cl^-)_2$ was inferred as an effective species of Cu(II) for the methyl displacement reaction. This proposal is consistent with the known stability constant ($Cu^{2+}(H_2O)_6+Cl^- \rightleftharpoons Cu^{2+}(H_2O)_5Cl^-$, $K=1.6\text{M}^{-1}$ (25°))[7] as well as with the similarity of a spectrum of $CuCl_2$ in acetonitrile[8] to that of Cu(II) in the aqueous solution containing a high concentration of LiCl (9.7M).

When EtOH was substituted for water as the solvent, the reaction took place with $CuCl_2$ only; the addition of 1.5M LiCl caused the reaction rather to surpress in contrast to the case in the aqueous solution. The result would imply that Cu(II) is coordinated with Cl^- in the EtOH solution. The rates of reaction are shown against various concentrations of $CuCl_2$ in Fig. 1(b). The reaction order appeared to be quite complex perhaps due to the adduct formation of Cu(II) with the periphery of Me-Co. Precipitates were actually formed at high concentrations of Me-Co and $CuCl_2$ (>2 mM).

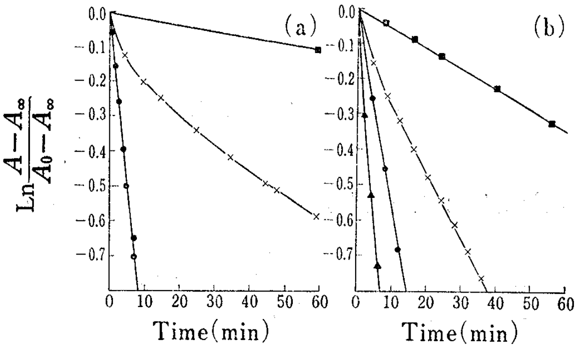

Fig. 1. Semilogarithmic Plots of Absorbance Change (355 nm) against Time (min) for the Reaction of Me–Co with $CuCl_2$

(a): aqueous solution
—■—: 4.0 M LiCl, 1.0 mM $CuCl_2$
—×—: 5.9 M LiCl, 0.8 mM $CuCl_2$
—●—: 8.8 M LiCl, 0.6 mM $CuCl_2$
50 μM Me-Co, pH 4.8, 23°
(b): EtOH solution
—■—: 66 μM $CuCl_2$ —×—: 164 μM $CuCl_2$
—●—: 323 μM $CuCl_2$ —▲—: 476 μM $CuCl_2$
13 μM Me-Co, 28°

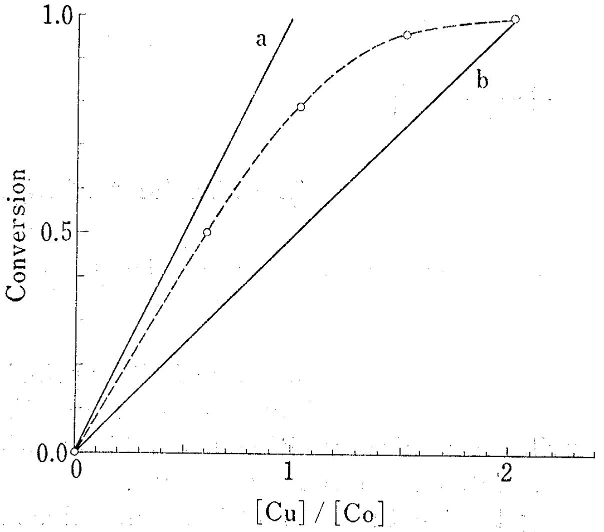

Fig. 2. Stoichiometry of the Cu(II)–cleavage of Co–C Bonds in EtOH

---○---: observedvalues, a and b: see text

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[7] Z. Libus, *Inorg. Chem.*, **12**, 2972 (1973).
[8] S.E. Manahan and R.T. Iwamoto, *Inorg. Chem.*, **10**, 1409 (1965).

The stoichiometry of the reaction was then investigated in the EtOH solution. The degree of methyl displacement after 110 hours reaction (conversion) was plotted against the initial molar ratio of $CuCl_2$ to Me-Co ([Cu]/[Co]) in Fig. 2. The open circles indicate the average values of runs performed with three different initial concentrations of Me-Co (60, 85 and 150 μM) and adjusted amounts of $CuCl_2$ at room temperature. The observed results preferred a 1:1 ratio of $CuCl_2$ to Me-Co (solid line a), to a 2:1 ratio (solid line b), in support of eq. 1.

The equimolar mixture of Me-Co and $CuCl_2$ in EtOH was then subjected to electron spin resonance measurements at suitable time intervals at liquid nitrogen temperature, and it was found that the concentration of Cu(II) decreases with time while no Co(II) (cobalamin r) signal can be detected at any stage of the reaction. The gas-liquid chromatography analysis of the product indicated that methyl chloride and methyl ethyl ether are equally formed. Neither hydrogen nor methane was perceptible. On the basis of these observations, it is assumed that the Cu(II)-promoted cleavage of Me-Co proceeds principally through the methyl transfer reaction between Me-Co and $CuCl_2$ as expressed by

$$\text{Me-Co} + \text{CuCl}_2 \longrightarrow \text{Me-CuCl} + \text{Cl-Co} \qquad (1)$$

The methylcopper intermediates (Me-CuCl) produced are so unstable that they would have decomposed to methyl chloride and copper or methyl ethyl ether when reacted with EtOH, although metallic copper was seemingly absent probably due to its low concentrations.

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The Structures of Nigakihemiacetals E and F

The structures of two new bitter principles, nigakihemiacetals E and F, isolated from *Picrasma ailanthoides* Planchon were shown to be **1** and **2**, respectively.

The isolation of a number of bitter principles from *Picrasma ailanthoides* Planchon (=*P. quassioides* Bennett; Simaroubaceae) and the determination of their structures have been described.[1,2] Two additional new bitter principles, nigakihemiacetals E and F, have now been isolated from the same plant. In this communication, we wish to report evidence leading to structures **1** and **2** for nigakihemiacetals E and F, respectively.

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[1] *a*) T. Murae, T. Tsuyuki, T. Ikeda, T. Nishihama, S. Masuda, and T. Takahashi, *Tetrahedron*, **27**, 1545 (1971); *b*) T. Murae, T. Tsuyuki, T. Ikeda, T. Nishihama, S. Masuda, and T. Takahashi, *Tetrahedron*, **27**, 5147 (1971); *c*) T. Murae, A. Sugie, T. Tsuyuki, S. Masuda, and T. Takahashi, *Tetrahedron*, **29**, 1515 (1973). And the references cited therein.

[2] H. Hikino, T. Ohta, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **19**, 2651 (1971). And the references cited therein.