CHEMISTRY LETTERS, pp. 1693-1696, 1993.

A New Transformation, α-Hydroxylation, of Pyridoxylidene-Amino Acid Mediated on Co(III) Complex

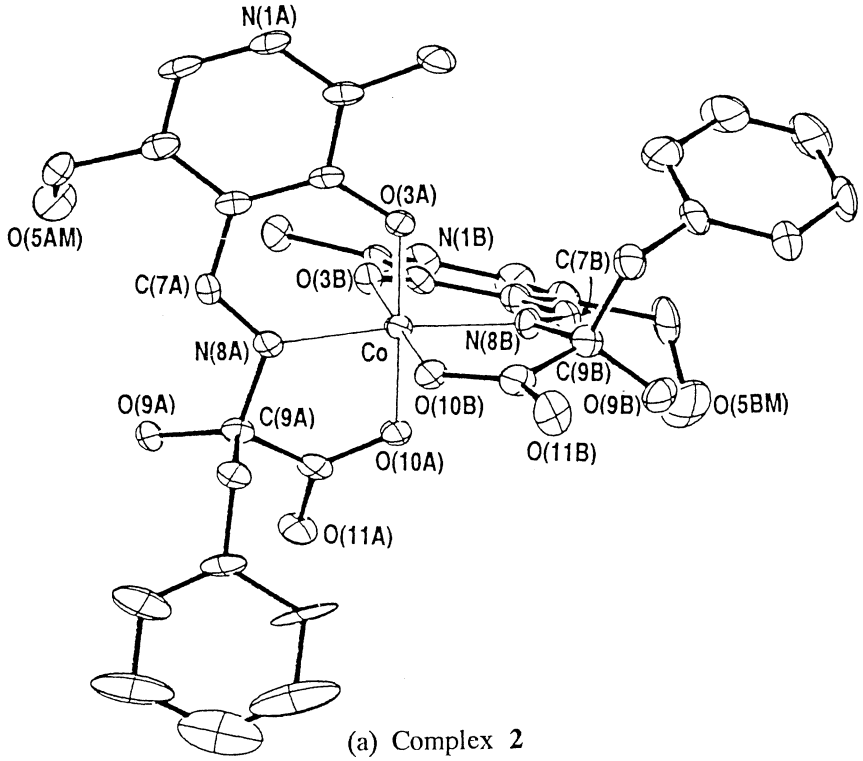
Koichiro JITSUKAWA,* Tetsuya YAMAMOTO, Hideki MASUDA,* and Hisahiko EINAGA
Department of Applied Chemistry, Nagoya Institute of Technology, Nagoya 466

Transformation of amino acid to α-hydroxy amino acid has been discovered in the Co(III) complex with pyridoxylidene-amino acid ligand, which has been discussed on the basis of the ^{1}H-NMR and FAB mass spectroscopic data and X-ray crystallographic analysis.

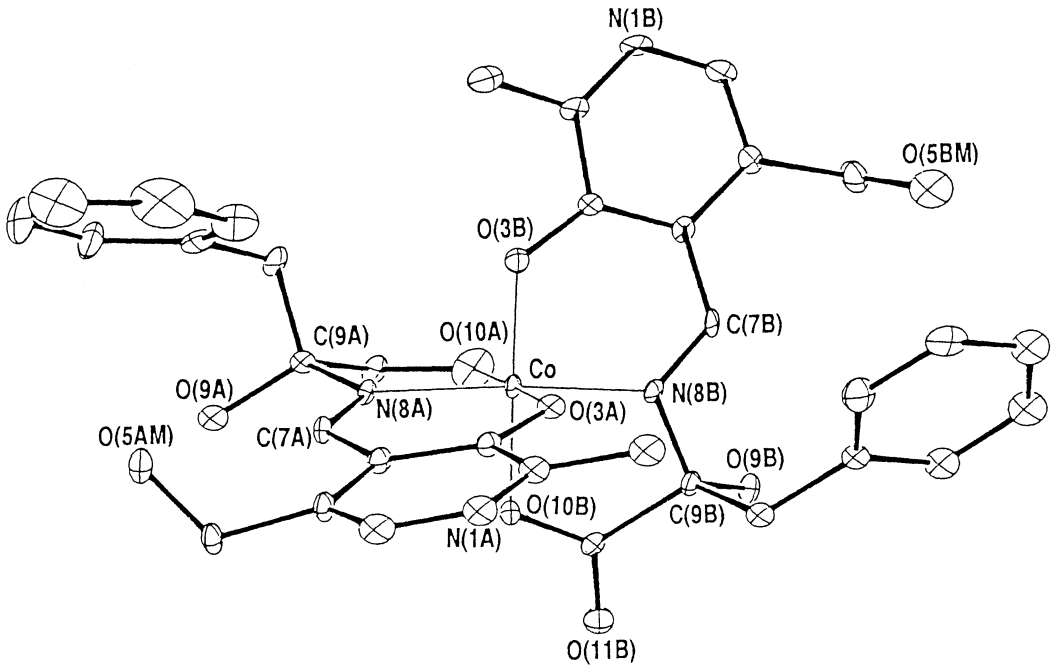
Pyridoxal and the related derivatives are well known to catalyze a large variety of amino acid transformation.[1] Many model reactions have been mimicked by use of metal complexes with Schiff base ligands composed of amino acid and pyridoxal or salicylaldehyde, a coordination analogue of pyridoxal, which proceed through aldimine and/or ketimine forms.[2-6] In the course of the study on amino acid transformation mediated with the substitutionally inert Co(III) ion and pyridoxal, we discovered a novel transformation, α-hydroxylation, of amino acid, which was characterized on the basis of ^{1}H-NMR and FAB mass spectra and X-ray structure analysis. Such an α-hydroxylation has never been detected using salicylidene-amino acid ligands. This is a new type oxygenation of amino acid in the model systems of the pyridoxal reactions to our knowledge.[7]

The α-hydroxylation in Co(III) complex with pyridoxylidene-amino acids was performed as follows. To 150 mL of an aqueous solution of L-phenylalanine (1.65 g, 10 mmol) and NaOH (0.40 g, 10 mmol) was added pyridoxal hydrochloride (2.04 g, 10 mmol), and then carefully added a $K_3[Co(CO_3)_3]$ solution (5 mmol). Under an aerobic condition, the resulting mixture with adjustment to pH 6 was stirred for 3 h at room temperature, and then passed through an anion exchange column with QAE Sephadex A-25 (Cl^- form, 3.6 cmϕ × 25 cm). The adsorbed compounds were separated into three main bands with the ratio of about 5:1:2, which were named complexes **1**, **2**, and **3** for the first, second, and third eluates, respectively; the total yield of **1**−**3** being 86% in 1.55×10^{-3} M aqueous solution based upon the starting material. Each eluate fractionated with 1M NaCl solution was allowed to stand for several days to give single crystals suitable for X-ray structure analysis.

^{1}H-NMR spectrum of the complex **1** gave a quartet peak assignable to an α-proton of phenylalanine of the Schiff base ligand at 5.24 ppm (d-d, J = 9.6 and 4.0 Hz), whereas no peaks were observed on the complexes **2** and **3** in the same spectral region.[8] In order to elucidate the structural details of **1**−**3**, we tried to analyze their X-ray structures. All the crystal structures for **1**, **2**, and **3** showed that the two Schiff base ligands coordinated to the Co(III) ion in mer-trans(N)-configuration, which were characterized to be aldimine form judging from the sp^3-character of α-carbon C(9) and the double bond character of azomethine C(7)=N(8) bond (1.30(1) and 1.30(1) Å for **1**, 1.290(8) and 1.267(8) Å for **2**, and 1.281(2) and 1.287(2) Å for **3**, respectively).[9,10] The most



(a) Complex **2**

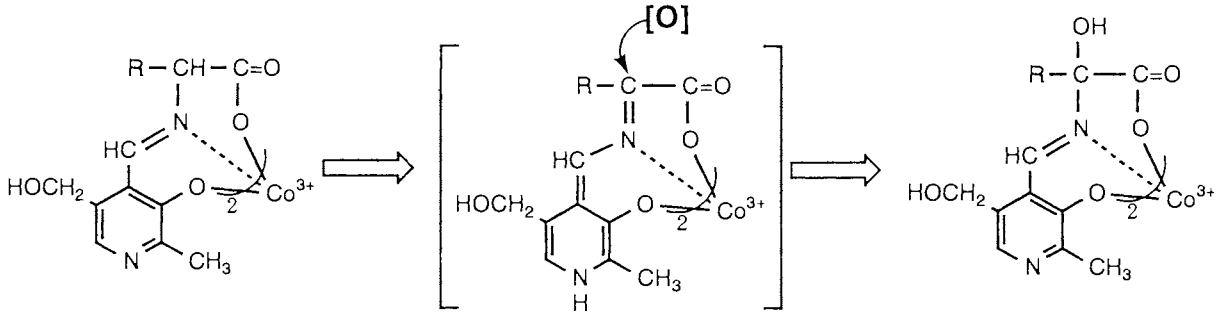


(b) Complex **3**

Fig. 1. Molecular structures of the bis(pyridoxylidene-α-hydroxyphenylalaninato)cobalt(III) complexes: (a), $[Co(phfs)_2]^-$ (**2**) (Λ(S,R)-configuration); (b), $[Co(phfs)_2]^-$ (**3**) (Λ(R,R)-configuration).

striking structural feature of **2** and **3** is indicated in Fig. 1. The α-protons of L-phenylalanines of the Schiff base ligands in both **2** and **3** were replaced by other substituent groups, as suggested from their ^{1}H-NMR spectra, although those in **1** remained unchanged. The substituents were assigned to be hydroxyl groups from the electron density of the located atoms and the bond lengths; the bond lengths between α-carbons and the substituted atoms in **2** and **3** were 1.382(7) and 1.400(8) Å, and 1.400(2) and 1.403(2) Å, respectively, which were within the range of C-O bond length known for tertiary alcohols. The hydroxyl substituent was identified by positive-ion FAB mass spectra; parent ion peak for **1** showed at $m/z = 685$ assignable to bis(pyridoxylidene-phenylalaninato)cobalt(III) complex ion, $[H[Co(pfs)_2)]+H]^+$, while those for **2** and **3** were both detected at $m/z = 717$ corresponding to bis(pyridoxylidene-α-hydroxyphenylalaninato)cobalt(III) complex ions, ($[H[Co(phfs)_2]+H]^+$), indicating that two oxygen atoms were incorporated into the α-position of Co(III) pyridoxylidene-amino acid complexes. The α-hydroxylation of the cobalt(III) complex with pyridoxylidene-L-alaninato ligands was observed in the same way as described above, which was confirmed on the basis of ^{1}H-NMR and positive-ion FAB mass spectra. The hydroxylation proceeded in the presence of cobalt(III) ion, but all attempts to separate free α-hydroxy amino acid from **2** and **3** were unsuccessful, which means that the α-hydroxy amino acid can exist only as the metal complex.[7)]

The reaction of cobalt(III) complex with pyridoxamine and phenylpyruvic acid, as the reverse pathway of the above reaction of pyridoxal and L-phenylalanine, gave the corresponding complexes **1**, **2**, and **3** with the ratio of about 1:1:1. Using salicylidene-amino acid instead of pyridoxylidene-amino acid, only aldimine type complex without α-hydroxyl group was formed in contrast to the present pyridoxal reaction. These facts presumably suggest that the hydroxylation of amino acid from ketimine form proceeds through the same transition state of quinoide form as that from the aldimine form. The oxygen in the α-hydroxyl group is expected to be originated from molecular oxygen, because the obvious absorption of oxygen was observed during the reaction. From the above results, the α-hydroxylation mechanism illustrated in Scheme 1 is proposed; further detailed comprehension will be reported elsewhere.



Scheme 1.

Thanks are due to the Chemical Material Center, the Institute for Molecular Science, for assistance in obtaining the positive FAB mass spectra. The present study was partly supported by a Grant-in-Aid for Scientific Research on Priority Areas (No.05209212) from the Ministry of Education, Science and Culture of Japan.

References

1) "Transaminases," ed by P. Christen and D. E. Metzler, John Wiley & Sons (1985).
2) A. E. Martell, *Acc. Chem. Res.*, **22**, 115 (1989).
3) D. E. Metzler, M. Ikawa, and E. E. Snell, *J. Am. Chem. Soc.*, **76**, 648 (1954).
4) H. Kuno, K. Okamoto, J. Hidaka, and H. Einaga, *Bull. Chem. Soc. Jpn.*, **62**, 2824 (1989).
5) V. M. Shanbhag and A. E. Martell, *Inorg. Chem.*, **29**, 1023 (1990).
6) I. I. Mathews, P. A. Joy, S. Vasudevan, and H. Manohar, *Inorg. Chem.*, **30**, 2181 (1991).
7) The α-hydroxylation of N-salicyloyl-glycine in the Cu(III)-molecular oxygen-pyridine ternary system has been reported as a peptidylglycine-α-amidating monooxygenase (PAM) model, but that in pyridoxal system has never been reported. P. Capdevielle and M. Maumy, *Tetrahedron Lett.*, **32**, 3831 (1991).
8) In 200 MHz ^{1}H-NMR spectra of both **2** and **3** the benzyl protons of phenylalanine moiety were observed as follows: **2** (two kinds of streoisomers) (CD_3OD, TMS) $\delta = 3.32$ (1H, d, J = 12.0 Hz), 3.48 (1H, d, J = 12.0 Hz) and $\delta = 3.57$ (1H, d, J = 12.0 Hz), 3.73 (1H, d, J = 12.0 Hz). **3** (D_2O, DSS): $\delta = 3.22$ (1H, d, J = 12.8 Hz), 3.56 (1H, d, J = 12.8 Hz). All of the corresponding protons in **2** and **3** exhibited doublet pattern, indicating the absence of α-proton of phenylalanine.
9) Crystal data for **1**: $C_{34}H_{32}CoN_4NaO_8 \cdot 8H_2O$, $M = 850.68$, triclinic, $P\bar{1}$, $a = 10.984(2)$ Å, $b = 13.822(3)$ Å, $c = 14.790(8)$ Å, $\alpha = 110.62(3)^\circ$, $\beta = 92.84(3)^\circ$, $\gamma = 102.71(2)^\circ$, $Z = 2$, $V = 2030.1$ Å^3, $Dc = 1.39$ gcm^{-3}, μ(Mo-K$_\alpha$) = 5.00 cm^{-1}, and 2644 unique reflections with $I_0 > 3\sigma(I)$ were used in the refinement to $R = 0.080$, $Rw = 0.098$. Crystal data for **2**: $C_{34}H_{33}CoN_4O_{10} \cdot 4H_2O$, $M = 788.63$, orthorhombic, *Pbca*, $a = 13.925(11)$ Å, $b = 24.258(5)$ Å, $c = 21.483(7)$ Å, $Z = 8$, $V = 7256.8$ Å^3, $Dc = 1.44$ gcm^{-3}, μ(Mo-K$_\alpha$) = 5.40 cm^{-1}, and 3970 unique reflections with $I_0 > 3\sigma(I)$ were used in the refinement to $R = 0.074$, $Rw = 0.092$. Crystal data for **3**: $C_{34}H_{32}CoN_4NaO_{10} \cdot 5H_2O$, $M = 764.64$, triclinic, $P\bar{1}$, $a = 10.959(2)$ Å, $b = 12.448(3)$ Å, $c = 14.866(2)$ Å, $\alpha = 80.75(2)^\circ$, $\beta = 80.11(2)^\circ$, $\gamma = 69.81(2)^\circ$, $Z = 2$, $V = 1867.3$ Å^3, $Dc = 1.36$ gcm^{-3}, μ(Mo-K$_\alpha$) = 5.41 cm^{-1}, and 6322 unique reflections with $I_0 > 3\sigma(I)$ were used in the refinement to $R = 0.035$, $Rw = 0.051$.
10) The crystal of the complex **2** contains two stereochemical isomers of Λ(S,R)- and Δ(S,R)-configurations, that is, Λ-Δ isomerism is helical configuration around the central metal atom and S-R isomerism is asymmetry at the α-position of the amino acid moieties; that of the complex **3** is composed of Λ(R,R)- and Δ(S,S)-configurations.

(Received July 5, 1993)