Metal Complexes of Amino Acids. VIII.[1] Carbon-13 Nuclear Magnetic Resonances of Cobalt(III) Complexes Containing L-Aspartic and L-glutamic Acids

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The ^{13}C NMR spectra of cobalt(III) complexes containing L-aspartic acid or L-glutamic acid as unidentate, bidentate, or bridged ligand were measured, and the relationship between the coordination types and the changes in chemical shift of carboxyl carbons in these amino acid complexes was discussed. Two structural isomers were newly separated for the *trans*(O)-[Co en_2(L-aspH_2 or L-gluH_2)$_2$]X_3 complex by a column chromatographic method. The structures of the earlier eluted isomers for the *trans*(O)-[Co en_2(L-aspH_2)$_2$]X_3 and -[Co en_2(L-gluH_2)$_2$]X_3 complexes were assigned to *trans*(O_0, O_γ) and *trans*(O_0, O_δ) (O_0, O_γ and O_δ indicate the coordinated oxygen atoms of C_0OO, $C_\gamma OO$, and $C_\delta OO$), respectively, and the structures of the later eluted isomers for the both complexes to *trans*(O_0, O_0), on the basis of the shift patterns of carboxyl carbons.

It is well known that the ^{13}C NMR technique gives many useful informations about structural analyses of organic compounds.[2,3] Recently this technique has been applied to the metal complexes.[4-7] In the previous paper[8] we studied the ^{13}C NMR of cobalt(III) complexes containing various amino acids.

In the present paper, the relationship between the coordination types and the changes in chemical shift of carboxylate groups in amino acid-cobalt(III) complexes containing L-aspartic and L-glutamic acids as unidentate, bidentate and bridged ligands will be discussed on the basis of their ^{13}C NMR spectra. Furthermore the structural assignments for the newly obtained structural isomers of the *trans*(O)-[Co en_2(L-aspH_2)$_2$]X_3 and [Co en_2(L-gluH_2)$_2$]X_3 complexes will be made.

Experimental

Preparation of Complexes. Trans(O_0,O_γ)- and trans(O_0, O_0)-[Co en_2(L-aspH_2)$_2$]$Br_3\cdot H_2O$: To an aqueous solution containing 5.2 g (0.01 mol) of [Co$(OH_2)_2en_2$]$(ClO_4)_3$ in 50 ml of water was added 3.2 g (0.024 mol) of L-aspartic acid, and the mixture was gradually evaporated to dryness on a steam-bath. The residue was dissolved in 20 ml of water, and the solution was evaporated again. This procedure was repeated three or four times more. The final residue was dissolved in about 300 ml of water, and the solution was poured into a SP-Sephadex cation-exchange column (C-25, K-form, $\phi 4.5\times 50$ cm). After two days two purplish-red bands were separated from the upper adsorbed band by sweeping with 0.03 M aqueous solution of KBr which was slightly acidified with hydrobromic acid. Each split band was taken out and transfered into a short column. The purplish-red solution obtained from each column by elution with concentrated KBr solution was concentrated by using a vacuum evaporator at 30—35 °C. The deposited KBr was removed by filtration and the filtrate was evaporated almost to dryness. The purplish-red complex was extracted from the residue with methanol and the solution was filtered. The crude complex was obtained by evaporation of the methanolic solution. Purification of the complex was carried out by adding acetone to a concentrated aqueous solution. The purplish-red powder (*trans*(O_0,O_γ) isomer) and crystals (*trans*(O_0,O_0) isomer) were obtained from the earlier and later eluted fractions, respectively, and yields were 0.20 g and 0.75 g.

Found: C, 20.85; H, 4.79; N, 11.88% for *trans*(O_0,O_γ) isomer and C, 20.76; H, 4.83; N, 12.02% for *trans*(O_0,O_0) isomer. Calcd for $C_{12}H_{32}N_6O_9Br_3Co$: C, 20.50; H, 4.60; N, 11.95%.

Trans(O_0,O_δ)- and trans(O_0,O_0)-[Co en_2(L-gluH_2)$_2$]Br_3. These isomers were obtained by a similar procedure to that for the L-aspartic acid complex except for use of 3.5 g of L-glutamic acid. In the case of L-glutamic acid, however, at the first stage three purplish-red bands were separated from the adsorbed band. All of the complexes eluted from these bands showed the absorption spectra pattern corresponding to the type *trans*(O)-[CoN_4O_2] complex,[9] but unfortunately no analytically pure complex was isolated from the first eluting band. The purplish-red powder (*trans*(O_0,O_δ) isomer) and crystals (*trans*(O_0,O_0) isomer) were obtained from the second and third eluted fractions by the same way as used for the L-aspartic complex, respectively, and yields were 0.15 g and 0.50 g.

Found: C, 23.82; H, 4.80; N, 12.10% for *trans*(O_0,O_δ) isomer, and C, 23.36; H, 4.59; N, 11.50% for *trans*(O_0,O_0) isomer. Calcd for $C_{14}H_{34}N_6O_8Br_3Co$: C, 23.57; H, 4.81; N, 11.79%.

[Co(NH_3)$_5$(L-glnH)]$Br_3\cdot H_2O$. This complex was prepared by modification of the method described in previous paper.[9,10] To an aqueous solution containing 4.8 g (0.01 mol) of [Co$(NH_3)_5(OH_2)$]$(ClO_4)_3\cdot H_2O$ in 50 ml of water was added 2.0 g (excess) of L-glutamine, and the mixture was gradually evaporated to 10 ml on a water-bath at 55—60 °C. The resulting solution was filtered and the filtrate was diluted to 100 ml with water and poured into a short column ($\phi 7\times 5$ cm) containing SP-Sephadex cation-exchanger (C-25, Na-form). The orange eluting solution obtained by elution with 0.03—1.0 M aqueous solution of KBr was evaporated by using a vacuum evaporator. The deposited KBr was removed by filtration and the filtrate was evaporated again. The crude complex obtained was recrystallized from an aqueous solution by adding methanol. The orange complex deposited as plate crystals was filtered and washed with 90% methanol, 99% methanol and then ether, and dried in air.

Found: C, 10.86; H, 5.17; N, 18.22%. Calcd for $C_5H_{27}N_7O_4Br_3Co$: C, 10.95; H, 4.98; N, 17.89%.

[Co(NH_3)$_5$(L-asnH)]$(ClO_4)_3$.[11] This complex was obtained as orange needle crystals by the same method as for the L-glutamine complex.

(+)$_D$-[Co(L-asp)en_2]$ClO_4\cdot 2H_2O$, (+)$_D$-[en_2Co(L-asp)Co(NH_3)$_5$]$(ClO_4)_4\cdot H_2O$, (+)$_D$-[en_2Co{(L-asp)Co en_2}$_2$]$(ClO_4)_5\cdot 5H_2O$, (+)$_D$-[Co(L-glu)en_2]ClO_4, (+)$_D$-[en_2Co(L-glu)Co(OH_2)-en_2]$(ClO_4)_4\cdot 2H_2O$, and (+)$_D$-[en_2Co{(L-glu)Co en_2}$_2$]$(ClO_4)_5\cdot 4H_2O$. Preparations of these complexes are described in

the previous study.[12]

Measurements. The absorption spectra were obtained by using a Hitachi Model EPS-3T spectrophotometer. The ^{13}C NMR spectra were recorded on a JEOL-MFT-100 spectrometer with JEC-6 spectrum computer in pulse Fourier transform/proton noise decoupled mode at 25.15 MHz and at room temperature. An internal D_2O rocking form was used for the measurements. Peak positions were measured relative to external benzene, and D_2O is used as solvent of the samples. Chemical shifts are reported relative to TMS using the relation $\delta_{TMS}=\delta_{benzene}-128.5$ ppm.[3] Experimental errors were ± 0.1 ppm. Some samples were converted to the chloride to obtain higher solubility in D_2O.

Results and Discussion

Structural Isomers of trans*(O)-[Co en$_2$(L-aspH$_2$ or L-gluH$_2$)$_2$]X$_3$ Complex.* L-Aspartic and L-glutamic acids have three functional groups, two carboxylates and one amino. Therefore, these amino acids will provide the complexes of three coordination types containing them as unidentate, bidentate,[13-17] and terdentate[18,19] ligands It is well known that a large number of amino acids form easily unidentate complexes in which the amino acids coordinate to cobalt(III) with only carboxylates.[9-11] However, the unidentate complexes containing L-aspartic and L-glutamic acids have not been isolated so far. In this study we attempted to prepare the type *trans*(O)-[Co en$_2$(L-aaH$_2$)$_2$]X$_3$ complex containing L-aspartic acid or L-glutamic acid as unidentate ligand. There are three structural isomers possible in this type complex, that is, *trans*(O_γ,O_γ), *trans*(O_0,O_γ) and *trans*-(O_0,O_0) isomers for L-aspartic acid complex, and *trans*-(O_δ,O_δ), *trans*(O_0,O_δ) and *trans*(O_0,O_0) isomers for L-glutamic acid complex, as shown in Fig. 1. Indeed the existence of three structural isomers for the L-glutamic acid complex was confirmed on a column chromatogram. These isomers showed the absorption spectra corresponding to the typical *trans*(O)-[Co N_4O_2] complex[9] as shown in Fig. 2, but unfortunately it was unsuccessful to isolate the analytically pure complex from the earliest eluted fraction (perhaps *trans*(O_δ,O_δ) isomer) of three split bands because of its unstability in aqueous solution. On the other hand in the L-aspartic acid complex the bands corresponding to two structural isomers, *trans*(O_0,O_γ) and *trans*(O_0, O_0), were confirmed on a column chromatogram, but another one (*trans*(O_γ, O_γ) was not detected on it.

The structural assignment of each isomer will be mentioned in section of ^{13}C NMR spectra. The notations of all carbon atoms in L-aspartic and L-glutamic acids are shown below.

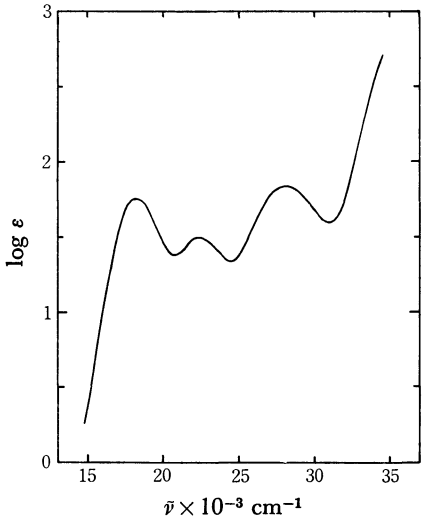

Fig. 2. Absorption spectra of *trans*(O)-[Co en$_2$(L-aspH$_2$ or L-gluH$_2$)$_2$]$^{3+}$ ion.

NH_3^+
$^-OOC_0-C_\alpha H-C_\beta H_2-C_\gamma OOH$
L-Aspartic Acid (L-aspH$_2$)

NH_3^+
$^-OOC_0-C_\alpha H-C_\beta H_2-C_\gamma H_2-C_\delta OOH$
L-Glutamic Acid (L-gluH$_2$)

^{13}C NMR Spectra. The observed ^{13}C NMR shifts of L-aspartic and L-glutamic acids and of their cobalt(III) complexes are listed in Table 1, and their shift patterns are shown in Figs. 3 and 4. The resonance of carboxyl carbons, α-carbons and methylene carbons (C_β and C_γ) of the free and coordinated L-aspartic and L-glutamic acids, and ethylene carbons of ethylenediamine were observed in the ranges of 171.9—187.4, 49.8—58.1, 24.5—39.7, and 42.8—45.9 ppm, respectively.

As described in the previous paper[8] the carboxyl carbon resonances of various α-amino acids show upfield shifts in the range of 2.5—3.0 ppm in acidic solution, compared with those in neutral solution. Quite similar behaviors in ^{13}C chemical shift are also observed for two carboxyl carbons in L-aspartic and L-glutamic acids of the present study, as shown in Figs. 3 and 4. The changes in chemical shifts of these carboxyl groups can be related to the changes of their chemical environments. Horsley[20] and Quirt[21] have demonstrated from the pH titration studies for various amino acids that the carboxyl carbon resonance exhibits large shift with pH

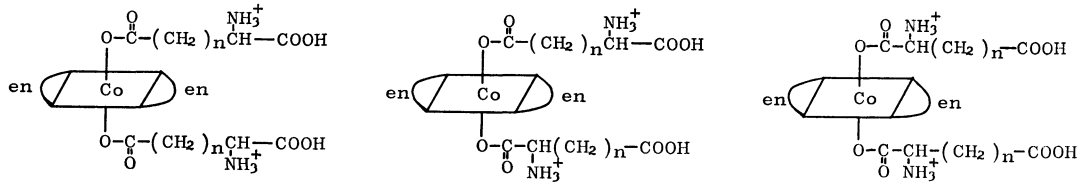

Fig. 1. The structures of three possible isomers in *trans*(O)-[Co en$_2$(L-aspH$_2$ or L-gluH$_2$)$_2$]X$_3$ complex.

TABLE 1. OBSERVED ^{13}C NMR SHIFTS (IN ppm FROM TMS) OF L-ASPARTIC AND L-GLUTAMIC ACID COMPLEXES

Label	Compound	C_0OO	$C_\gamma OO$	$C_\delta OO$	C_α	C_β	C_γ	C_{en}			
I	L-asp$H_3^{+a)}$	171.9	174.9		49.8	33.5					
II	L-aspH^-	176.0	179.3		52.5	36.8					
III	L-asp^{2-}	184.4	182.3		54.1	43.3					
IV	$(+)_D$-[Co(L-asp)en_2]$^+$	187.4	179.2		55.1	39.1		45.6	44.8	42.8	
V	$(+)_D$-[Co(L-aspH)en_2]$^{2+}$	186.9	176.6		54.4	36.5		45.9	45.1	43.6	
VI	$(+)_D$-[en_2 Co(L-asp)Co$(NH_3)_5$]$^{4+}$	187.0	183.8		54.9	39.7		45.8	44.9	43.3	
VII	$(+)_D$-[en_2 Co{(L-asp)Co en_2}$_2$]$^{5+}$	186.7	184.4		54.5	39.0		45.9	45.5	44.5	43.0
VIII	[Co$(NH_3)_5$ (L-asnH)]$^{3+}$	179.1	175.6		51.0	34.5					
IX	*trans*(O_0,O_γ)-[Co en_2(L-aspH_2)$_2$]$^{3+}$	178.7	182.4		50.4	36.5		45.1			
		172.8	175.1		50.1	34.1					
X	*trans*(O_0,O_0)-[Co en_2(L-aspH_2)$_2$]$^{3+}$	178.7	175.1		50.0	34.0		45.0			
XI	L-glu$H_3^{+a)}$	172.5		178.4	52.8	24.5	29.2				
XII	L-gluH^-	176.2		183.0	54.8	26.8	33.4				
XIII	L-glu^{2-}	185.0		184.7	56.3	32.0	34.5				
XIV	$(+)_D$-[Co(L-glu)en_2]$^+$	186.5		183.5	58.1	28.5	34.0	45.5	45.0	44.7	43.6
XV	$(+)_D$-[Co(L-gluH)en_2]$^{2+}$	186.5		178.7	57.4	27.3	30.1	45.6	45.0	44.7	43.5
XVI	$(+)_D$-[en_2 Co(L-glu)Co$(OH_2)en_2$]$^{4+}$	185.8		185.8	57.9	28.4	34.1	45.3	44.8	44.4	43.6
								43.2			
XVII	$(+)_D$-[en_2 Co{(L-glu)Co en_2}$_2$]$^{5+}$	186.1		187.4	57.4	28.2	33.7	45.5	44.8	43.4	
XVIII	[Co$(NH_3)_5$(L-glnH)]$^{3+}$	179.5		179.0	54.4	25.9	31.2				
XIX	*trans*(O_0,O_δ)-[Co en_2(L-gluH$_2$)$_2$]$^{3+}$	179.5		186.4	53.5	25.5	32.9	45.0			
		172.9		177.9	52.4	25.0	29.9				
XX	*trans*(O_0,O_0)-[Co en_2(L-gluH$_2$)$_2$]$^{3+}$	179.7		178.0	53.6	25.1	30.1	45.4			

a) Dissolved in 30% D_2SO_4 solution. b) Dissolved in 10% NaOD Solution.

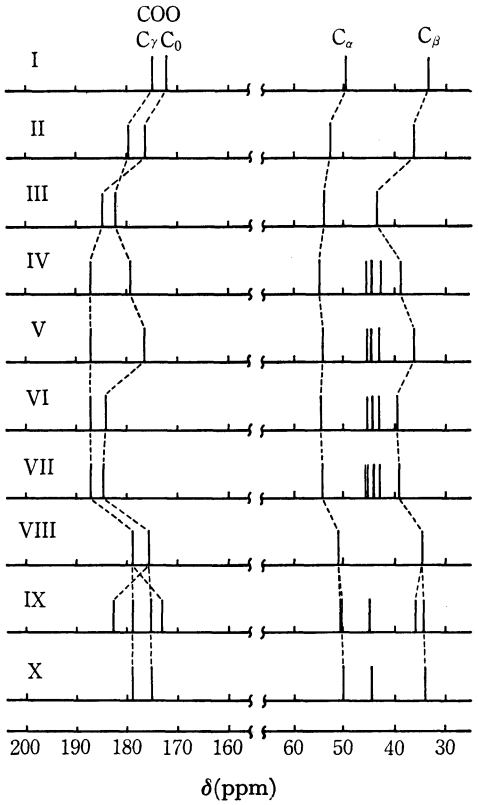

Fig. 3. ^{13}C NMR patterns of L-aspartic acid and its complexes.

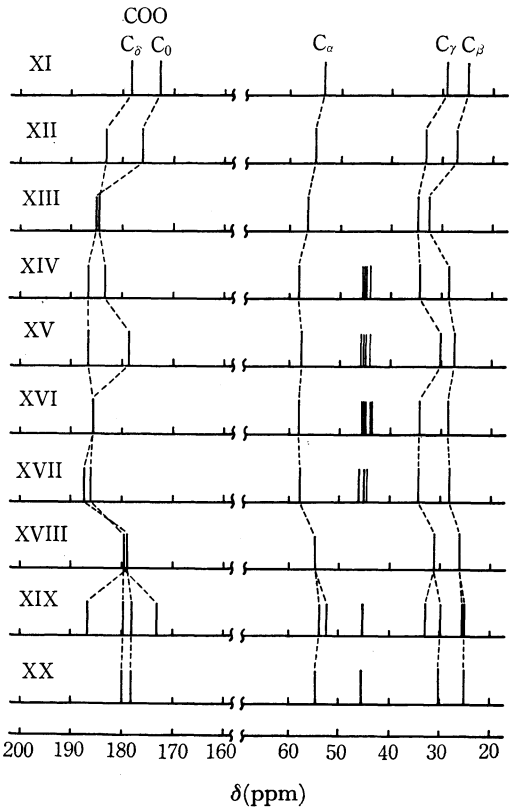

Fig. 4. ^{13}C NMR patterns of L-glutamic acid and its complexes.

change. Generally the protonation (acidic solution) and deprotonation (alkaline solution) of the amino acid provide upfield and downfield shift changes for the carboxyl carbon resonance, respectively. Such upfield shift changes also appear on protonations of uncoordinated carboxyl groups ($C_\gamma OO$ and $C_\delta OO$) in IV and XIV complexes. On the other hand the α-carboxyl carbons in chelated complexes (IV, V, XIV, and XV)

show downfield shifts, relative to those in unidentate complexes and free amino acids,[8] and their chemical shifts are almost constant within the range of 186.3—187.4 ppm. This constant shift can be applied to assignments of the chelated α-carboxyl carbons in the μ-L-aspartato (VI and VII) and μ-L-glutamato (XVI and XVII) complexes.

The structural assignments of two newly obtained isomers for the type *trans*(O)-[Co en_2(L-aaH_2)$_2$]X_3 complex can be easily made from the shift patterns of the carboxyl carbons. These isomers show considerably different shifts patterns from each other, that is, the earlier eluted isomers (IX and XIX) exhibit double peaks for each of two carboxyl carbons (C_0 and C_γ for L-aspartic acid complex, and C_0 and C_δ for L-glutamic acid complex), α-methine, β- and γ-methylene carbons, while the later eluted isomers (X and XX) do single peak. On the other hand for four carbons of two chelated ethylenediamines in these isomers only one resonance peak was observed. This means that four carbons are equivalent in chemical environment, and suggests that two isomers are structural ones taking *trans*(O) structure.

The peaks at 178.7 ppm of IX and X correspond well to the peak at 179.1 ppm of the coordinated carboxyl C_0 in VIII where L-asparagine coordinates with α-carboxyl group as a unidentate ligand, while the peaks at 175.1 ppm of IX and X do to the peak at 174.9 ppm of carboxyl C_γ in the protonated L-aspartic acid (I). Therefore the former peak of 178.7 ppm can be assigned to the coordinated carboxyl carbon (C_0) and the latter one (175.1 ppm) to the uncoordinated and protonated carbon (C_γ). The peaks at 172.8 and 182.4 ppm of IX correspond to the peaks of the protonated α-carboxyl C_0 in I (171.9 ppm) and of the coordinated carboxyl C_γ in VI and VII (183.8 and 184.4 ppm), respectively. From these results it is concluded that one of two L-aspartic acids contained in IX coordinates to cobalt(III) with C_0OO group and the other with $C_\gamma OO$ group as a unidentate ligand, while those in X coordinate with C_0OO groups only.

The same discussion can be made about two structural isomers (XIX and XX) of the L-glutamic acid complex. The peak at 179.5 ppm of XIX and that at 179.7 ppm of XX may correspond to that at 179.5 ppm of the coordinated α-carboxyl C_0 in XVIII where L-glutamine coordinates to cobalt(III) as a unidentate ligand. The peak at 177.9 ppm of XIX and that at 178.0 ppm of XX correspond to the peak at 178.4 ppm of the protonated carboxyl C_δ in XI, and the peaks at 172.9 and 186.4 ppm of XIX to that at 172.5 ppm of the protonated α-carboxyl C_0 in XI and to those at 185.8 and 187.4 ppm of the coordinated carboxyl C_δ in XVI and XVII, respectively. Therefore, for two structural isomers (XIX and XX) of L-glutamic acid complex the structures of (B) and (C) shown in Fig. 1 can be assigned.

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