January, 1979] BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 52 (1), 79—83 (1979) 79

# Metal Complexes of Amino Acids. XI.[1] Preparation and $^{13}C$ NMR Spectra of the Cobalt(III) Complexes Containing β-Alanine and Glycine

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(Received June 17, 1978)

A series of complexes or geometrical isomers, $[Co(β\text{-ala or gly})(en)_2]^{2+}$, $[Co(β\text{-ala or gly})_2(en)]^+$, $[Co(β\text{-ala or gly})(ox)_2]^{2-}$, and $[Co(β\text{-ala or gly})_2(ox)]^-$, were prepared and their $^{13}C$ NMR spectra were measured. Assignments of their structures and $^{13}C$ NMR signals were made. The β-alaninate carbons in the complexes resonate at 33.6±0.5, 38.3±0.6, and 183.0±1.5 ppm and the glycinate carbons at 46.3±1.3 and 187.4±1.4 ppm. The $^{13}C$ chemical shifts of the mixed (β-alaninato)(glycinato) complexes consist of those of the corresponding isomers of bis(β-alaninato) and bis(glycinato) complexes.

$^1H$ NMR has been widely used to study the structures and reactions of the cobalt(III) complexes containing amino carboxylate as the chelate ligands. Recently, $^{13}C$ NMR has been applied to the study of cobalt(III) complexes, and it has been found that the $^{13}C$ NMR is useful to determine the geometrical structures of the cobalt(III) complexes with organic ligands.[2–15] Determination of the geometrical structure of the complex by $^1H$ NMR is sometimes difficult because of the complicated signals caused by spin-spin couplings. In most case, since carbon atoms are closer to cobalt(III) atom than hydrogen atoms in the complexes, $^{13}C$ NMR makes it possible to obtain more useful information than $^1H$ NMR concerning the structures of the cobalt(III) complexes. However, $^{13}C$ NMR data of the cobalt(III) complexes are few for satisfactory discussion of the relation between the chemical shifts and the stereochemical structure of the complex.

In the present study, we attempted the preparation of the complexes, $[Co(β\text{-ala or gly})(en)_2]^{2+}$, $[Co(β\text{-ala or gly})_2(en)]^+$, $[Co(β\text{-ala or gly})(ox)_2]^{2-}$, and $[Co(β\text{-ala or gly})_2(ox)]^-$ ($C_2$- and $C_1$-*cis*(*O*)-$[Co(β\text{-ala})_2(en)]^+$ and *trans*(*O*)-, *cis*(*O*)*trans*($N_g$,*O*)-, *cis*(*O*)*trans*(*N*)-, and *cis*(*O*)*trans*($N_β$,*O*)-$[Co(β\text{-ala})(gly)(en)]^+$ are new complexes), and measured their $^{13}C$ NMR spectra. In this paper we report on their preparation, absorption and $^{13}C$ NMR spectral data, and assignments of the structures.

## Experimental

*Preparation of the Complexes.* *Isomers of Bis(β-alaninato)(ethylenediamine)cobalt(III) Chloride*: An aqueous solution containing 19 g of β-alanine, 6 g of ethylenediamine, and 4 g of sodium hydroxide in 100 cm³ of water was added to an aqueous solution containing 24 g of cobalt(II) chloride hexahydrate in 80 cm³ of water. The mixed solution was oxidized with 20 g of lead dioxide at 75 °C for about 30 min with stirring, and cooled to room temperature. After removal of insoluble matter by filtration, the filtrate was poured into a column (30 mm×800 mm) containing a cation exchange resin (Dowex 50WX8, 200—400 mesh, $K^+$ form). After the column had been swept with 3 dm³ of water, the adsorbed band was eluted with 0.05 M aqueous solution of potassium chloride. The band separated into three bands, another violet-red band remaining at the top of the column. The eluted solution from each band was concentrated to a few milliliters in a vaccum evaporator at 30—35 °C. To the concentrated solution was added a large amount of methanol, potassium chloride deposited being filtered off. Acetone was added to the filtrate in order to deposit crude complex. Each crude complex obtained was recrystallized from aqueous solution by addition of methanol and acetone. The first eluted band was confirmed to be *trans*(O)-$[Co(β\text{-ala})_2(en)]Cl$ from its absorption spectrum.[16] Isomer from the second eluted band: Found: C, 27.12; H, 6.44; N, 15.89%. Calcd for $[Co(β\text{-ala})_2(en)]Cl \cdot 1.5H_2O$: C, 26.86; H, 6.48; N, 15.66%. Isomer from the third eluted band: Found: C, 26.22; H, 6.64; N, 15.33%. Calcd for $[Co(β\text{-ala})_2(en)]Cl \cdot 2H_2O$: C, 26.20; H, 6.60; N, 15.28%.

*Isomer of (β-Alaninato)(glycinato)(ethylenediamine)cobalt(III) Chloride*: 10 cm³ of 60% perchloric acid was gradually added to a suspension of 5.0 g of *mer*(*N*)-(carbonato)(glycinato)(ethylenediamine)cobalt(III) monohydrate in 50 cm³ of water. After vigorous evolution of gas had subsided, the solution was adjusted to pH 9 by addition of 2 M NaOH, and then 5 g of β-alanine was added to the alkaline solution. The solution was kept at 65 °C for 3 h and then cooled to room temperature. The resulting solution was poured into a column (30 mm×800 mm) containing a cation-exchange resin (Dowex 50WX8, 200—400 mesh, $K^+$ form). After the column had been swept with 3 dm³ of water, the adsorbed band was eluted with 0.05 M KCl. The band separated gradually into four violet-red ones, a red-violet band remaining at the top of the column. Each eluted solution was concentrated to a few milliliters in a vacuum evaporator at 35—40 °C. A large amount of methanol was added to the concentrated solution, potassium chloride deposited being filtered off. Acetone was added to the filtrate and then crude complex deposited was collected. Each crude complex was recrystallized from aqueous solution by addition of methanol and acetone. Isomer from the first eluted band: Found: C, 24.97; H, 5.98; N, 16.56%. Calcd for $[Co(β\text{-ala})(gly)(en)]Cl \cdot H_2O$: C, 25.12; H, 6.02; N, 16.74%. Isomer from the second eluted band: Found: C, 25.11; H, 6.00; N, 16.61%. Calcd for $[Co(β\text{-ala})(gly)(en)]Cl \cdot H_2O$: C, 25.12; H, 6.02; N, 16.74%. Isomer from the third eluted band: Found: C, 27.07; H, 6.07; N, 16.87%. Calcd for $[Co(β\text{-ala})(gly)(en)]Cl \cdot 0.5MeOH$: C, 27.08; H, 6.06; N, 16.84% (methanol containing in this ratio was checked by $^1H$ NMR). Isomer from the fourth eluted band: Found: C, 26.22; H, 5.55; N, 17.67%. Calcd for $[Co(β\text{-ala})(gly)(en)]Cl$: C, 26.55; H, 5.73; N, 17.70%.

$C_1$-*cis*(*O*)-*(Glycinato-2,2-d$_2$)(glycinato)(ethylenediamine)cobalt(III) Chloride*: $C_1$-*cis*(*O*)-bis(glycinato)(ethylenediamine)cobalt(III) chloride (150 mg) was dissolved in 9 cm³ of deuterium oxide, and then deuterium oxide solution of sodium carbonate (1 M, 1 cm³) was added to the solution. The solution was allowed to stand at 40 °C until the $^1H$

NMR signal at 3.44 ppm, assigned to the methylene proton of I-position glycine (see Results and Discussion), disappeared (about 3 h).[17] To the solution was added 0.5 cm$^3$ of 2 M deuteriochloric acid, and a large amount of acetone. The red crystals deposited were collected and recrystallized from deuterium oxide solution by addition of acetone.

*$C_1$-cis(O)-, $C_2$-cis(O)-, and trans(O)-Bis(glycinato-2,2-$d_2$)-(ethylenediaamine)cobalt(III) Chloride and (Glycinato-2,2-$d_2$)-bis(ethylenediamine)cobalt(III) Dichloride*: The complexes were prepared in a similar way to that described above. Chloride of each complex (150 mg) was dissolved in 10 cm$^3$ of 0.1 M sodium cabonate-deuterium oxide solution. The solution was allowed to stand at 50 °C for *ca.* 3 h. To this was added 0.5 cm$^3$ of 2 M deuteriochloric acid, followed by a large amount of acetone, and the deuterated complex deposited was collected. $^1$H NMR spectra of the deuterated glycinato complexes are shown in Fig. 1 with those of the non-deuterated complexes.

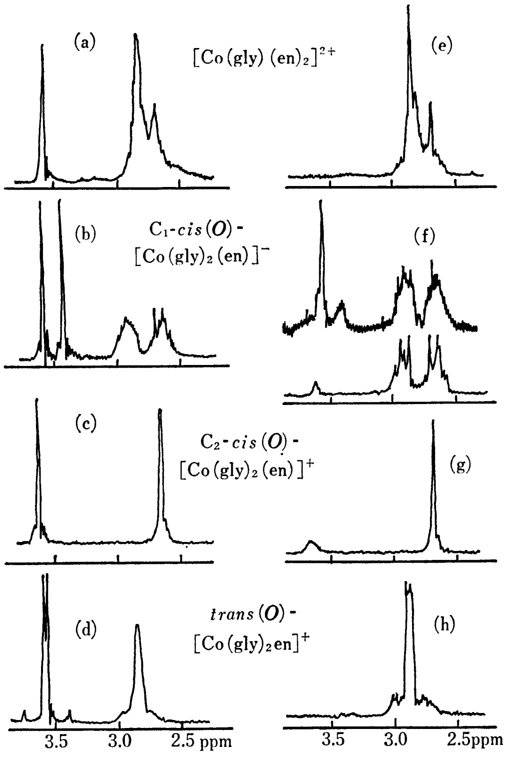


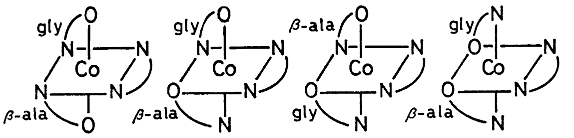
Fig. 1. $^1$H NMR spectra of the glycinato complexes. (a), (b), (c), and (d): Non-deuterated complexes, (e), (f), (g), and (h): deuterated complexes.

The other complexes examined were prepared by the methods reported.[1,18–20)]

*Measurements.* The electronic absorption spectra of the complexes were measured with a Hitachi EPS-3T spectrophotometer in aqueous solution. The $^{13}$C NMR spectra were measured on a JEOL Model MFT-100 spectrometer in pulse Fourier transform/proton noise decoupled mode at 25.15 MHz in deuterium oxide solution. The $^{13}$C chemical shifts were measured relative to benzene (in capillary) and converted into chemical shifts from TMS by means of the relation $\delta_{\mathrm{TMS}} = \delta_{\mathrm{benzene}} - 128.5$ ppm. The errors were about ±0.15 ppm. The $^1$H NMR spectra were recorded on a JEOL Model MH-100 spectrometer with DSS as an internal standard.

## Results and Discussion

Both $[Co(gly)_2(en)]^+$ and $[Co(\beta\text{-ala})_2(en)]^+$ ions provide three geometrical isomers, *trans(O)*, $C_1$-*cis(O)*, and $C_2$-*cis(O)*. On the other hand, the mixed complex ion, $[Co(\beta\text{-ala})(gly)(en)]^+$, exists in four geometrical isomers of *trans(O)*, *cis(O)trans*$(N_\beta,O)$, ($N_\beta$ represents the coordinated nitrogen atom in β-alanine), *cis(O)*-*trans*$(N_g,O)$ ($N_g$ represents the coordinated nitrogen atom in glycine), and *cis(O)trans*$(N_\beta,N_g)$ (Fig. 2). Absorption spectral data are given in Table 1.



*trans(O)* *cis(O)trans*$(N_\beta O)$ *cis(O)trans*$(N_g O)$ *cis(O)trans(N)*

Fig. 2. The geometerical structures of possible four isomers in the $[Co(\beta\text{-ala})(gly)en)]^+$ complex ion.

*Absorption Spectra.* The visible absorption band at lower energy side (so-called first absorption band) of *trans(O)* isomers of $[Co(O)_2(N)_4]$ type complexes shows a marked split as compared with that of the corresponding *cis(O)* isomers. The marked split of the first absorption band was observed for the complex obtained from the first eluted band in chromatographical separation of the $[Co(\beta\text{-ala})_2(en)]^+$, the absorption spectrum of the complex quite agreeing with that of the *trans(O)*-$[Co(\beta\text{-ala})_2(en)]^+$ in the Ref. 16. Thus, we concluded that the isomer from the first eluted band takes the *trans(O)* configuration. On the other hand, the second and third eluted isomers are expected to take the *cis(O)* configuration since no marked split in the first absorption band was observed.

Matsuoka *et al.*[21)] found that $D_{\mathrm{I}}/D_{\mathrm{II}}$ value in $C_2$-*cis(O)* isomer of bis(amino acidato)(ethylenediamine)-cobalt(III) is smaller than that in $C_1$-*cis(O)* one, where $D_{\mathrm{I}}$ and $D_{\mathrm{II}}$ are the optical densities at the absorption maxima of the first and second absorption bands, respectively. As shown in Table 1, $D_{\mathrm{I}}/D_{\mathrm{II}}$ value of the second eluted isomer of bis(β-alaninato)(ethylenediamine) complex is smaller than that of the third eluted isomer. Thus, we assigned the geometrical structures of the second and third eluted isomers to $C_2$-*cis(O)* and $C_1$-*cis(O)*, respectively. These assignments are in line with those from the $^{13}$C NMR data.

The structure of the first eluted isomer in chromatographical separation of $[Co(\beta\text{-ala})(gly)(en)]^+$ can be assigned to *trans(O)*, since a marked split was observed in the first absorption band, which is characteristic of the complex of *trans(O)*-$[Co(O)_2(N)_4]$ type. The structure of the third eluted isomer of $[Co(\beta\text{-ala})(gly)(en)]^+$ might be assigned to *cis(O)trans(N)* ($C_2$ symmetry if the chelate rings of β-ala and gly are neglected), since the $D_{\mathrm{I}}/D_{\mathrm{II}}$ value of the third eluted isomer is smaller than that of the second and fourth eluted isomers (Table 1).

*$^{13}$C NMR Spectra.* The $^{13}$C chemical shifts of carboxy carbons ($C_0$), α-carbons ($C_\alpha$), and β-carbons ($C_\beta$) of the glycinato and β-alaninato complexes are

TABLE 1. ABSORPTION SPECTRAL DATA OF BIS(AMINO ACIDATO)COBALT(III) COMPLEXES

| Elution order | | Complex ion | I Band $10\ \mathrm{cm^{-1}}$ (log $\varepsilon$) | II Band $10\ \mathrm{cm^{-1}}$ (log $\varepsilon$) | Intensity ratio of I band to II band ($D_{\mathrm{I}}/D_{\mathrm{II}}$) |
|---|---|---|---|---|---|
| GG-1 | **10** | *trans*(*O*)-[Co(gly)$_2$(en)]$^+$ | 18.87 (1.94) | 27.77 (2.12) | |
| | | | *ca.* 22 (*ca.* 1.64) | | |
| GG-2 | **6** | C$_1$-*cis*(*O*)-[Co(gly)$_2$(en)]$^+$ | 19.97 (2.09) | 27.70 (2.13) | 0.91 |
| GG-3 | **3** | C$_2$-*cis*(*O*)-[Co(gly)$_2$(en)]$^+$ | 19.93 (2.04) | 28.00 (2.11) | 0.85 |
| AG-1 | **11** | *trans*(*O*)-[Co(β-ala) (gly) (en)]$^+$ | 18.61 (1.97) | 27.78 (2.06) | |
| | | | *ca.* 22 (*ca.* 1.68) | | |
| AG-2 | **8** | *cis*(*O*)*trans*($N_{\mathrm{g}}$,*O*)-[Co(β-ala) (gly) (en)]$^+$ | 19.85 (2.16) | 27.71 (2.06) | 1.24 |
| AG-3 | **4** | *cis*(*O*)*trans*(*N*)-[Co(β-ala) (gly) (en)]$^+$ | 19.88 (2.06) | 27.78 (1.99) | 1.17 |
| AG-4 | **7** | *cis*(*O*)*trans*($N_\beta$,*O*)-[Co(β-ala) (gly) (en)]$^+$ | 19.43 (2.18) | 27.68 (2.03) | 1.43 |
| AA-1 | **12** | *trans*(*O*)-[Co(β-ala)$_2$(en)]$^+$ | 18.40 (2.00) | 27.60 (1.94) | |
| | | | 21.93 (1.61) | | |
| AA-2 | **5** | C$_2$-*cis*(*O*)-[Co(β-ala)$_2$(en)]$^+$ | 19.96 (2.10) | 27.47 (1.83) | 1.88 |
| AA-3 | **9** | C$_1$-*cis*(*O*)-[Co(β-ala)$_2$(en)]$^+$ | 19.47 (2.20) | 27.31 (1.90) | 2.02 |

TABLE 2. $^{13}$C CHEMICAL SHIFTS OF THE COORDINATED GLYCINATE AND β-ALANINATE

| | Complex ion | Position of the chelate ring | Carboxy carbon | | Methylene carbon | | |
|---|---|---|---|---|---|---|---|
| | | | Glycinate | β-Alaninate | Glycinate | β-Alaninate | |
| **1** | [Co(gly) (en)$_2$]$^{2+}$ | — | 186.7[a] | — | 45.8[a] | — | — |
| **2** | [Co(β-ala) (en)$_2$]$^{2+}$ | — | — | 182.0 | — | 33.3 | 38.5 |
| **3** | C$_2$-*cis*(*O*)-[Co(gly)$_2$(en)]$^+$ | O | 187.4[b] | — | 46.5[b] | — | — |
| **4** | *cis*(*O*)*trans*(*N*)-[Co(β-ala) (gly) (en)]$^+$ | O | 187.4 | 184.2 | 46.0 | 33.3 | 38.4 |
| **5** | C$_2$-*cis*(*O*)-[Co(β-ala)$_2$(en)]$^+$ | O | — | 184.1 | — | 33.1 | 38.0 |
| **6** | C$_1$-*cis*(*O*)-[Co(gly)$_2$(en)]$^+$ | I | 186.0[b,c] | — | 46.0[b,c] | — | — |
| | | O | 187.2[b] | — | 46.0[b] | — | — |
| **7** | *cis*(*O*)*trans*($N_\beta$,*O*)-[Co(β-ala) (gly) (en)]$^+$ | I | — | 182.0 | — | 33.3 | 37.8 |
| | | O | 187.2 | — | 46.5 | — | — |
| **8** | *cis*(*O*)*trans*($N_{\mathrm{g}}$,*O*)-[Co(β-ala) (gly) (en)]$^+$ | I | 186.0 | — | 45.9 | — | — |
| | | O | — | 183.7 | — | 33.6 | 38.4 |
| **9** | C$_1$-*cis*(*O*)-[Co(β-ala)$_2$(en)]$^+$ | I | — | 181.8 | — | 33.5 | 37.7 |
| | | O | — | 184.0 | — | 33.3 | 38.2 |
| **10** | *trans*(*O*)-[Co(gly)$_2$(en)]$^+$ | — | 187.6[b] | — | 45.0[b] | — | — |
| **11** | *trans*(*O*)-[Co(β-ala) (gly) (en)]$^+$ | — | 187.2 | 181.9 | 45.0 | 33.5 | 38.2 |
| **12** | *trans*(*O*)-[Co(β-ala)$_2$(en)]$^+$ | — | — | 181.5 | — | 33.1 | 38.0 |
| **13** | *trans*(*N*)-[Co(gly)$_2$(ox)]$^-$ | — | 188.6 | — | 46.1 | — | — |
| **14** | *trans*(*N*)-[Co(β-ala) (gly) (ox)]$^-$ | — | 188.7 | 184.4 | 45.9 | 33.7 | 38.1 |
| **15** | *trans*(*N*)-[Co(β-ala)$_2$(ox)]$^-$ | — | — | 184.4 | — | 33.9 | 38.3 |
| **16** | C$_1$-*cis*(*N*)-[Co(gly)$_2$(ox)]$^-$ | I* | 186.8 | — | 47.6 | — | — |
| | | O* | 187.2 | — | 46.1 | — | — |
| **17** | *cis*(*N*)*trans*($O_\beta$,*N*)-[Co(β-ala) (gly) (ox)]$^-$ | I* | — | 184.3 | — | 34.1 | 38.8 |
| | | O* | 187.2 | — | 46.1 | — | — |
| **18** | *cis*(*N*)*trans*($O_{\mathrm{g}}$,*N*)-[Co(β-ala) (gly) (ox)]$^-$ | I* | 186.7 | — | 47.5 | — | — |
| | | O* | — | 183.1 | — | 33.7 | 37.9 |
| **19** | C$_1$-*cis*(*N*)-[Co(β-ala)$_2$(ox)]$^-$ | I* | — | 184.2 | — | 33.7 | 38.6 |
| | | O* | — | 183.1 | — | 33.7 | 38.0 |
| **20** | C$_2$-*cis*(*N*)-[Co(gly)$_2$(ox)]$^-$ | O* | 187.0 | — | 46.2 | — | — |
| **21** | C$_2$-*cis*(*N*)-[Co(β-ala)$_2$(ox)]$^-$ | O* | — | 183.3 | — | 33.6 | 38.0 |
| **22** | [Co(gly) (ox)$_2$]$^{2-}$ | — | 187.2 | — | 46.5 | — | — |
| **23** | [Co(β-ala) (ox)$_2$]$^{2-}$ | — | — | 183.7 | — | 33.4 | 37.7 |

a) Weakened in the $[\mathrm{Co(ND_2CD_2COO)(en)_2}]^{2+}$ complex ion. b) Weakened in the $[\mathrm{Co(ND_2CD_2COO)_2(en)}]^{+}$ complex ion. c) Weakened in the C$_1$-*cis*(*O*)-$[\mathrm{Co(ND_2CD_2COO)(ND_2CH_2COO)(en)}]^{+}$ complex ion.

summarized in Table 2. The chemical shifts of $C_o$, $C_\alpha$, and $C_\beta$ in the complexes (in which the glycinate and β-alaninate coordinate to cobalt(III) in the chelated form) are represented by $\delta_{\mathrm{ch}}(C_o)$, $\delta_{\mathrm{ch}}(C_\alpha)$, and $\delta_{\mathrm{ch}}(C_\beta)$, respectively. Symbols $\delta(C_o)$, $\delta(C_\alpha)$, and $\delta(C_\beta)$ represent the chemical shifts of $C_o$, $C_\alpha$, and $C_\beta$, respectively, of free glycine and β-alanine in acidic solutions.

The carboxy carbon resonances of the chelated glycines ($\delta_{\mathrm{ch}}(C_o)$) were observed in the range 186.0—

188.7 ppm. On the other hand, the $\delta(C_o)$ of free glycine was 171.1 ppm. Thus, the $\Delta_{ch}(C_o)$ values ($\delta_{ch}(C_o)$ minus $\delta(C_o)$) of these glycinato complexes are found in the range 14.9—17.6 ppm. The $\delta(C\alpha)$ of free glycine was 40.4 ppm and the $\delta_{ch}(C\alpha)$ of glycinato complexes were observed in the range 45.0—47.6 ppm. Consequently, the $\Delta_{ch}(C\alpha)$ values of the glycinato complexes are found in the range 4.6—7.2 ppm. The $\Delta_{ch}(C_o)$ and $\Delta_{ch}(C\alpha)$ values of glycinato complexes are comparable to those of the other α-amino acids.[2)]

The $\delta(C_o)$, $\delta(C\alpha)$, and $\delta(C\beta)$ values of free β-alanine are 175.9, 31.2, and 35.6 ppm, respectively. The $\delta_{ch}(C_o)$, $\delta_{ch}(C\alpha)$, and $\delta_{ch}(C\beta)$ values of the chelated β-alanine were observed in the ranges 181.5—184.4 ppm, 33.1—33.9 ppm, and 37.7—38.8 ppm, respectively. The $\Delta_{ch}(C_o)$ values of β-alaninato complexes are *ca.* 7 ppm, smaller than those of α-amino acidato complexes. Similarly, the $\Delta_{ch}(C\alpha)$ values (1.9—2.7 ppm) of β-alaninato complexes are smaller than those of the α-amino acidato complexes. In this manner, the chemical shift changes caused by chelation can be used to differentiate β- and α-amino acids.[2)]

In $[Co(\beta\text{-ala})_2(ox)]^-$ complex ion, one and two resonance lines of the carboxy carbons were observed for the isomers **21** and **19** (Table 2), respectively, since the chemical environments of the carboxy carbons of the two β-alaninate are equivalent in the former isomer but not in the latter one.[1)] A similar relationship can be applied to the structural assignments of the two isomers of **5** and **9** in the $[Co(\beta\text{-ala})_2(en)]^+$ complex ion. The second and third eluted isomers in chromatographical separation of $[Co(\beta\text{-ala})_2(en)]^+$ show respectively one and two resonance lines arising from the carboxy carbons of β-alaninates. Thus, the structures can be assigned to $C_2$-*cis*(*O*) for the former (**5**) and $C_1$-*cis*(*O*) for the latter (**9**), respectively.

Yoneda *et al.*[22)] pointed out the importance of the effect of magnetic anisotropy induced on the central cobalt(III) chromophore. Application of this magnetic anisotropy to the **6** and **3** complexes leads to the following prediction. Two chelated glycinates in the **6** complex do not occupy equivalent positions; one is present in the plane formed by the coordinated oxygen atoms of glycinates and cobalt(III) (I-position), and the other is out of plane (O-position). On the other hand, the chelated glycinates in **3** at the O-position and their steric relation to the cobalt(III) chromophore ($[Co(O)_2(N)_4]$) are identical with the O-position glycinate of **6**. The O-position glycinate in **6** is expected to show nearly equal chemical shifts of the carboxy carbon to those in **3**. A similar relation is expected between **9** and **5**. In **16**, one glycinate is in the plane formed by the nitrogen atoms of the glycinates and cobalt(III) (I*-position), and another glycinate out of the N–Co(III)–N plane (O*-position). Two glycinates in **20** present at the O*-positions and their relation to the cobalt(III) chromophore (*cis*(*N*)-$[Co(O)_4(N)_2]$) are identical with the O*-position glycinate of **16**. In this manner, the following assignments are possible. In **6**, the carbon resonances at 46.0 and 187.2 ppm are for the O-position glycinate, at 46.0 and 186.0 ppm for the I-position glycinate. In **9**, those at 33.5, 37.7, and 181.8 ppm are for the I-position β-alaninate. In **16**, those at 46.1 and 182.2 ppm are for the O*-position glycinate, at 47.8 and 186.8 ppm for the I*-position glycinate. In **19**, those at 33.7, 38.0, and 183.1 are for the O*-position β-alaninate, at 33.7, 38.6, and 184.2 ppm for the I*-position β-alaninate.

The chemical shifts of the glycinate carbons in **11** are in fair agreement with those of the glycinate carbons in **10**. The chemical shifts of the β-alaninate carbons in **11** agree with those of the β-alaninate carbons in **12**. Similar agreements in chemical shifts were observed among the same geometrical isomers of bis(β-alaninato), bis(glycinato), and (β-alaninato)(glycinato) complexes. In the case of the **17** or **18** complex ion, the following consideration, in line with the above observations, is possible. When the I*-position (O*-position) glycinate of **16** is exchanged by β-alaninate, forming **17** (**18**), the carbon resonances of the I*-position (O*-position) glycinate will disappear and those of the I*-position (O*-position) β-alaninate will appear. As we expected, the glycinate carbons in **17** resonates at 187.2 and 46.1 ppm and these chemical shifts consist of those of the O*-position glycinate in **16** (187.2 and 46.1 ppm). The chemical shifts of β-alaninate carbons in **17** are nearly equal to those of the I*-position β-alaninate in **19**. A similar relation can be expected for the *cis*(*O*) of the second eluted isomer (**8**) in chromatographical separation of $[Co(\beta\text{-ala})(gly)(en)]^+$; the resonance peaks at 186.0 and 45.9 ppm for glycinate carbons and at 33.6, 38.4, and 183.7 ppm for the β-alaninate carbons consist of those of the O-position β-alaninate in **9** and I-position glycinate in **6**, respectively. The structure of the second eluted isomer can be assigned to *cis*(*O*)*trans*($N_g$,*O*). As the chemical shifts of the fourth eluted isomer (**7**), resonating at 46.5 and 187.2 ppm (glycinate), and at 33.3, 37.8, and 182.0 ppm (β-alaninate), consist of those of O-position glycinate and I-position β-alaninate. The structure of this isomer can be assigned to *cis*(*O*)-*trans*($N_\beta$,*O*).

In the present work, the $^{13}C$ chemical shifts were measured in $^1H$ noise decoupled mode. Since the signal of the carbon bonding to hydrogen is enhanced due to the nuclear Overhauser effect from $^1H$ atom and no splitting resonance is observed in noise decoupled mode, the resonance peak of the methylene carbon (α-carbon) of the glycinate is expected to be fairly intense. On the other hand, the resonance of the deuterated methylene carbon of the glycine will be very weak, since the signal of the carbon bonding to deuterium is split due to the carbon-deuterium coupling, no enhancement due to the NOE from $^1H$ atom being expected.[8)] In the chelated glycine, the resonance of the carboxy carbon (which neighbors methylene carbon) is enhanced by the NOE from the methylene protons, but the deuteration of the methylene group results in weakening of the carboxy carbon resonance. Consequently, it is possible to confirm which glycinate possesses the deuterated methylene group by comparing the $^{13}C$ NMR spectra of the deuterated complex with that of the non-deuterated complex.

The methylene protons in the chelated glycine are easily deuterated in basic deuterium oxide solution.[23] We succeeded in preparing five glycinato-2,2-$d_2$ complexes, ($[Co(ND_2CD_2COO)(en)_2]^{2+}$, $C_1$-*cis*(*O*)-$[Co(ND_2CD_2COO)(ND_2CH_2COO)(en)]^+$, and $C_1$-*cis*(*O*)-, $C_2$-*cis*(*O*)-, and *trans*(*O*)-$[Co(ND_2CD_2COO)_2(en)]^+$, by leaving each corresponding glycinato complex in basic deuterium oxide solution to stand for an appropriate time.

It is difficult to distinguish the methylene carbon of glycinate from that of the ethylenediamine, since the chemical shifts of the methylene carbons in chelated ethylenediamine are close to those of the methylene carbon of the chelated glycinate. The $^{13}$C NMR measurements of these deuterated glycinato complexes make it possible to distinguish the signals of the carbons in the deuterated glycinate ring from those of the carbons in chelated ethylenediamine.

Yoneda *et al.*[22] showed for the **6** complex that the $^1$H NMR signal resonating at 3.44 ppm arises from the methylene protons of the I-position glycinate. It was found that the I-position methylene protons are deuterated more rapidly than the O-position methylene protons (Fig. 1 (b)).[17] Since the methylene carbon resonance at 46.0 ppm and the carboxy carbon resonance at 186.0 ppm in **6** are weakened in the $C_1$-*cis*(*O*)-$[Co(ND_2CD_2COO)(ND_2CH_2COO)(en)]^+$, it is concluded that these methylene and carboxy carbons belong to the I-position glycinate, and methylene carbon at 46.0 ppm and carboxy carbon at 187.2 ppm in **6** to the O-position glycinate. The assignments of the $^{13}$C NMR spectra based on the deuteration method are consistent with the assignments concluded from the comparison of the bis(glycinato) complexes with (β-alaninato)(glycinato) complexes.

The $^{13}$C chemical shifts of the coordinated glycinate in bis(glycinato) complexes remained unchanged by the replacement of one of the two glycinates with β-alaninate. The $^{13}$C chemical shifts of the β-alaninate in bis(β-alaninato) complexes also remained unchanged by the replacement of one of the β-alaninates with glycinate. These observations suggest that the main factor which determines the chemical shifts of the coordinated glycinate or β-alaninate is the steric position of the ligands to the central cobalt(III) chromophore. This recalls the theory of the magnetic anisotropy arising from the cobalt(III) chromophore.[22] However, the Co(III) magnetic anisotropy does not account for all the behavior of the chemical shifts of the ligands. It is expected from the Co(III) magnetic anisotropy in complex **6** that the $^{13}$C chemical shift of the O-position methylene carbon of glycinate is located at lower magnetic field than that of the I-position methylene carbon, but really both carbon resonances were observed at 46.0 ppm as an overlapping signal. The carboxy carbon resonances of **10** should be located at much higher magnetic field than the observed ones, according to the cobalt(III) magnetic anisotropy.

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