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Metal Complexes of Peptides. IV. Cobalt(III) Complexes with β-Alanyl-L-histidine (Carnosine) Functioning as a Quadridentate Ligand

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Three cobalt(III) complexes containing β-alanyl-L-histidine (or carnosine: H_2car), $[Co(car)(L)]^+$ (L=en) and $[Co(car)(L)]^-$ (L=ox^{2-} or CO_3^{2-}), were newly prepared and characterized on the basis of their ^{13}C NMR, UV and visible absorption, and circular dichroism spectra. In these complexes, the carnosine coordinates to a cobalt(III) ion as a quadridentate ligand in a meridional form with respect to the peptide chain. In the pH range of 0—6, these complexes exhibited the visible absorption spectral changes with pH. These changes are attributed to the protonation on the oxygen in β-alanyl moiety of the coordinating carnosine and the pK_a values of $[Co(car)(en)]^+$ and $[Co(car)(ox)]^-$ were estimated as ca. 1.5. The spectral behaviors of the present complexes were compared with that of $[Co(Hcar)(en)(OH_2)]^{2+}$, in which the carnosine functions as a terdentate ligand.

Some studies on the cobalt(III) complexes containing peptides have been reported.[1-16] However, there are few studies on the cobalt(III) complexes of β-alanyl-L-histidine (or carnosine: H_2car), in spite that the complexes are important compounds in a biological sense. Only one coordination mode is reported for the carnosine–cobalt(III) complex,[1] in which the carnosine functions as a terdentate with the uncoordinated amino group. However, it is expected that the carnosine also functions as a quadridentate in a cobalt(III) complex, as found in a chromium(III) complex by Murdoch et al.[17] This expection led us to the experiments to prepare some cobalt(III) complexes containing a quadridentate carnosine under different synthetic conditions from those reported for the terdentate carnosine complex.[1] In the present paper, we will describe the preparation of new carnosine–cobalt(III) complexes and discuss their structures and properties on the basis of the ^{13}C NMR, UV and visible absorption, and CD spectral data.

Experimental

Preparation of Complexes. [Co(car)(en)]Cl (Complex 1): Carnosine (0.1 g) was dissolved in 40 cm^3 of water, and the pH of the solution was adjusted to 9.0 with 1 mol dm^{-3} KOH. To this solution, $K[Co(en)(CO_3)_2]\cdot H_2O$ (1.3 g)[18] was added with stirring. After stirring at 70 °C for 16 h, the solution was cooled to room temperature and then filtered. The filtrate was charged on an SP-Sephadex column (K^+ form) and flushed with water to remove a violet-brown band which was elutable with water, and then developed with 0.2 mol dm^{-3} KCl. A red orange band appeared on the development, a violet-brown band remaining on the top of the column. The red orange band was transferred to another SP-Sephadex column and then repeatedly developed with 0.2 mol dm^{-3} KCl using a micropump. After the red orange band separated into pink and orange bands, an eluant from the orange band was collected and concentrated to small volume with a vacuum evaporator. To the concentrated solution, a large amount of methanol was added in order to make deposit KCl, and then the solution was filtered. The filtrate was evaporated under reduced pressure to a few milliliters, again. The concentrated solution was loaded on a Sephadex G-10 column to eliminate KCl and eluted with water. The eluate from the orange band was concentrated to a small volume. A crude complex was obtained by adding methanol to the concentrate. It was recrystallized from water by addition of methanol (Complex 1; yield: 100 mg). Found: C, 31.44; H, 5.58; N, 20.21%. Calcd for $[Co(car)(en)]Cl\cdot 2H_2O = C_{11}H_{24}N_6O_5ClCo$: C, 31.93; H, 5.85; N, 20.31%.

Na[Co(car)(ox)] (Complex 2): Carnosine (0.5 g) was dissolved in 40 cm^3 of water. After the pH of the solution was adjusted to 8.5 with 2 mol dm^{-3} KOH, $K_3[Co(ox)_3]\cdot 3H_2O$ (0.96 g)[19] was added to the solution with constant stirring. The temperature of the solution was kept 40 °C for 10 h. The solution was cooled to room temperature, and then insoluble materials were filtered off. The filtrate was charged on a QAE-Sephadex column (Cl^- form, 4 cm×50 cm). After a violet-brown band elutable with water was eluted out with 1 dm^3 of water, the adsorbed band was developed with 0.1 mol dm^{-3} KCl. A violet band was appeared on the development, a green band remaining on the top of the column. The eluate from the violet band was concentrated to a few milliliters by using a vacuum evaporator and then KCl which deposited was filtered off. To the filtrate, methanol was added to deposit excess of KCl. The solution was filtered and a crude complex (K[Co(car)(ox)]) was precipitated by addition of acetone to the filtrate. The solution obtained by dissolving the crude complex in a small amount of water was treated with an SP-Sephadex column (Na^+ form; eluent: water) to convert the potassium salt to the sodium salt. After the eluate was concentrated, ethanol, saturated with $NaClO_4$, was added to the concentrated solution. A crude complex of Na[Co(car)(ox)] thus deposited was recrystallized from water by addition of ethanol containing $NaClO_4$ (Complex 2; yeild: 350 mg). Found: C, 23.28; H, 3.22; N, 9.96%. Calcd for $Na[Co(car)(ox)]\cdot NaClO_4\cdot 3H_2O = C_{11}H_{18}N_4ClNa_2O_{14}Co$: C, 23.15; H, 3.18; N, 9.82%.

K[Co(car)(CO_3)] (Complex 3): Carnosine (0.5 g) was dissolved in 40 cm^3 of water and $Na_3[Co(CO_3)_3]\cdot 3H_2O$ (0.8 g)[20] was added to the solution. The solution was stirred at 45—50 °C for 24 h. After being cooled to room temperature, the solution was filtered and the filtrate was charged on a QAE-Sephadex (Cl^- form) column. The column was swept with 1 dm^3 of water to remove the violet-brown band which was

elutable with water. The adsorbed band was developed with 0.1 mol dm^{-3} KCl. A violet band appeared on the development, a brown band remaining on the top of the column. The eluate from the violet band was concentrated to a few milliliters by using a vacuum evaporator and then methanol was added to the concentrated solution. White precipitates (KCl) were filtered off. The filtrate was again evaporated almost to dryness and then the residue was dissolved in a few milliliters of water. The solution was treated with a Sephadex G-10 column in order to remove KCl. The crude complex, which was obtained from the violet eluate by addition of ethanol, were recrystallized from water by addition of ethanol (Complex **3**; yield: 250 mg). Found: C, 31.35; H, 4.23; N, 12.85%. Calcd for $K[Co(car)(CO_3)]\cdot 0.5C_2H_5OH\cdot 2H_2O=C_{11}H_{17}N_4O_{7.5}KCo$: C, 31.21; H, 4.05; N, 13.25%.

[Co(Hcar)(en)(OH_2)]$Cl_2\cdot H_2O$ (Complex 4): This complex was prepared by the method described in a previous paper.[1]

Absorption Spectral Changes with pH. In Lower pH Region: 5 cm^3 of the solution containing complex **1** (0.2004 g, 4.8×10^{-4} mol dm^{-3}) in 50 cm^3 of water was placed in a measuring flask (10 cm^3), and then a prescribed volume of 2 mol dm^{-3} HCl solution was added to it. The volume of the acidic solution was adjusted to 10 cm^3 with water. By the same procedure, seven solutions with different pH's (0.1—6.0) were prepared. Absorption spectrum and pH of the each solution were recorded at room temperature. In the same manner as above, the absorption spectral changes with pH's were examined for Complexes **2** and **4**.

In Higher pH Region: The spectral changes in the range of pH 5—10 were examined by the method similar to that described above, except for using 1 mol dm^{-3} NaOH instead of 2 mol dm^{-3} HCl.

Measurements. The absorption and CD spectra were measured by a HITACHI 557 spectrophotometer and a JASCO J-22 spectropolarimeter, respectively. The ^{13}C NMR spectra were recorded on a HITACHI R-90H spectrometer. The chemical shifts in ^{13}C NMR were measured relative to internal dioxane (δ=67.40).

Results and Discussion

^{13}C NMR Spectra. The ^{13}C NMR measurements at various pH provide useful information on the coordination modes of ligand. Figure 1 shows the ^{13}C NMR shift patterns of carnosine and its complexes, together with those of the related complexes. The numbering of all carbons in carnosine and their signal assignments adopted those described in the report of Viola et al.[21] (Fig. 1).

As Fig. 1 shows, the ^{13}C chemical shifts of the free carnosine vary with pH of the solution. The C_5 resonance peak of free carnosine in a pH 5 solution locates at more down-field than that in a 1 M DCl solution. This down-field shift arises from deprotonation on the carboxyl group. The C_7 and C_9 peaks of free carnosine at pH 9 shift to more down-field than those at pH 5, and the C_1 and C_2 peaks of free carnosine at pH 12 shift to more down-field than those at pH 9. The down-field shifts for the former and

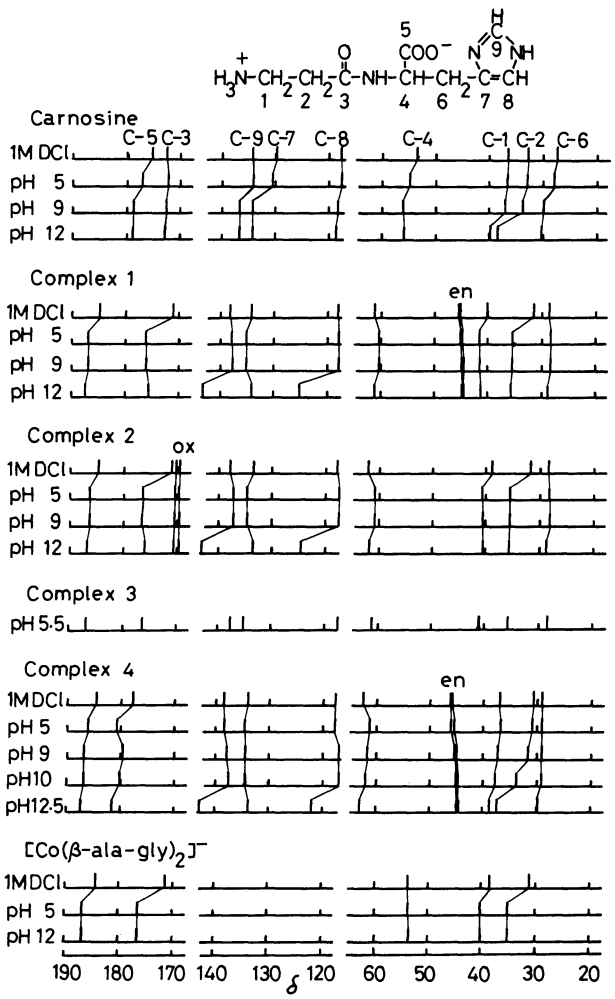

Fig. 1. ^{13}C NMR spectral patterns of carnosine and cobalt(III) complexes at various pH.

latter are related to deprotonations on the imidazole NH and on the amino groups, respectively.

The C_5 resonance peaks of the present complexes at pH 5—7 are observed in more down-field region than that of free carnosine at pH 5. These down-field shifts results from the coordination of the carboxyl groups to cobalt(III). Such down-field shifts due to the coordination of the carboxyl groups to cobalt(III) are also well recognized for the amino acidato and aliphatic acidato complexes.[22]

The ^{13}C chemical shifts of the carnosine-carbons in the present Complexes **1**, **2**, and **3** coincide well with each other for their neutral solutions, suggesting that the coordination mode of carnosine is the same in these complexes. In addition, the ^{13}C chemical shifts of the C_1, C_2, C_3, and C_5 carbons in Complexes **1**, **2**, and **3** are almost equal to those of the corresponding carbons of the terdentate β-ala-gly in $[Co(\beta\text{-ala-gly})_2]^-$. These results suggest that the coordination mode of carnosine in the present complexes is similar to that of β-ala-gly in $[Co(\beta\text{-ala-gly})_2]^-$ with respect to the peptide chain. The C_7 and C_9 resonance peaks of

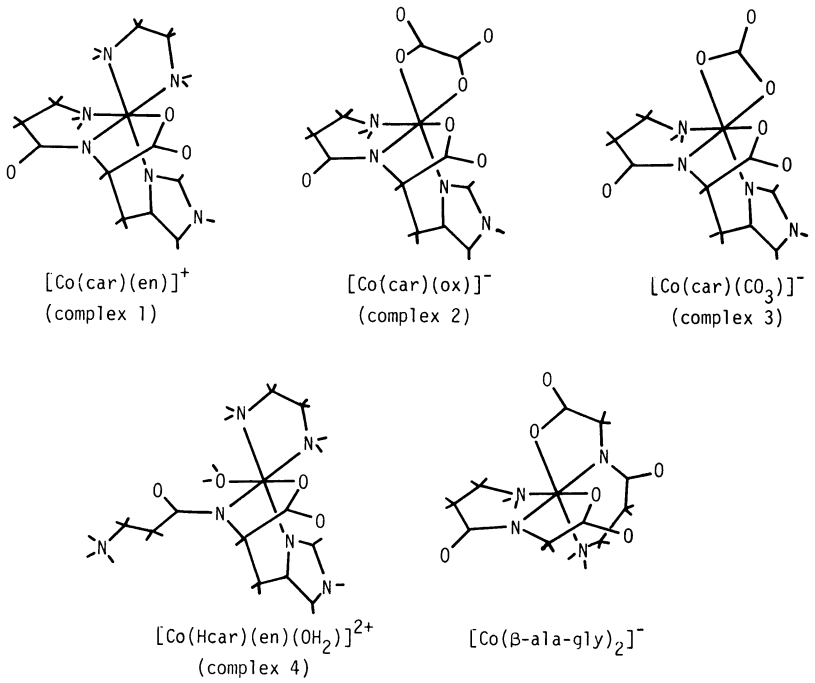

Fig. 2. Structures of the cobalt(III) complexes containing carnosine.

Complexes **1**, **2**, and **3** at pH 5 (or 5.5) are observed at more down-field than those of free carnosine at pH 5. These down-field shifts show that the coordination of imidazole moiety to cobalt(III) occurs. Consequently, the structures shown in Fig. 2 are proposed for Complexes **1**, **2**, and **3**. In these complexes, the marked up-field shifts of C_3 and C_2 at low pH (1 M DCl) are attributed to protonation on the oxygen in β-alanyl moiety of the coordinating carnosine,[23] and the down-field shifts of C_8 and C_9 at high pH (pH 12) are attributed to deprotonation of NH on the coordinating imidazole moiety.

Absorption and Circular Dichroism Spectra. The absorption and CD spectra of the newly prepared carnosine complexes are shown in Fig. 3, together with those of Complex **4** reported in our previous study.[1] The absorption spectral behaviors of complexes **2** and **3** resemble to each other, indicating that these complexes have the same chromophore of the *fac*-$[Co(N)_3(O)_3]$ type[24] and the same coordination mode of carnosine, that is, carnosine coordinate to Co(III) as quadridentate with carboxyl O, amino N, amide N, and imidazole N. The replacement of oxalato in Complex **2** with ethylenediamine gives Complex **1**, whose first absorption band shifts to higher energy side than that of Complex **2**. Moreover, the first absorption band of Complex **1** has characteristics of the $[Co(N)_5(O)]$ type chromophore.[24] These results based on the absorption spectra coincide with the assignment based on the ^{13}C NMR mentioned in the previous section.

While the band splitting in the first absorption band of Complex **1**, **2**, or **3** is not obvious, that of Complex **4** is obvious. This spectral pattern of Complex **4** is characteristic to the *trans*(*O*)-$[Co(N)_4(O)_2]$ type chromophore.[24] This result is consistent with the structure

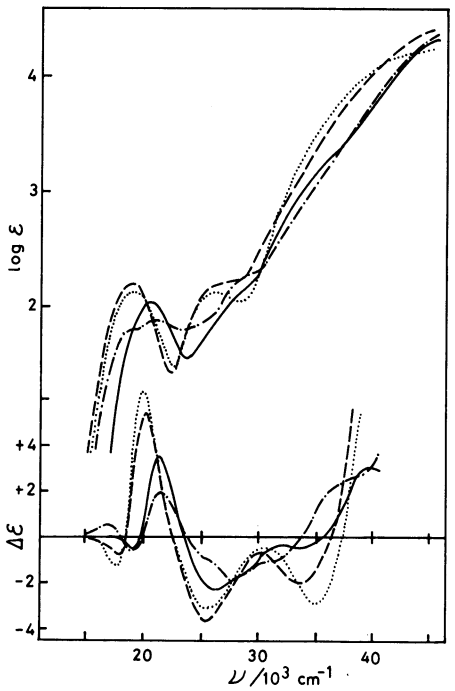

Fig. 3. Absorption and CD spectra of the complexes. $[Co(car)(en)]^+$ (Complex **1**) (——), $[Co(car)(ox)]^-$ (Complex **2**) (— — —), $[Co(car)(CO_3)]^-$ (Complex **3**) (·····), and $[Co(Hcar)(en)(OH_2)]^{2+}$ (Complex **4**) (—·—·—). (The ε and Δε values are given in $mol^{-1}\,dm^3\,cm^{-1}$).

determined by means of X-ray crystal analysis.[1]

The CD behaviors of Complexes **2** and **3** quite resemble each other. Both the complexes show two CD peaks with opposite signs in the first absorption band region (a weak (−) sign at lower energy side and an intense peak with (+) sign at higher energy side) and a (−) CD peak in the second band region. Similar spectral pattern also appears in Complex **1**. This similarlity in CD pattern suggests that these complexes have the same absolute configuration (Fig. 2). The CD pattern of Complex **4**, however, is different from those of Complexes **1**, **2**, and **3**, that is, three CD peaks ((+), (−), and (+) signs from lower energy side) appear in the first absorption band region. Such difference in CD patterns is attributed to difference in the coordination modes of carnosine.

Spectral Change with pH. The absorption spectral changes with pH were observed for the present complexes. Figure 4(A) shows the spectral change of Complex **1** in the range of pH 0.53—5.84. The isosbestic points appear at 463 and 426 nm. These spectral changes result from the protonation on the oxygen in the β-alanyl moiety of the coordinating carnosine.[23] The pK_a value estimated from the spectral change is 1.46. Analogous spectral change in an acidic solution (pH range: 1.12—6.19) was also observed for Complex **2**, with four isosbestic points at 523, 448, 335, and 350 nm. The pK_a value was estimated as 1.78. These pK_a values (1.46 and 1.78) are larger than 0.75 for [Co(mida)(L-ala-gly)]$^-$ (mida=*N*-methyl iminodiacetato), but smaller than 2.56 for [Co(mida)(β-ala-gly)]$^-$.[3]

The spectral change of Complex **4** in the range of pH 0.13—5.12 provided three isosbestic points at 565, 389, and 366 nm. The pK_a value estimated is 0.51 and smaller than those of Complexes **1** and **2**. This small value results from that the coordination mode of carnosine in Complex **4** (terdentate with uncoordinated amino moiety) is different from that in Complex **1** or **2** (quadridentate). The X-ray crystal data of Complex **4** imply that hydrogen bond is present between β-alanyl O^- in carnosine and coordinating H_2O. This hydrogen bond makes it difficult for another proton to attach to β-alanyl O^-, and lowers the pK_a value of Complex **4**. In addition, the hydrogen bond makes the amino group on the β-alanine moiety keep away from the cobalt center and stabilizes the unique structure containing cis-peptide bond[25] (C_2 and C_4 carbons are in cis position with respect to C_3–N bond) (Figs. 1 and 2).

Complex **4** shows marked absorption spectral change in the range of pH 5.27—9.10, as shown in Fig. 5 (the spectral curve at pH 10.03 coincides with that at pH 9.10 in the range 600—320 nm). However, ^{13}C chemical shifts of Complex **4** at pH 9 are nearly equal to those at pH 5. These results indicate that no deprotonation occurs on the carnosine ligand at pH 9.10 (that is, no deprotonation occurs on either amino or imino group at this pH) but deprotonation occurs on coordinating H_2O to give a hydroxo complex. The pK_a value estimated from the spectral changes is 7.87.

In Complex **4** the absorption spectral curve at pH 9.10 coincides with that at pH 10.03 in the range 600—320 nm, as mentioned above. However, the ^{13}C resonance peaks of the β-alanine moiety in carnosine at pH 9 shift to more down-field than those at pH 10.

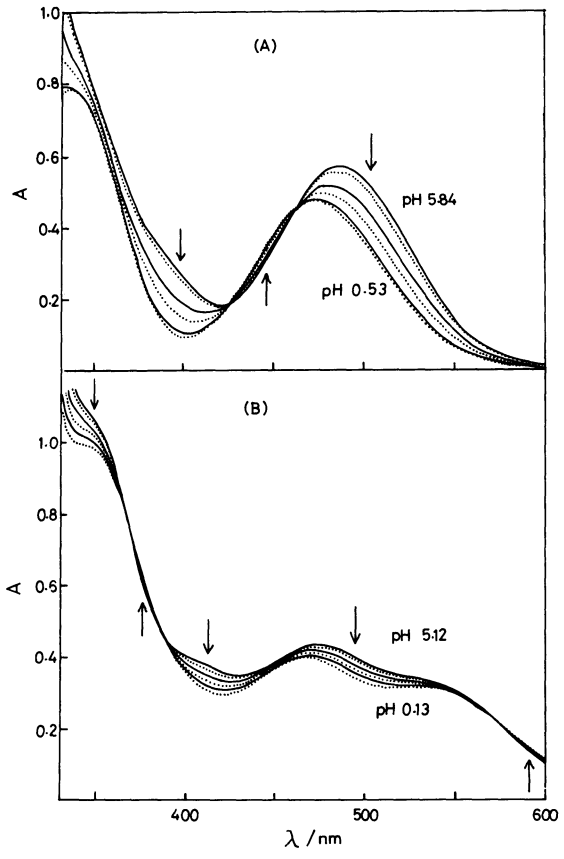

Fig. 4. Absorption spectral changes of the complexes with pH in acidic region. (A) Complex **1** (pH=5.84, 2.29, 1.60, 1.27, 0.79, and 0.53). (B) Complex **4** (pH=5.12, 0.78, 0.55, 0.40, 0.30, and 0.13).

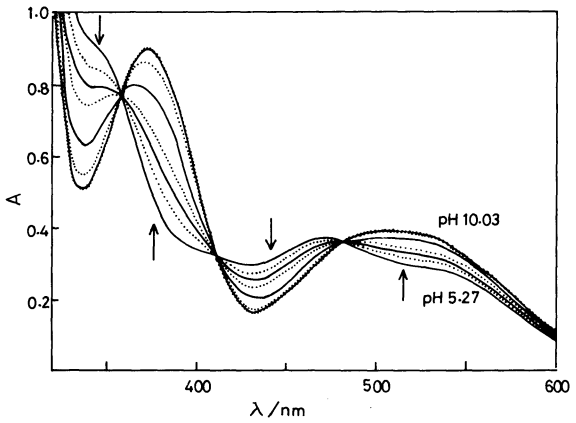

Fig. 5. Absorption spectral change of Complex **4** with pH in basic region. (pH=5.27, 7.20, 7.56, 7.87, 8.35, 8.99, 9.10, and 10.03).

This down-field shift is attributed to the deprotonation on $-NH_3^+$ of the β-alanyl moiety. The C_7 and C_9 resonance peaks of Complex **4** at pH 12.5 locate at more down-field than those at pH 10. The down-field shifts are attributed to the deprotonation of NH in the imidazole moiety. It is noteworthy that the basic solution of Complex **4** (containing carnosine as terdentate with uncoordinated $-NH_3^+$ moiety) does not produce easily Complex **1** (containing carnosine as quadridentate); replacement of the coordinating hydroxy ligand by the uncoordinating amino group does not occur easily. This results from that the cis-peptide bond in Complex **4** is stabilized by the hydrogen bond described above and that the structural conversion from cis- to trans-peptide is not easy at room temperature.

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