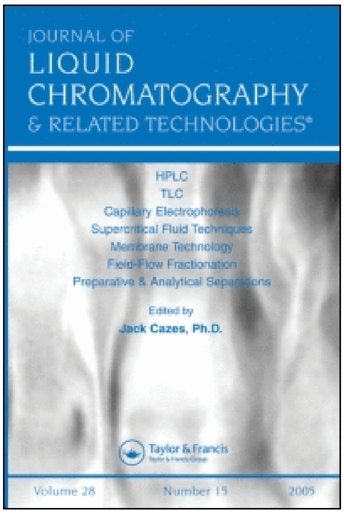

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Chromatographic Separation of Isomers of Meridional Tris(amino-acidato) Cobalt(III) Homochelates and Their Absolute Configuration

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CHROMATOGRAPHIC SEPARATION OF ISOMERS OF MERIDIONAL TRIS(AMINOACIDATO)COBALT(III) HOMOCHELATES AND THEIR ABSOLUTE CONFIGURATION[1]

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ABSTRACT

Chromatographic separation of enantiomers or diastereomers was undertaken for four kinds of meridional $[Co(AA)_3]$ complexes through the column packed with antimony d-tartrate form of the anion exchange resin, where AA stands for D-alanine, L-alanine, glycine and β-alanine. From the CD spectra of the separated isomers, it was revealed that the Λ-isomer was eluted first for the three α-aminoacidato chelates. As to the case of mer-$[Co(\beta\text{-ala})_3]$ a single crystal X-ray analysis was made for the second eluted isomer and determined its absolute configuration to be Δ. Thus, it was concluded that in all four cases the Δ-isomer has greater affinity toward antimony d-tartrate anion than the Λ-isomer. This makes a contrast to the case of the corresponding facial $[Co(AA)_3]$ in which the Λ-isomer has greater affinity toward antimony d-tartrate than the Δ-isomer. Based on the

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values of the separation factors for the four enantiomeric pairs, the association mode effective for chiral discrimination was discussed.

INTRODUCTION

Since complete resolution of fac-[Co(β-ala)$_3$] was achieved by chromatography(2), detailed studies have been done for facial tris(aminoacidato)cobalt(III) chelates, such as [Co(L/D-ser)$_n$(β-ala)$_{3-n}$] (3) and [Co(L/D-ala)$_n$(β-ala)$_{3-n}$] (4), using d-tartrate and antimony d-tartrate which is abbreviated hereafter as [Sb$_2$d-tart$_2$]$^{2-}$ as a chiral selector. The conclusion drawn in these studies was that the Λ-isomer of each chelate associates more firmly with these two chiral selectors than the Δ-isomer.

On the other hand, optical resolution of the corresponding meridional isomer was considered to be difficult, because the complex has only a small dipole moment in contrast to the facial isomer, so that it would flow through the column without noticeable adsorption. This difficulty was overcome by eluting the complex through the column packed with the [Sb$_2$d-tart$_2$]$^{2-}$ form of the anion-exchange resin. Thus, complete resolution was achieved for mer-[Co(β-ala)$_3$] as reported in the fourth paper of this series.(5) However, a problem remains unsolved which enantiomer Δ or Λ is eluted first in this chromatography.

Usually the absolute configuration of a chiral metal complex can be determined from its CD spectrum by comparing with that of a chiral complex having a similar structure whose absolute configuration is already established by X-ray analysis. In the case of fac-[Co(β-ala)$_3$], the CD spectrum of the isomer which was eluted first through the column packed with a chiral selector anionic form of an anion-exchange resin differs considerably from that of the first eluted isomer of fac-[Co(L-ala)$_3$] whose absolute configuration is already established to be Δ.(6) Comparison of these two CD spectra has no suggestion for determination of the absolute configuration of fac-[Co(β-ala)$_3$]. However, we can provide mixed ligand chelates, fac-[Co(β-ala)$_2$(L-ala)] and fac-[Co(β-ala)(L-ala)$_2$] which fill a gap between fac-[Co(β-ala)$_3$] and fac-[Co(L-ala)$_3$]. Thus, we have four CD curves for the first eluted isomers of a series of fac-[Co(L-ala)$_n$(β-ala)$_{3-n}$] which show a regular stepwise variation in the CD spectral pattern. It is natural to assume that the first eluted isomers of the four chelates have the same absolute configuration Δ.(4)

The same procedure can not be applied to the case of mer-[Co(β-ala)$_3$]. In the meridional chelate, each of mixed ligand chelates, mer-[Co(L-ala)(β-ala)$_2$] and mer-[Co(L-ala)$_2$(β-ala)] has three isomers each of which exists in two diastereomers Δ and Λ. It is practically very difficult at present to isolate them in a chemically pure state. There is no other way than X-ray analysis to determine the absolute configuration of the resolved enantiomers of mer-[Co(β-ala)$_3$]. We have determined the crystal structure of the second eluted isomer, $(+)^{500}_{\mathrm{CD}}$-mer-[Co(β-ala)$_3$]·4H$_2$O. This paper describes the chromatographic behaviors and absolute configurations of the four kinds of meridional tris chelates having D-ala, L-ala, gly and β-ala as ligands.

EXPERIMENTAL

Preparation of Compounds

The aminoacidato cobalt(III) chelates were prepared in the same way as reported previously.(7) The solution containing amino acid (L-alanine, D-alanine, glycine and β-alanine) and $CoCl_2 \cdot 6H_2O$ was oxidized with PbO_2 under stirring at 80°C for half an hour. The reaction mixture was filtered to remove insoluble material, and the filtrate was poured on a column packed with the Na^+ form of SP-sephadex C-25 (ϕ = 3 x 40 cm). The adsorbed chelates were eluted with water. Three bands were separated, a violet band (meridional isomer) came out first, then a red violet band (facial isomer) second, and another red violet band (cationic complexes) remained on a top of the column. The eluate containing the meridional isomer was concentrated in a vacumm evaporator and violet powder was obtained on addition of ethanol and ether.

Separation of Δ and Λ Isomers

The violet powder was dissolved in a small amount of water to prepare a nearly saturated solution. Two milliliters of the solution was loaded on a column packed with the $[Sb_2(d\text{-tart})_2]^{2-}$ form of QAE-Sephadex A-25 (ϕ = 2 x 108 cm), and eluted very slowly with water. Three days were spent for each run. Two bands were clearly separated for mer-$[Co(L\text{-ala})_3]$, mer-$[Co(D\text{-ala})_3]$ and mer-$[Co(\beta\text{-ala})_3]$, respectively, except for mer-$[Co(gly)_3]$. The eluate containing each of these two bands was separately collected, checked by the CD spectrum and concentrated to a nearly saturated solution. The concentrated solution thus obtained was used for the measurement of the retention volume by HPLC. In spite of having spent six days for elution, mer-$[Co(gly)_3]$ gave still only a broad single band. However, the front and the rear fractions of the band gave the enantiomeric CD spectra. These fractions (only small fractions) and the middle part (a main fraction) of the elution band were collected separately. The middle fraction was concentrated to a nearly saturated solution and loaded again on a column for elution. The procedure was repeated several times to collect a sufficient amount of the front and rear fractions. These front and rear fractions were concentrated to a nearly saturated solution and were used for measurement of the retention volume by HPLC without further purification.

Retention Volume Measurement

The retention volume was measured on a laboratory-built chromatographic unit that consisted of a JASCO LCP pump, PM-150 pressure gauge, PC-150 pump controller, injector, column and a Shimadzu UV-140 double-beam spectrophotometer. A dual penstrip-chart recorder was used. The detector was operated at 525 nm in each run. The column was a 25 cm long precision stainless steel tube of I.D. 4mm and was packed with the $[Sb_2d\text{-}tart_2]^{2-}$ form of TSK-220 anion-exchange resin.

The flow-rate was set at 4 x 10^{-2} ml/min. Since the flow-rate was so small that the elution was affected by variation of the pressure, and the value of the retention volume was fluctuated. Thus, the measurement was done several times for one sample and an average value was taken. The void volume was assumed to be 1.375 ml(this value is equal to that of the TSK cation-exchange resin having the same particle size packed in the same column).

Preparation of Single Crystals of $(+)^{500}_{CD}$-mer-$[Co(\beta\text{-}ala)_3]\cdot 4H_2O$

To obtain a sufficient amount of each enantiomer, chromatographic separation was repeated several times. Each of the two elution bands was collected separately and evaporated for crystallization. In the present study, a single crystal which was occasionally picked up from the second eluate was used for x-ray analysis. The crystal is a small plate(0.4x0.4x0.2 mm^3)

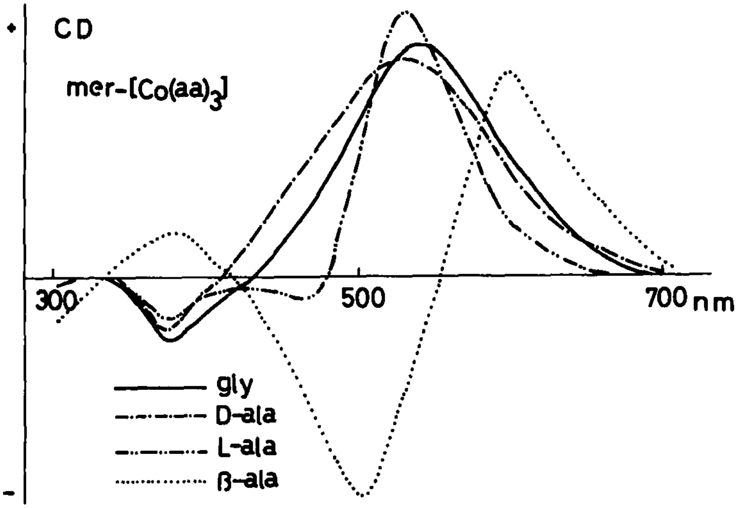

Fig. 1. The CD spectra of the first eluted isomers of the mer-[Co(AA)$_3$].

and was found to have four waters of crystallization from the x-ray analysis.(8)

RESULTS AND DISCUSSION

Chromatographic Behavior and Absolute Configuration

Figure 1 shows the CD spectra of the first eluted isomers (diastereomers or enantiomers) of four mer-[Co(AA)$_3$] in the present chromatographic runs where the chiral selector was held in the stationary phase. The CD spectrum of the first eluted diastereomer of mer-[Co(L-ala)$_3$] proved to be identical with that of Λ-mer-[Co(L-ala)$_3$](7) whose absolute configuration had been established by Drew et al.(9) The spectrum of the first eluted diastereomer of mer-[Co(D-ala)$_3$] proved to be similar in shape and opposite in sign to that of Δ-mer-[Co(L-ala)$_3$]

reported by Denning and Piper(7) and confirmed by Herak et al.(10), so that the absolute configuration of this isomer can be assigned also to Λ.

The spectrum of the first eluted isomer of mer-$[Co(gly)_3]$ shows a big similarity to those of the above two complexes. Thus, it can also be assigned to Λ. Since the first eluted isomer of mer-$[Co(\beta\text{-}ala)_3]$ shows a CD spectrum considerably different from other three spectra, its absolute configiuration can not be determined from the CD spectral pattern. However, its absolute configuration could be determined as Λ, because the absolute configuration of the second eluted enantiomer proved to be Δ by x-ray crystal structure analysis as shown in Figure 2.(8) Therefore, it is concluded that the Λ isomer is eluted first in the four mer-$[Co(AA)_3]$ complexes, that is, the Δ isomer is retained more firmly by the stationary phase than the Λ. In other words, the Δ isomer is favored more by $[Sb_2d\text{-}tart_2]^{2-}$.

Chromatographic Behavior and Chiral Discrimination

Above conclusion is in conflict with the prediction by the L-J model.(11) According to the L-J model, the Λ isomer having L-shaped channels should be favored more by the $[Sb_2d\text{-}tart_2]^{2-}$ anion. A new association model for chiral discrimination should be constructed in the present meridional complexes. A precise measurement of retention volumes might provide some clue to find out the association model for chiral discrimination. Since separation of the two isomers of each mer-$[Co(AA)_3]$ chelates was so small that the precise value of the retention volume for each of the two isomers could not be measured in the chromatographic run of the diastereomeric or enantiomeric mixtures of these mer-$[Co(AA)_3]$. Thus, individual isomers were separated in advance

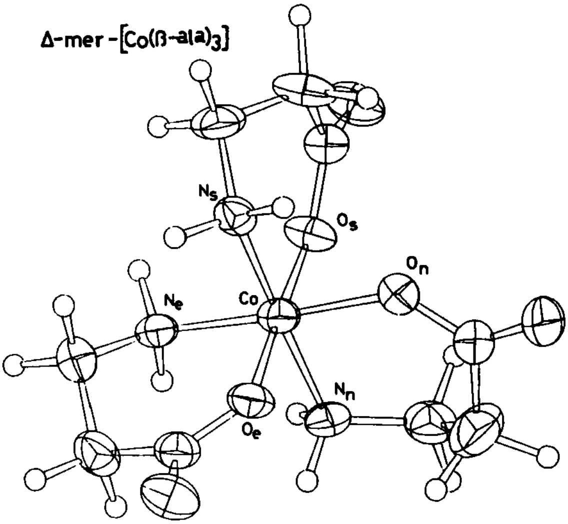

Fig. 2. The absolute configuration of the second eluted enantiomer of mer-[Co(β-ala)$_3$] ($(+)^{500}_{CD}$ for Δ).

and used to measure their retention volumes. The separation factors for four kinds of enantiomeric pairs were calculated by combining these retention volumes.

In Table 1 are listed values of the retention volumes and the separation factors for the four enantiomeric pairs of mer-[Co(AA)$_3$]. From this Table, two noteworthy points can be drawn: (1) The separation factors for the enantiomeric pairs of the gly and β-ala complexes are almost equal (1.138 and 1.175 respectively). (2) As to the separation factors for two enantiomeric pairs of the D- and L-ala complexes, the value is 1.512 for the (Λ-D...Δ-L) pair and 1.089 for the (Λ-L...Δ-D) pair. The former value is much larger than those for the gly and β-ala cases, which suggests a big influence of the CH$_3$ group on chiral discrimination in the (Λ-D...Δ-L) pair. On the contrary, the

TABLE 1

VALUES OF RETENTION VOLUMES AND SEPARATION FACTORES FOR FOUR ENANTIOMERIC PAIRS OF MER-$[Co(AA)_3]$

Sample complex	First eluted (ml)	Second eluted (ml)	Separation factor
mer-$[Co(gly)_3]$	1.525(Λ)[a)]	1.735(Δ)	1.138
mer-$[Co(\beta\text{-ala})_3]$	0.652(Λ)	0.766(Δ)	1.175
mer-$[Co(\alpha\text{-ala})_3]$	0.605(Λ-D)	0.915(Δ-L)	1.512
mer-$[Co(\alpha\text{-ala})_3]$	0.675(Λ-L)	0.735(Δ-D)	1.089

a) Absolute configuration of the complex and that of the ligand.

latter value is near to those for the gly and β-ala cases, which suggests that the existence of the CH_3 group has almost no influence on chiral discrimination in the (Λ-L...Δ-D) pair. Based on these points, the association mode effective in chiral discrimination by the $[Sb_2d\text{-}tart_2]^{2-}$ can be deduced.

From the pure electrostatic point of view, the most probable direction of access toward mer-$[Co(AA)_3]$ for the chiral selector anion is considered to be the direction indicated by arrow 0 in Fig. 3. The anion coming along this direction should receive the least electrostatic repulsion from the negative charges of the three meridionally arranged oxygen atoms. However, the anion coming along this direction can not discriminate the chirality of the complex because it associates with the complex by one-point attachment at the N_e atom. In other words, the anion can get in contact with the complex at only one of its three chelate rings. The anion can not recognize the configuration of the two remote chelate rings in such an association.

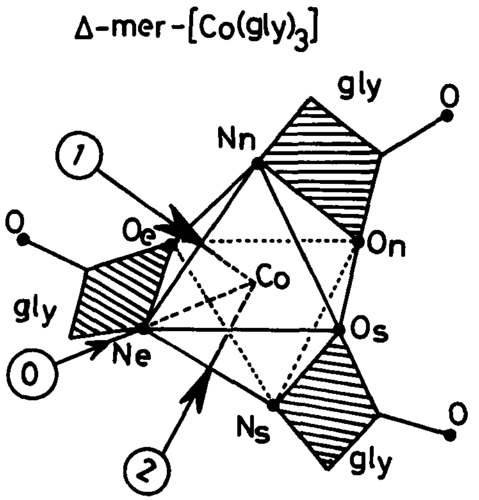

Fig. 3. The most probable direction of access towards mer-[Co(AA)$_3$].

Since chiral discrimination does take place in the present cases, it is quite reasonable to imagine that the anion approaches the complex along either one of the two directions indicated by arrows 1 and 2 in Fig. 3. Here, the association takes place in such a way that the anion gets in contact with the complex at either one of the two edges of the octahedron, N_n- N_e and N_e- N_s. Both approaches allow the anion to touch two chelate rings of the complex so that the anion can recognize the absolute configuration of the complex. The characteristic features of these two association modes 1 and 2 can be seen in the two structures of mer-[Co(L-ala)$_3$], viewed along the two directions, 1 and 2, as shown in Fig. 4a and 4b.(10) The schematic illustrations of these figures with their enantiomers are shown in Fig. 5a(Δ-L and Λ-D) and their diastereomeric pairs are also shown in Fig. 5b(Δ-D

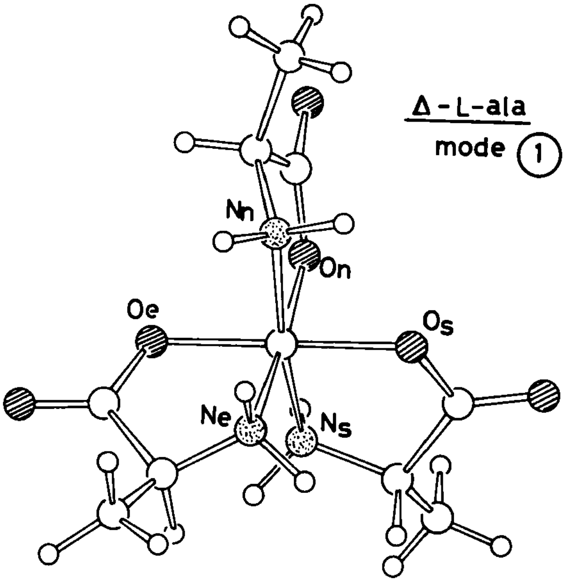

Fig. 4a. Structure of Δ-mer-[Co(L-ala)$_3$] viewed along the direction 1.

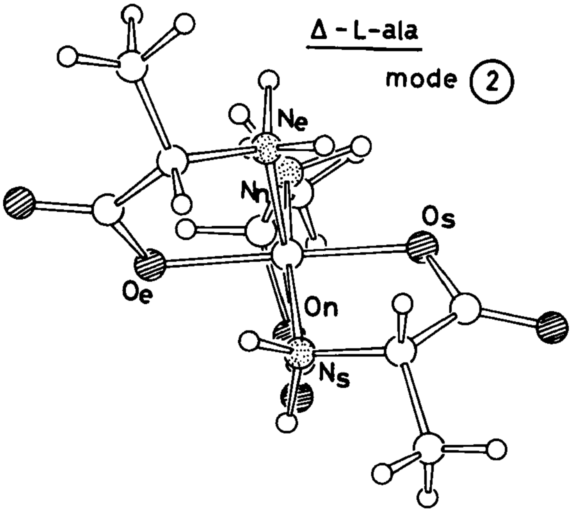

Fig. 4b. Structure of Δ-mer-[Co(L-ala)$_3$] viewed along the direction 2.

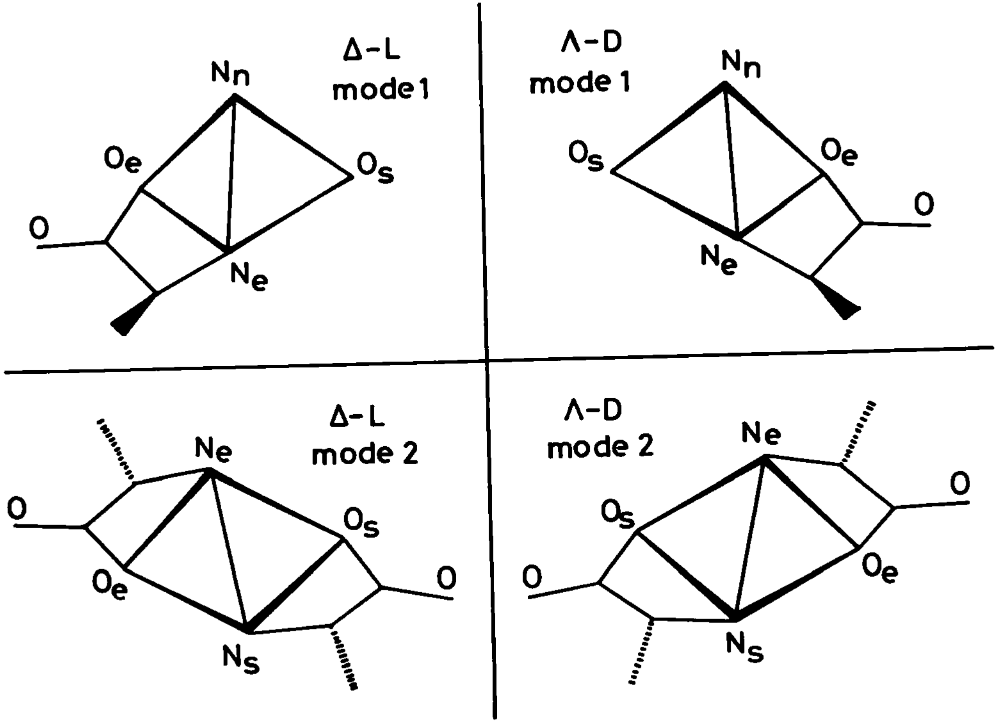

Fig. 5a. Schematic representation of the structure of Δ-mer-[Co(L-ala)$_3$] and its enantiomer viewed along the directions 1 and 2.

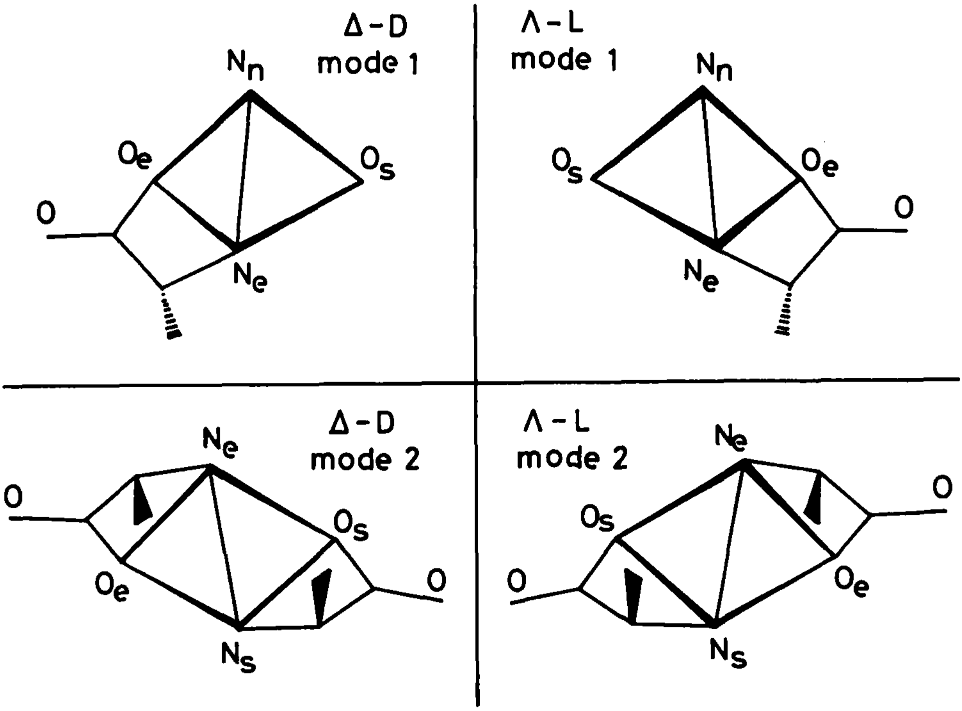

Fig. 5b. Schematic representation of the structure of Δ-mer-[Co(D-ala)$_3$] and its enantiomer viewed along the directions 1 and 2.

and Λ-L). The corresponding association modes in mer-[Co(β-ala)$_3$] are shown in Fig. 6.

As is clearly seen in Fig. 5a, the CH$_3$ group in Λ-D and Δ-L are projecting toward the approaching anion in mode 1 and projecting backward from the approaching anion in mode 2. Since the separation factor of this pair is 1.512 which is much larger than the separation factors 1.138 and 1.175 of the corresponding gly and β-ala complex pairs, the effect of the CH$_3$ group is evident. Therefore, it can be presumed that the association

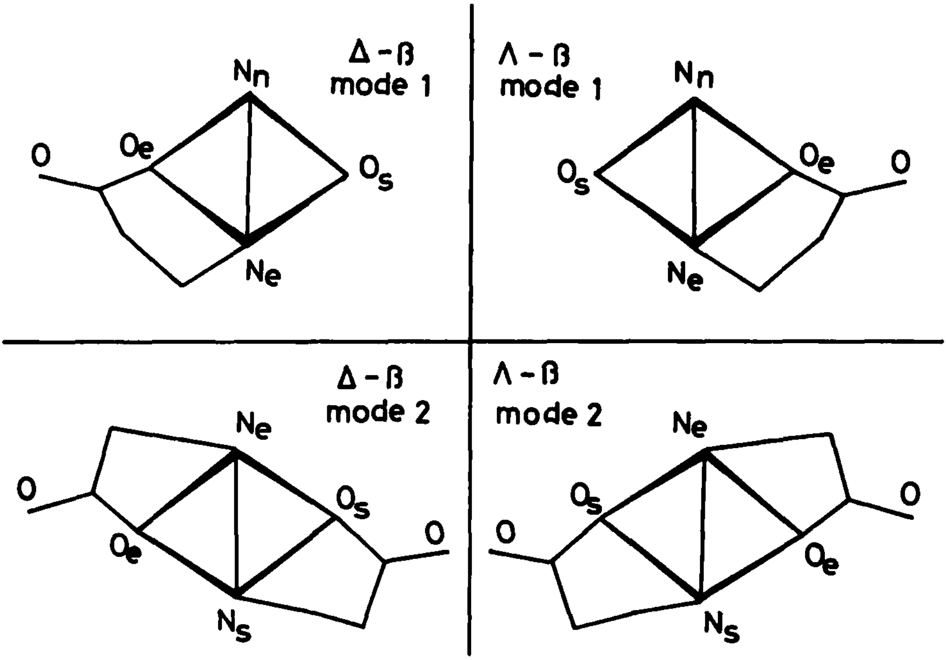

Fig. 6. Schematic representation of the structure of Δ-mer-[Co(β-ala)$_3$] and its enantiomer viewed along the directions 1 and 2.

mode 1 is predominant. If the association mode 2 were predominant, the CH_3 groups stretching backward from the associating $[Sb_2d\text{-}tart_2]^{2-}$ anion could have no significant effect on chiral discrimination, so that the separation factor should be quite close to those for mer-[Co(gly)$_3$] and mer-[Co(β-ala)$_3$].

On the contrary, the separation factor for the (Λ-L,Δ-D) pair in mer-[Co(α-ala)$_3$] is 1.089 which is fairly close to the separation factors 1.138 and 1.175 for the corresponding gly and β-ala complex pairs. Thus, it can be presumed that the association mode 1 is also predominant here, because the CH_3 group is projecting backward from the associating anion and has no significant effect on the association mode as shown in Fig 5b. If the association mode 2 were predominant, the separation factor would differ largely from those of the gly and β-ala complexes, because two CH_3 groups are projecting toward the approaching anion.

Thus, we can conclude that the association mode 1 is most effective in chiral discrimination in mer-$[Co(\alpha\text{-ala})_3]$ and $[Sb_2d\text{-tart}_2]^{2-}$system.

Chromatographic Behavior of cis-mer-[Co(edda)(AA)]

In the above reasoning, we can not decide which association mode, 1 or 2, is actually effective on chiral discrimination of mer-$[Co(gly)_3]$ and mer-$[Co(\beta\text{-ala})_3]$ because these complexes have no CH_3 group in their chelate rings. To decide this point, the chromatographic behaviors of two types of geometric isomers(sym- and unsym-) cis-mer-[Co(edda)(gly)] and cis-mer-[Co(edda)(β-ala)] have been investigated.(12) As shown in Fig. 7, these symmetric and unsymmetric isomers are presumed to accept the oncoming anion only through either one of the two association modes 1 and 2, because the ethylene bridge of the edda ligand blocks either one of the two edges Nn-Ne and Ne-Ns of the octahedron in Fig. 3.

The retention volumes of the enantiomers and their ratios (separation factors) are listed in Table 2. All first eluted complexes are estimated to have a Λ-configuration from these CD spectral patterns. There are, however, no difference in the retention volumes and in the separation factors practically between the symmetric and the unsymmetric complexes. Therefore, we can estimate that the two association modes 1 and 2 are equally effective on the chiral discrimination in mer-$[Co(gly)_3]$

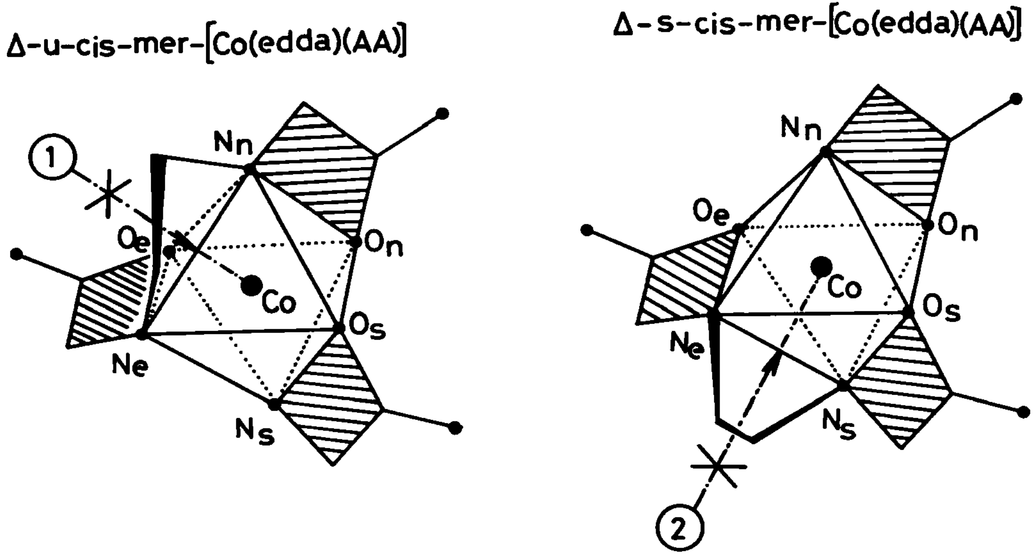

Fig. 7. Schematic representation of the structure of sym-cis-mer-[Co(edda)(AA)] and unsym-cis-mer-[Co(edda)(AA)].

TABLE 2

VALUES OF RETENTION VOLUMES AND SEPARATION FACTORS OF FOUR ENANTIOMERIC PAIRS OF MER-[Co(edda)(AA)]

Sample complex	First eluted (ml)[a]	Second eluted (ml)	Separation factor
s-cis-mer-[Co(edda)(gly)]	55.6(Λ)[b]	63.6(Δ)	1.14
u-cis-mer-[Co(edda)(gly)]	56.1(Λ)	64.6(Δ)	1.15
s-cis-mer-[Co(edda)(β-ala)]	47.9(Λ)	53.1(Δ)	1.13
u-cis-mer-[Co(edda)(β-ala)]	45.1(Λ)	53.1(Δ)	1.17

a) A column of ϕ = 1.0 cm was filled with a 82.5 cm length of QAE-Sephadex A-25 treated with 0.1M of $K_2[Sb_2d\text{-}tart_2]$. The elution was done with water at a flow-rate 7.8 x 10^{-2}ml/min.

b) Absolute configuration estimated from the CD pattern.(12)

(edda = ethylenediamine-N,N'-diacetato(2-) anion.)

and mer-[Co(β-ala)$_3$] with using $[Sb_2d\text{-}tart_2]^{2-}$ as a chiral selector.

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