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CHROMATOGRAPHIC STUDY OF OPTICAL RESOLUTION

VI. SEPARATION OF ISOMERS OF FACIAL TRIS(AMINOACIDATO) MIXED LIGAND CHELATES WITH *d*-TARTRATE AND ANTIMONY *d*-TARTRATE ADSORBED ON AN ANION-EXCHANGE RESIN

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SUMMARY

A systematic study was made of the chromatographic separation of enantiomers of a series of fac-[Co(β-ala)$_n$(α-ala)$_{3-n}$] through an anion-exchange column adsorbing chiral and achiral anions such as *d*-tartrate, antimony *d*-tartrate, chloride and sulphate. It was found that both the retention volume and the separation factor of the enantiomeric pair increase with increasing number of β-ala ligands when eluted through the *d*-tartrate form of the resin. On the other hand, when eluted through the antimony *d*-tartrate form of the resin, the trend of the retention volumes and the separation factors is the reverse of that for the *d*-tartrate form. These results are discussed in comparison with the results obtained for the corresponding series of fac-[Co(β-ala)$_n$(gly)$_{3-n}$].

INTRODUCTION

It is difficult to obtain the different enantiomers of non-charged complexes in an optically pure form. Chromatography may be one of the most effective means for this purpose, but it usually leads to a partial separation. In Part I[1] we showed that fac-[Co(β-ala)$_3$] is separated into its pure enantiomers through a CM-Sephadex column using a solution of sodium *d*-tartrate in ethanol–water as eluent. This success was followed by a study of the resolution mechanism in chromatography for a series of mixed aminoacidato chelates of the type fac-[Co(β-ala)$_n$(D/L-ser)$_{3-n}$] using an aqueous solution of Na_2(*d*-tart) and $Na_2[Sb_2$(*d*-tart)$_2]$ as eluent[2]. It was found that the separation factor of the enantiomeric pair of these complexes increases or decreases with increasing number of β-ala groups when they are eluted with an aqueous solution of Na_2(*d*-tart) or $Na_2[Sb_2$(*d*-tart)$_2]$, respectively. However, no regular increase or decrease in the retention volume could be found with variation in the number of β-ala groups.

This seems to be due to the effect of the substituent CH_2OH group of the

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chelating serinato ligand. The CH_2OH group may exert a steric repulsion effect upon the oncoming anion (resolving agent); however, at the same time it may provide an additional contact point for the oncoming anion to form the hydrogen bond and to strengthen the association with the complex. Which of these antagonistic effects predominates is a delicate problem and may be dependent on the structure of the complex. Thus, there is no monotonous increase or decrease in the retention volume from $n = 0$ to $n = 3$ in a series of fac-[Co(β-ala)$_n$(D/L-ser)$_{3-n}$]. A study of a series of mixed aminoacidato chelate complexes of α-ala which contain CH_3 instead of CH_2OH may simplify the separation mechanism, so that we could obtain a regular trend in the retention volume as well as the separation factor with variation of the number of β-ala groups.

In this study, the chromatographic run was carried out through a column packed with various anionic forms (Cl^-, SO_4^{2-}, *d*-tart^{2-} and [Sb_2(*d*-tart)$_2$]$^{2-}$) of an anion-exchange resin using pure water as the eluent. This type of chromatography may be more suitable for the separation of uncharged complexes than chromatography in which the resolving agent is contained in an eluent solution, because sample complexes are eluted through the high concentration of the resolving agent anion in the stationary phase. Usually we cannot have such a high concentration of a resolving agent in an aqueous solution.

EXPERIMENTAL

Preparation of complexes

There are several reports concerning the preparation of mixed aminoacidato cobalt(III) chelates using a tricarbonato complex as a starting material. In the preparation of fac-[Co(β-ala)$_n$(D/L-ser)$_{3-n}$] we adopted another method in which lead dioxide is used as an oxidizing agent, because this method proved to give better yields of facial isomers. In this study, we adopted the same method.

Λ-fac-[Co(β-ala)$_2$(L-ala)] and Δ-fac-[Co(β-ala)$_n$(L-ala)$_{3-n}$] (n = 2, 1, 0). A 6.7-g amount of β-alanine and 6.7 g of L-alanine were dissolved in a solution of 11.9 g of $CoCl_2 \cdot 6H_2O$ in 150 ml of water. The solution was oxidized with 15 g of lead dioxide with stirring at 80°C. After stirring for 30 min, 7 g of lead dioxide were added and the solution was kept at 80°C for a further 30 min to complete the oxidation. The reaction mixture was filtered to remove insoluble material and the filtrate was poured into a column packed with the Na^+ form of SP-Sephadex C-25 (40 × 3 cm I.D.). The adsorbed complexes were eluted with water. Three bands were separated: a violet band (meridional isomers) eluted first, a red-violet band (facial isomers) was eluted second, and another red-violet band (cationic complexes) remained at the top of the column. The eluate containing facial complexes was concentrated in a vacuum evaporator and loaded again on a column (62 × 2 cm I.D.) packed with the SO_4^{2-} form of QAE-Sephadex A-25. The elution was carried out slowly with pure water. Four bands were separated, as shown in Fig. 1. The eluate of each of these four bands was concentrated in a vacuum evaporator, and in each instance the red-violet powder was obtained on addition of ethanol. The identification of these complexes was made on the basis of the electronic absorption (AB) and circular dichroism (CD) spectra.

Δ-fac-[Co(β-ala)$_2$(D-ala)] and Λ-fac-[Co(β-ala)$_n$(D-ala)$_{3-n}$] (n = 2, 1, 0). These complexes are the enantiomers of the above-mentioned complexes containing L-ala and were prepared from β-ala, D-ala and $CoCl_2 \cdot 6H_2O$ in the same way.

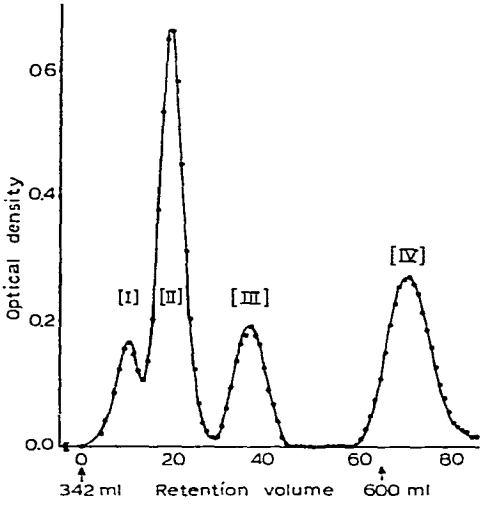

Fig. 1. Elution curve for the mixture of facial complexes $[Co(\beta\text{-ala})_n(\text{L-ala})_{3-n}]$ (n = 0–2): (I) Δ-[Co(L-ala)$_3$]; (II) Δ-[Co(β-ala)(L-ala)$_2$]; (III) Δ-[Co(β-ala)$_2$(L-ala)]; (IV) Λ-[Co(β-ala)$_2$(L-ala)].

Δ- and Λ-fac-[Co(β-ala)$_n$(gly)$_{3-n}$] (n = 2, 1, 0). The method of preparation was the same as above. However, for the separation of the enantiomers, the $[Sb_2(d\text{-tart})_2]^{2-}$ form of the resin was used instead of the SO_4^{2-} form.

Δ- and Λ-fac-[Co(β-ala)$_3$]. The separation was performed using the $[Sb_2(d\text{-tart})_2]^{2-}$ form of the resin.

Spectral measurements

The absorption (AB) and circular dichroism (CD) spectra of the complexes were recorded on a Shimadzu UV-200 spectrophotometer and a JASCO J-40CS spectropolarimeter, respectively.

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-pen strip-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. 4 mm packed with the Cl^-, SO_4^{2-}, d-tart^{2-} and $[Sb_2(d\text{-tart})_2]^{2-}$ forms of TSK-220 anion exchanger.

A saturated solution of each complex was used as a sample solution. The sample solution (*ca.* 5 μl) was injected into the column through a septum using a 50-μl pressure syringe (if the solubility of the complex was low, *ca.* 20 μl were injected). Water was used as the eluent. The flow-rate was set at 0.10 ml/min.

RESULTS AND DISCUSSION

Identification of complexes

Fig. 2 shows the AB spectra of the four complexes which were separated through a chromatographic column as shown in Fig. 1. To facilitate the identification of these complexes, the spectrum of fac-[Co(β-ala)$_3$] is also given. Complex I (the first eluate) is identified as fac-[Co(L-ala)$_3$] because the two maximum positions of the absorption bands and the spectral pattern as a whole are identical with those of fac-[Co(L-ala)$_3$]. Complexes II, III and IV are neither fac-[Co(β-ala)$_3$] nor fac-[Co(L-ala)$_3$]. Their spectral curves can be placed between those of fac-[Co(β-ala)$_3$] and fac-[Co(L-ala)$_3$] and hence they must be mixed ligand chelates of α- and β-alanines.

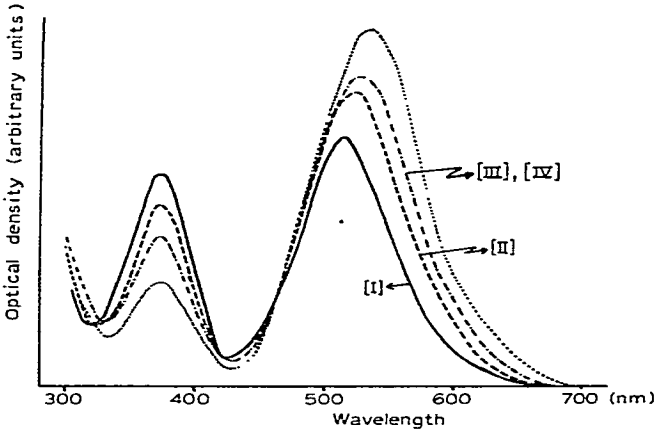

Fig. 2. AB spectra of a series of fac-[Co(β-ala)$_n$(L-ala)$_{3-n}$]: Δ-[Co(L-ala)$_3$] (I); Δ-[Co(β-ala)(L-ala)$_2$] (II); Δ- and Λ-[Co(β-ala)(L-ala)$_2$] (III) and (IV); Δ-[Co(β-ala)$_3$] (···).

Complexes III and IV must be diastereomeric to each other, because their AB spectra are similar, but their CD spectra have opposite signs, as shown in Fig. 3. Complexes III and IV must be fac-[Co(β-ala)$_2$(L-ala)], because the AB curve of complexes III and IV comes near to that of fac-[Co(β-ala)$_3$]. On the other hand, the AB curve of complex II comes near to that of fac-[Co(L-ala)$_3$]. Thus, complex II can be identified as fac-[Co(β-ala)(L-ala)$_2$].

With these assignments, we have a regular stepwise variation of the spectral pattern of the four complexes with variation of the number of β-ala groups from n =

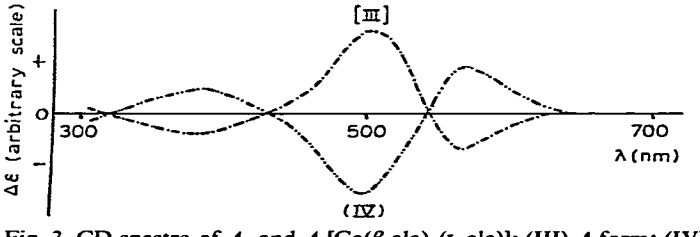

Fig. 3. CD spectra of Λ- and Δ-[Co(β-ala)$_2$(L-ala)]: (III) Δ-form; (IV) Λ-form.

0 to $n = 3$. That is, the ratio of the absorption coefficients of the first and second bands at their maximum positions show a stepwise decrease with increasing number of β-ala groups (six-membered chelate rings). With these assignments, the CD spectrum pattern also shows a stepwise variation with variation of the number of β-ala groups as shown in Fig. 4. That is, the major peak in fac-[Co(L-ala)$_3$] appearing at 530 nm decreases with decreasing number of L-ala groups, and a peak of the opposite sign appears at around 500 nm and increases with increasing number of β-ala groups. The trend is the same as that reported for the fac-[Co(β-ala)$_n$(L-ser)$_{3-n}$] series in Part III[2]. Thus, everything is consistent, which means that these assignments are valid. These trends observed in the AB and CD spectra are presumed to be common for a series of mixed aminoacidato chelates containing five- and six-membered chelate (planar and puckered) rings.

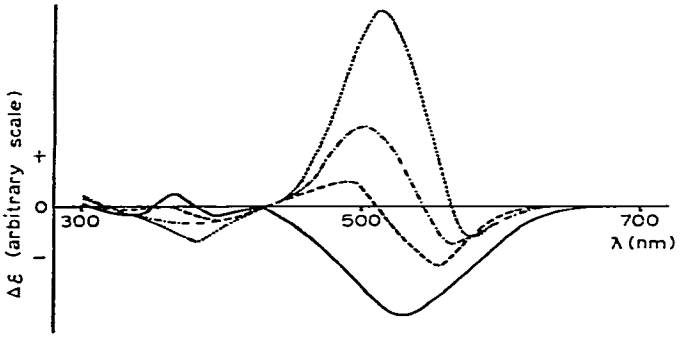

Fig. 4. CD spectra of a series of Δ-fac-[Co(β-ala)$_n$(L-ala)$_{3-n}$] (n = 0–3): Δ-[Co(L-ala)$_3$] (——); Δ-[Co(β-ala)(L-ala)$_2$] (– – –); Δ-[Co(β-ala)$_2$(L-ala)] (– · – · –); Δ-[Co(β-ala)$_3$] (· · ·).

Chromatographic behaviour

As α-ala exists in two forms, L and D, there are two pairs of enantiomers (Δ-L), (Λ-D) and (Δ-D), (Λ-L) for each "n" in the tris-type mixed aminoacidato chelates, fac-[Co(β-ala)$_n$(α-ala)$_{3-n}$]. Therefore, when we consider the trends of the retention volume and of the separation factor for the enantiomeric pair in a series of fac-[Co(β-ala)$_n$(α-ala)$_{3-n}$] from $n = 0$ to $n = 3$, we must work with the two series separately.

However, the enantiomeric pair Λ-fac-[Co(L-ala)$_3$] and Δ-fac-[Co(D-ala)$_3$] is too insoluble to be used for chromatographic experiments. The enantiomeric pair Λ-fac-[Co(β-ala)(L-ala)$_2$] and Δ-fac-[Co(β-ala)(D-ala)$_2$] could not be found in a separation column, probably because it remained in the insoluble material in the reaction mixture. Only the enantiomeric pair Λ-fac-[Co(β-ala)$_2$(L-ala)] and Δ-fac-[Co(β-ala)$_2$(D-ala)] was obtained as a member of this series and could be used for a chromatographic run.

Other series of enantiomeric pairs are all fairly soluble in water, so that we could obtain a complete series of their retention volumes and separation factors. Fig. 5 is a schematic representation of the trend of the retention volume of the latter series of complexes when eluted through the Cl^-, SO_4^{2-} and d-tart^{2-} forms of the resin. Whereas the enantiomers show the same retention volumes when eluted through the achiral anionic form of the resin (Cl^- and SO_4^{2-}), they show different retention volumes when eluted through the chiral anionic form of the resin (d-tart^{2-}).

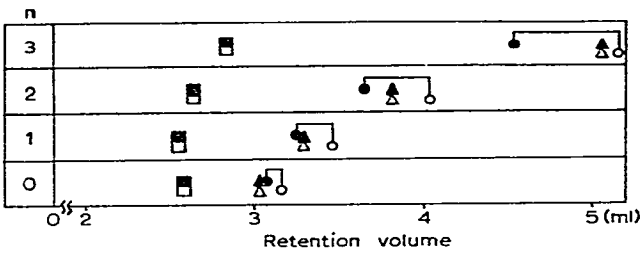

Fig. 5. Retention volumes of Δ-[Co(β-ala)$_n$(L-ala)$_{3-n}$] and Λ-[Co(β-ala)$_n$(D-ala)$_{3-n}$] (n = 0–3): ■ and □, elution through the Cl^- form of the resin; ▲ and △, elution through the SO_4^{2-} form of the resin; ● and ○, elution through the $tart^{2-}$ form of the resin (closed and open symbols correspond to the Δ- and Λ-enantiomers, respectively).

As shown in Fig. 5, the retention volume varies only slightly with variation of "n" when eluted through the Cl^- form of the resin. In contrast, when eluted through the SO_4^{2-} and d-$tart^{2-}$ forms of the resin, the retention volume decreases with decreasing "n". This suggests that a similar type of association takes place for SO_4^{2-} and d-$tart^{2-}$ (see Fig. 6). The amino group of the chelated β-ala in fac-[Co(β-ala)$_3$] has axial and equatorial N–H bonds just like that of the chelated en in $[Co(en)_3]^{3+}$.

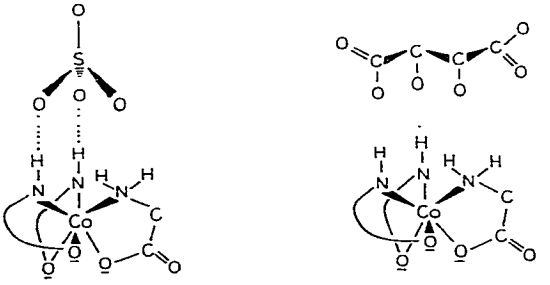

Fig. 6. Association modes of sulphate and d-tartrate ions along the pseudo C_3 axis of fac-[Co(β-ala)$_2$(α-ala)].

Three such axial N–H bonds in fac-[Co(β-ala)$_3$] are presumed to be used for hydrogen bonding to three oxygen atoms of SO_4^{2-} or d-$tart^{2-}$ anions coming in the direction of the three-fold axis of the complex, and a fairly stable association takes place. However, the situation will be changed when β-ala is replaced by α-aminoacidato ligands such as D- or L-ala or -gly, which form a five-membered chelate ring. As such a five-membered chelate ring is almost planar, the two N–H bonds of the amino group are stretched upwards and downwards from the plane at equal angles so that there are neither axial nor equatorial N–H bonds. Thus, substitution of α-ala or gly for β-ala leads to a decrease in the number of axial N–H bonds, which makes the association of SO_4^{2-} or d-$tart^{2-}$ with the complex less favourable. The stepwise decrease in the retention volume with the decreasing number of β-ala in fac-[Co(β-ala)$_n$(α-AA)$_{3-n}$] can thus be explained.

As the monovalent anion Cl^- has little tendency toward the hydrogen-bond-assisted association, the degree of association may not be varied with variation of the

number of β-ala groups in fac-[Co(β-ala)$_n$(α-AA)$_{3-n}$] so that the retention volume is kept almost constant when the complexes are eluted through the Cl^- form of the resin.

As for the optical resolution by *d*-tart^{2-}, the mechanism of discrimination should be the same with that in the case of $[Co(en)_3]^{3+}$. Therefore, when eluted through the *d*-tart^{2-} form of the resin, the *Λ* enantiomer is retained more strongly than the *Δ* enantiomer. The stepwise decrease in the separation factor with decreasing number of β-ala groups can also be understood when we consider that the discrimination between *Λ* and *Δ* is effective only through the association of *d*-tart^{2-} with the complex.

In order to elucidate the effect of the substituent CH_3 group of α-ala on the chromatographic behaviour, the retention volumes of the enantiomeric pairs of three bis-β-alaninato complexes, (A) *Λ*- and *Δ*-fac-[Co(β-ala)$_2$(gly)], (B) *Λ*-fac-[Co(β-ala)$_2$(L-ala)] and *Δ*-fac-[Co(β-ala)$_2$(D-ala)] and (C) *Λ*-fac-[Co(β-ala)$_2$(D-ala)] and *Δ*-fac-[Co(β-ala)$_2$(L-ala)], were compared (Fig. 7). The retention volumes of the two kinds of enantiomeric pairs of α-ala complexes, (B) and (C), are smaller than that of the gly complex, (A), which can be explained as a result of the steric hindrance of the CH_3 group in (B) and (C). That is, the CH_3 group in (B) and (C) prevents stronger association with SO_4^{2-} and *d*-tart^{2-} on the resin. The difference in the retention volumes for (B) and (C) can be easily understood by comparing the molecular models of these complexes. Viewed in the direction perpendicular to the quasi-three-fold axis of the complex, the CH_3 group in (B) is stretched equatorially, while that in (C) is stretched toward the anion (see Fig. 8). Thus, SO_4^{2-} which is coming in the direction of the quasi-three-fold axis of the complex will experience stronger steric repulsion by the CH_3 group in (C) than in (B). The smaller retention volume for (C) than that for (B) can thus be understood.

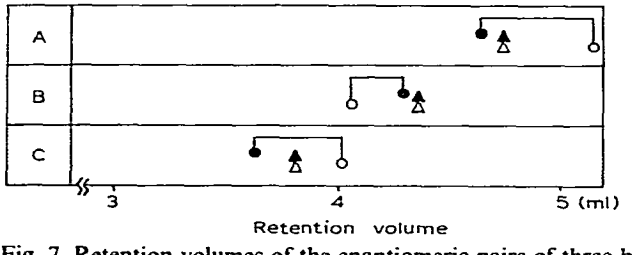

Fig. 7. Retention volumes of the enantiomeric pairs of three bis-β-alaninato complexes eluted through a column containing sulphate and *d*-tartrate: A, *Λ*- and *Δ*-[Co(β-ala)$_2$(gly)]; B, *Λ*-[Co(β-ala)$_2$(L-ala)] and *Δ*-[Co(β-ala)$_2$(D-ala)]; C, *Λ*-[Co(β-ala)$_2$(D-ala)] and *Δ*-[Co(β-ala)$_2$(L-ala)]. Closed and open symbols correspond to the *Δ* and *Λ* enantiomers, respectively.

As *d*-tart^{2-} associates with the complex in a similar manner to SO_4^{2-}, the trends in the retention volume from (A) to (C) should be similar to each other for both anions. It should be noted for (B) that the *Λ* form is eluted faster than the *Δ* form, contrary to expectation. We assume that the retention volume for the *Λ* form of (B) is decreased abnormally by some unknown effect of the CH_3 group.

Fig. 9 shows the retention volume trend for two series of enantiomeric pairs of fac-[Co(β-ala)$_n$(gly)$_{3-n}$] and fac-[Co(β-ala)$_n$(α-ala)$_{3-n}$] when eluted through the [Sb$_2$(*d*-tart)$_2$]$^{2-}$ form of the resin. For a series of enantiomeric pairs of glycinato complexes,

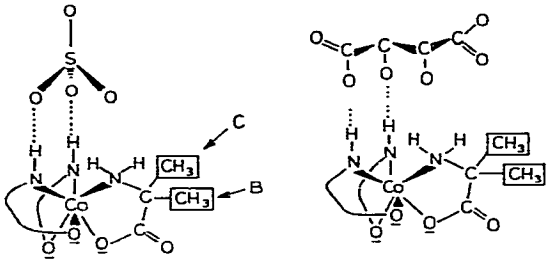

Fig. 8. Steric repulsion by the methyl group towards the association anion.

the retention volume increases with decrease in the number of gly ligands (five-membered chelate rings). The separation of the two elution peaks for the enantiomers also shows the same trend, that is, both the retention volume and the separation of the two elution peaks increase with the increasing number of five-membered chelate rings. In contrast, for a series of α-alaninato complexes, the retention volume decreases with increasing number of α-ala groups (five-membered chelate rings), but the separation of the two elution peaks increases. This means that the CH_3 group behaves so as to prevent the association with the anion and at the same time helps to make the discrimination between Δ and Λ more effective.

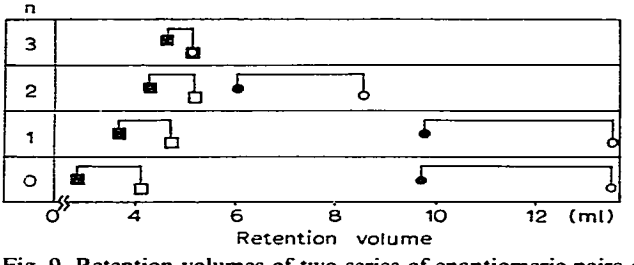

Fig. 9. Retention volumes of two series of enantiomeric pairs of fac-[Co(β-ala)$_n$(gly)$_{3-n}$] (● and ○) and fac-[Co(β-ala)$_n$(α-ala)$_{3-n}$] (■ and □) eluted through a column containing antimony *d*-tartrate. Closed and open symbols correspond to the Δ and Λ enantiomers, respectively.

As pointed out in Part II[3], the tris-type chelate exists in two enantiomeric forms, Λ and Δ, according to the mode of chelation (see Fig. 10). The mode of chelation of the Λ form produces L-shaped channels, and the mode of chelation of the Δ form produces J-shaped channels. A chiral anion which fits the L-shaped channel

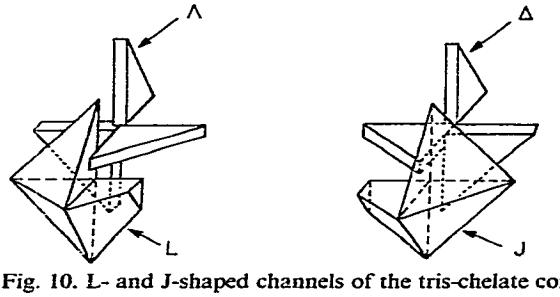

Fig. 10. L- and J-shaped channels of the tris-chelate complex.

does not fit the J-shaped channel of the *Δ*-enantiomer well. Such a chiral anion associates more firmly with the *Λ*- than the *Δ*-enantiomer, so that it can be used as a resolving agent. We assume that the $[Sb_2(d\text{-tart})_2]^{2-}$ anion has a special skeleton which fits an L-shaped channel. If this association mode is valid in the present case also, the *Λ*-enantiomer, having L-shaped channels, should associate more favourably with $[Sb_2(d\text{-tart})_2]^{2-}$ than the *Δ*-enantiomer, so that the *Λ*-enantiomer should be retained more firmly than the *Δ*-enantiomer in the resin phase containing $[Sb_2(d\text{-tart})_2]^{2-}$. With this idea concerning the discrimination mechanism of the tris-type chelate, the above-mentioned trends in the retention volume and in the peak separation for a series of fac-$[Co(\beta\text{-ala})_n(\alpha\text{-ala})_{3-n}]$ (n = 3–0) can be understood as a result of narrowing of the space of the L-shaped channel by the methyl group: the methyl group of α-ala in the complex narrows the space of the L-shaped channel so that the association with $[Sb_2(d\text{-tart})_2]^{2-}$ becomes more difficult with increasing number of α-ala groups in the complex, and at the same time the discrimination of the *Λ*- from the *Δ*-form becomes more effective for such narrowed L-shaped channels.

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