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CHROMATOGRAPHIC STUDY OF OPTICAL RESOLUTION

III*. SEPARATION OF ISOMERS OF FACIAL TRIS(AMINOACIDATO)-COBALT(III) COMPLEXES WITH *d*-TARTRATE AND ANTIMONY *d*-TARTRATE SOLUTIONS

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SUMMARY

A systematic study was made of the chromatographic separation of a series of diastereomeric and enantiomeric pairs of facial tris(aminoacidato)cobalt(III) chelates of formula $[Co(\text{L- or D-ser})_{3-n}(\beta\text{-ala})_n]$ and $[Co(gly)_{3-n}(\beta\text{-ala})_n]$ (ser = serinato anion, β-ala = β-alaninato anion and gly = glycinato anion) using aqueous solutions of sodium *d*-tartrate and sodium antimony *d*-tartrate (abbreviated to Na_2*d*-tart and $Na_2[Sb_2(d\text{-tart})_2]$, respectively) as eluents. From the data, the separation factors of enatiomeric pairs were calculated for Δ-[C(DL-ser)$_3$] and Λ-[Co(D-ser)$_3$], Δ-[Co(D-ser)$_3$] and Λ-[Co(L-ser)$_3$], Δ-[Co(L-ser)$_2$(β-ala)] and Λ-[Co(D-ser)$_2$(β-ala)], etc. As a result, it was found that the separation factors of these enantiomeric pairs increase with increasing number of β-ala$^-$ ligands when they are eluted with aqueous Na_2*d*-tart solution. In contrast to this trend, the separation factors decrease with increase in the number of β-ala$^-$ ligands when they are eluted with $Na_2[Sb_2(d\text{-tart})_2]$ solution. These trends are discussed in relation to stereochemical association models.

INTRODUCTION

It is difficult to obtain each of the enantiomers of non-charged chiral complexes in an optically pure form. Chromatographic separation by the use of asymmetric adsorption has been reported as the only practical means for this purpose. However, most of such chromatographic separations lead only to partial separations, and only one example of complete resolution has been reported, in which facial $[Co(\beta\text{-ala})_3]$ was completely separated into enantiomers using a CM-Sephadex column with an ethanol–water solution of sodium *d*-tartrate (Na_2*d*-tart) as eluent.[1] This success was expected as the complex showed a similar NMR signal of the NH_2 groups to that of $[Co(en)_3]^{3+}$, which was known to be completely separated into enantiomers with

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aqueous Na_2*d*-tart solution as eluent[2]. Thus, the mode of association of *d*-tart^{2-} with fac-[Co(β-ala)$_3$] was considered to be similar to that with [Co(en)$_3$]$^{3+}$.

For the mode of association between *d*-tart^{2-} and [Co(en)$_3$]$^{3+}$, the face-to-face close contact structure of these two ions was established[3-5]. In such an association model, four carbon atoms in *d*-tart^{2-} make a plane perpendicular to the three-fold axis of the complex, and from this plane, four carbon atoms stretch towards the complex and three of them form hydrogen bonds with the three axial N–H groups of the complex. A similar association model can be imagined for fac-[Co[β-ala)$_3$] because this complex has also a facet made of three NH_2 groups. To confirm this association model and to obtain a more detailed idea of optical resolution, a series of complexes of formula fac-[Co(α-aminoacidato)$_{3-n}$(β-ala)$_n$] were prepared, and their chromatographic behaviours were studied using aqueous solutions of Na_2*d*-tart and sodium antimony *d*-tartrate {Na_2[Sb$_2$(*d*-tart)$_2$]} as eluents.

EXPERIMENTAL

Preparation of complexes

fac-[Co(L-ser)$_3$] [mixture of Λ(L) and Δ(L)]. One gram of L-serine and 0.8 g of $CoCl_2 \cdot 6H_2O$ were dissolved in 30 ml of water. The solution was oxidized with 7 g of lead(IV) oxide with stirring at 70°. After stirring for 1 h, a further 7 g of lead(IV) oxide were added, and the solution was kept at 70° for 0.5 h to complete the oxidation. The solution was filtered to remove insoluble material and the filtrate was poured into a column packed with SP-Sephadex C-25(H^+) (50 × 6 cm I.D.). The complexes produced made a band at the top of the column which was eluted with water. The absorbed band separated into four bands. A small amount of anionic complex came out first, followed by a violet band and a red-violet band, and a red band of cationic complexes remained at the top of the column. From the visible absorption spectra and the circular dichroism (CD) spectra of the second and third bands, it was confirmed that the second violet band contains mer-[Co(L-ser)$_3$] and the third red-violet band contains fac-[Co(L-ser)$_3$]. The eluate containing fac-[Co(L-ser)$_3$] was concentrated in a vacuum evaporator and several times its volume of ethanol was added. A red-violet powder was deposited, which was filtered, washed with ethanol–diethyl ether and dried in air.

fac-[Co(L-ser)$_2$(β-ala)] and fac-[Co(L-ser) (β-ala)$_2$] [both are mixtures of Λ(L) and Δ(L)]. L-Serine (1.5 g) and β-alanine (1.4 g) were dissolved in a solution of 2.4 g of $CoCl_2 \cdot 6H_2O$ in 100 ml of water. Oxidation with lead(IV) oxide and chromatographic separation were carried out as in the first procedure. The reaction products were developed into six bands, as shown in Fig. 1. They consisted of a thin band of a small amount of anionic complexes, a very broad violet band of meridional complexes, three red violet bands of facial complexes and a red band of cationic complexes. The visible absorption spectra and the CD spectra together with elemental analyses (Table I) revealed that the complexes in the three red-violet bands are fac-[Co(L-ser)$_2$ (β-ala)], fac-[Co(L-ser) (β-ala)$_2$] and fac-[Co(β-ala)$_3$]. The eluate of each of these three bands was concentrated in a vacuum evaporator, and in each instance a red-violet powder was obtained on addition of ethanol.

Facial isomers containing D-serine or glycine with β-alanine were also prepared in the same way.

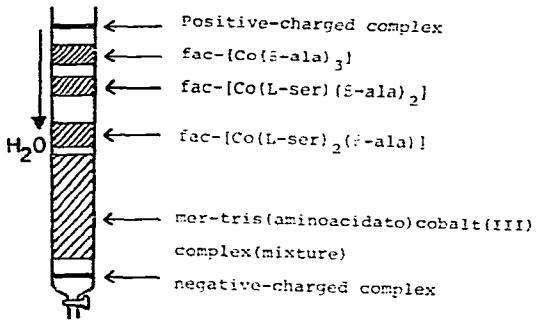

Fig. 1. Preparative elution on an SP-Sephadex column.

TABLE I

ELEMENTAL ANALYSES OF COMPLEXES

Complex	*C (%)*		*H (%)*		*N (%)*	
	Found	*Calculated*	*Found*	*Calculated*	*Found*	*Calculated*
fac-[Co(L-ser)$_3$]	28.65	29.12	4.99	4.85	10.35	11.32
fac-[Co(L-ser)$_2$(β-ala)]	28.82	30.43	5.03	5.07	11.29	11.83
fac-[Co(L-ser) (βala)$_2$]	29.93	31.87	5.51	5.31	11.54	12.39
fac-[Co(β-ala)$_3$]	29.59	33.45	5.82	5.57	11.45	13.01
fac-[Co(gly) (β-ala)$_2$]	30.80	31.07	5.32	5.23	13.65	13.59
fac-[Co(gly)$_2$(β-ala)]	26.02	28.48	4.64	4.79	13.39	14.24

Absorption and circular dichroism spectral measurements

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

Retention volume measurement

Retention volumes were measured on a laboratory-built chromatographic unit which consisted of a Jasco LCP-pump, PM-150 pressure gauge, PC-150 pump controller, injector, column and Shimadzu UV-140 double-beam spectrophotometer.

The column was a 25-cm precision stainless-steel tube of 4 mm I.D. packed with TSK 211 strongly acidic cation exchanger (Toyo Soda, Tokyo, Japan) [for collecting the eluate for the CD measurement, a larger column size (50 × 0.8 cm I.D.) was used]. A dual-pen strip-chart recorder was used. The detector was operated at 525 nm in each run. Blue Dextran 2000 was used as a marker for measuring the void volume of the column. Sample solutions were prepared by dissolving 10 mg of the complex and a small amount of Blue Dextran 2000 in a few millilitres of the eluent. The sample solution (10 μl) was injected into the column through a septum using a 100-μl pressure syringe. The elution was performed with aqueous 0.05 *M* Na_2SO_4, 0.1 *M* Na_2*d*-tart and 0.1 *M* $Na_2[Sb_2(d\text{-tart})_2]$ solutions. The flow-rate was set at 0.2 ml/min with Na_2SO_4 solution and at 0.1 ml/min for the other two solutions.

RESULTS AND DISCUSSION

Identification of each of the separated complexes

Facial complexes separated on an SP-Sephadex column, [Co(L-ser)$_3$], [Co(L-

ser)$_2$(β-ala)] and [Co(L-ser)(β-ala)$_2$], contain the chiral L-ser$^-$ ligand and are presumed to be a mixture of diastereomers, Δ(L) and Λ(L) [Δ(L) = the complex with Δ configuration containing the L-aminoacidato anion]. The separation of diastereomers is, in general, not as difficult as that of enantiomers. In the present instance, each of these facial complexes was chromatographed through the TSK 211 column using 0.05 *M* Na_2SO_4 solution as eluent. As expected, each elution curve consists of two peaks, a large first peak and a much smaller second peak, which indicates that the complex is in fact a mixture of Δ(L) and Λ(L) diastereomers (Fig. 2). By repeating the elution procedure several times, fractions of the first and the second peaks were collected and used for CD measurements. The problem was whether the isomer eluted first had the Δ or Λ configuration. To determine this, we began with fac-[Co(L-ser)$_3$].

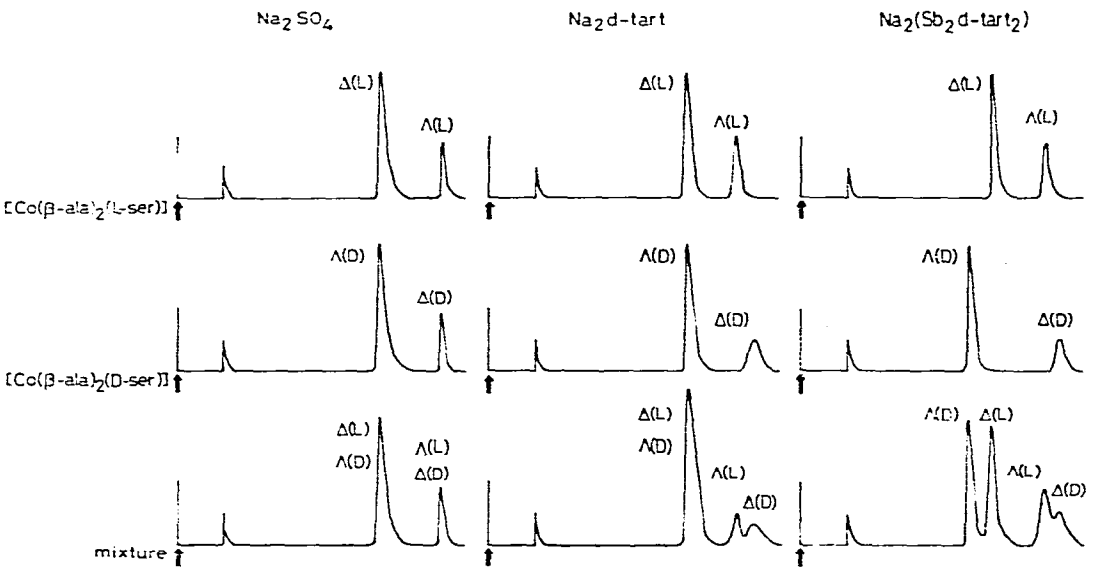

Fig. 2. Elution curves of fac-[Co(L-ser)$_2$(β-ala)] and fac-[Co(D-ser)$_2$(β-ala)].

As the CD spectrum(Fig. 3, Ia) of the peak eluted first with fac-[Co(L-ser)$_3$] resembles that of Δ-fac-[Co(L-ala)$_3$], whose absolute configuration has been established[6], the isomer eluted first is concluded to have the Δ configuration. Therefore, the configuration of the isomer eluted second should be Λ (Fig. 3, IIa). With fac-[Co(L-ser)$_2$(β-ala)], the CD spectrum pattern of the isomer eluted first (Fig. 3, Ib) is very similar to that of the isomer eluted first with fac-[Co(L-ser)$_3$] in that the major CD band lies in the longer wavelength region (about 525 nm). Therefore, the isomer eluted first with fac-[Co(L-ser)$_2$(β-ala)] is also assigned the Δ configuration. It is worth noting that a new minor CD component of opposite sign to the major CD band appears at about 500 nm, which may be attributed to the effect of replacement of L-ser$^-$ with β-ala$^-$. This effect should be more marked with fac-[Co(L-ser)(β-ala)$_2$]. In fact, in the CD spectrum (Fig. 3, Ic) of the isomer eluted first with the third complex, the component in the longer wavelength region diminishes and the component at about 500 nm is enhanced. As the isomer eluted first is a major component of the third complex mixture {Λ- and Δ-[Co(L-ser)(β-ala)$_2$]} as it is of the first {Λ- and Δ-[Co(L-ser)$_3$]}

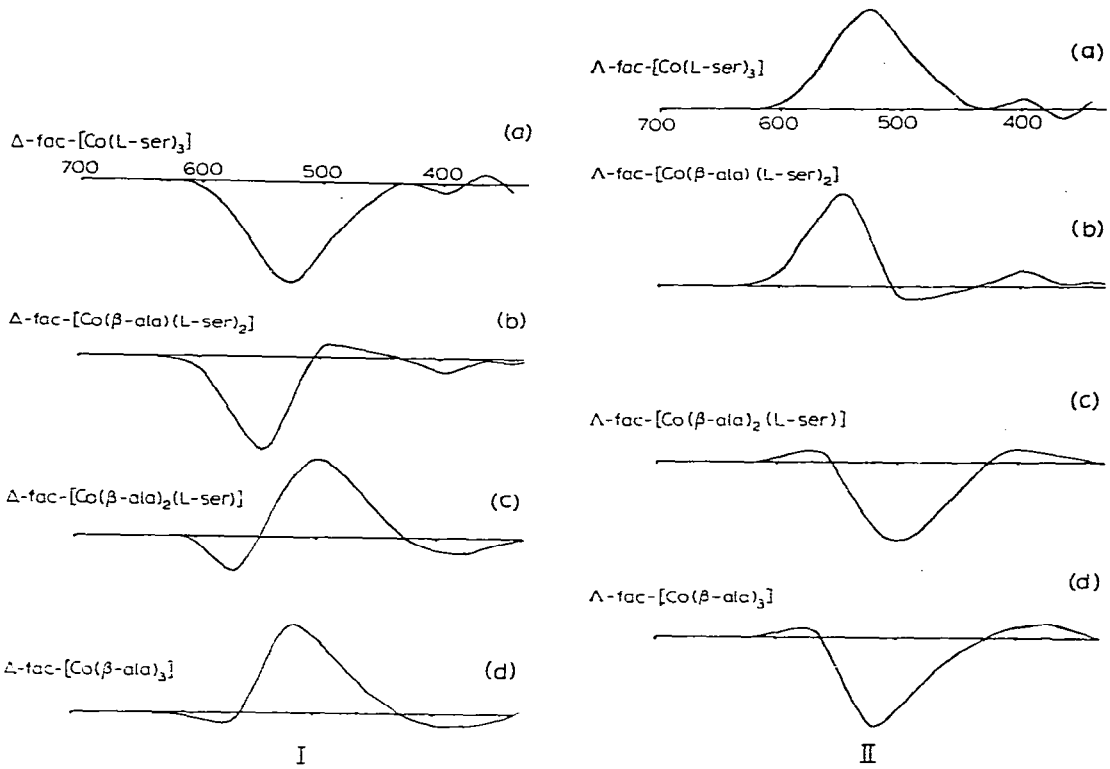

Fig. 3. CD spectra of Δ-fac-[Co(L-ser)$_{3-n}$(β-ala)$_n$] (I) and Λ-fac-[Co(L-ser)$_{3-n}$(β-ala)$_n$] (II).

and the second complex {Λ- and Δ-[Co(L-ser)$_2$ (β-ala)]} mixtures, the isomer eluted first with the third complex must also have the Δ- configuration.

As fac-[Co(β-ala)$_3$] was not separated into two peaks with 0.05 *M* Na_2SO_4 solution, 0.1 *M* Na_2*d*-tart solution was used as the eluent and optical resolution was achieved. Fig. 3, Id, shows the CD spectrum of the isomer eluted second. The CD pattern is similar to that of the isomer eluted first with fac-[Co(L-ser) (β-ala)$_2$]. Therefore, it can be concluded that the isomer eluted first with [Co(β-ala)$_3$] has the Λ configuration and that the isomer eluted second has the Δ configuration. This assignment coincides with the assignment made by analogy with Λ-[Co(en)$_3$]$^{3+}$-*d*-tart^{2-} (see Part I of this series[2]). The CD spectra of the isomers eluted second with three complexes (Fig. 3, II), [Co(L-ser)$_{3-n}$(β-ala)$_n$] (n = 0, 1 and 2), are not identical with but are similar to those of the corresponding isomers eluted first, except that the CD sign is reversed. From these CD spectra, the CD spectra of Λ- and Δ-fac-[Co(D-ser)$_{3-n}$ β-ala)$_n$] were estimated, as they could not be measured directly because only small amounts of D-serine complexes were available. When eluted with 0.05 *M* Na_2SO_4 solution, the elution curve of fac-[Co(D-ser)$_{3-n}$(β-ala)$_n$] is the same as that of fac-[Co(L-ser)$_{3-n}$(β-ala)$_n$] (see Fig. 2). Therefore, the configuration of the isomer eluted first in the D-serine complex must be Λ. Thus, we have obtained two series of retention volumes of diastereomeric pairs, Δ- (major product) and Λ-fac-[Co(L-ser)$_{3-n}$(β-ala)$_n$] and Λ- (major product) and Δ-fac-[Co(D-ser)$_{3-n}$(β-ala)$_n$]. These retention volumes were rearranged to make the enantiomeric pairs Δ-fac-[Co(L-ser)$_{3-n}$(β-ala)$_n$] and Λ-

TABLE II

ADJUSTED RETENTION VOLUMES AND SEPARATION FACTORS OBTAINED ON ELUTION WITH 0.05 *M* Na_2SO_4 SOLUTION

Complex	*Retention volume (ml)*	*Separation factor*
fac-Λ-[Co(D-ser)$_3$]	3.25	1.003
fac-Δ-[Co(L-ser)$_3$]	3.24	
fac-Δ-[Co(D-ser)$_3$]	4.20	1.002
fac-Λ-[Co(L-ser)$_3$]	4.19	
fac-Δ-[Co(D-ser)$_2$(β-ala)]	4.05	1.000
fac-Λ-[Co(L-ser)$_2$(β-ala)]	4.05	
fac-Δ-[Co(D-ser)$_2$(β-ala)]	5.43	1.004
fac-Λ-[Co(L-ser)$_2$(β-ala)]	5.41	
fac-Δ-[Co(D-ser) (β-ala)$_2$]	4.52	1.002
fac-Λ-[Co(L-ser) (β-ala)$_2$]	4.51	
fac-Δ-[Co(D-ser) (β-ala)$_2$]	6.30	1.006
fac-Λ-[Co(L-ser) (β-ala)$_2$]	6.20	
fac-Δ,Λ-[Co(β-ala)$_3$]	4.74	—

fac-[Co(D-ser)$_{3-n}$(β-ala)$_n$]. When 0.05 *M* Na_2SO_4 solution was used, the ratio of the retention volumes of each enantiomeric pair was always close to unity, as shown in Table II, indicating that they are in fact enantiomeric pairs.

As stated before, the CD spectrum of Δ-fac-[Co(L-ser)$_{3-n}$(β-ala)$_n$] shows a gradual change with increasing number of β-ala⁻ groups. If we changed the configurational assignment of one of these complexes from Δ to Λ, this gradual change in the CD pattern would be broken. In addition, Δ-fac-[Co(L-ala)$_3$] is fairly soluble in water, whereas Λ-fac-[Co(L-ala)$_3$] is insoluble. Therefore, it is natural to assume that the more soluble diastereomer of the facial tris(aminoacidato)metal chelate is Λ(L) or Λ(D), and that the less soluble diastereomer is Δ(L) or Δ(D). Considering that the second eluted isomer Λ(L) or Λ(D) has a very low solubility, most of this isomer is presumed to be removed at the filtration stage of the preparation. The very small amount of this isomer observed can be thus understood.

Hence, we can conclude that, in all respects, the present assignment is correct.

Trend of the separation factors of enantiomeric pairs eluted with d-tartrate

The separation of each diastereomeric pair was also complete when 0.1 *M* Na_2*d*-tart solution was used in place of 0.05 *M* Na_2SO_4 solution (see Fig. 2). The diasteromeric mixture obtained by the preparation procedure was dissolved in the eluent (0.1 *M* Na_2*d*-tart) and the solution was stirred and filtered to remove the undissolved portion. The filtrate thus obtained was used as the sample solution, and was injected into the column for the determination of the retention volume of the diastereomers. For each run of a series of L-serine complexes we obtained the retention volumes of Δ(L) and Λ(L), and for each run of a series of D-serine complexes we obtained the retention volumes of Δ(D) and Λ(D). Thus, we could combine the data for Δ(L) with Λ(D), and Δ(D) with Λ(L), and calculate the ratio of the retention volumes of each enantiomeric pair, that is, the separation factor of each enantiomeric pair. The separation factor of an enantiomeric pair fac-[Co(gly)$_2$(β-ala)] and fac-[Co(gly) (β-ala)$_2$] was obtained simply from the retention volumes of two peaks in each elution curve, because these complexes exist originally as an enantiomeric pair. The results

TABLE III

ADJUSTED RETENTION VOLUMES (ml) AND SEPARATION FACTORS OBTAINED ON ELUTION WITH Na_2*d*-tart AND WITH $Na_2[Sb_2(d\text{-tart})_2]$

Complex	*Na_2d-tart*		*$Na_2[Sb_2(d\text{-tart})_2]$*	
	Retention volume	*Separation factor*	*Retention volume*	*Separation factor*
fac-⊿-[Co(D-ser)$_3$]	2.86	1.005	1.71	1.702
fac-⊿-[Co(L-ser)$_3$]	2.88		2.91	
fac-⊿-[Co(D-ser)$_3$]	3.66	1.007	2.40	1.510
fac-⊿-[Co(L-ser)$_3$]	3.69		3.63	
fac-⊿-[Co(D-ser)$_2$(β-ala)]	3.75	1.035	2.58	1.349
fac-⊿-[Co(L-ser)$_2$(β-ala)]	3.63		3.48	
fac-⊿-[Co(D-ser)$_2$(β-ala)]	4.90	1.032	3.75	1.193
fac-⊿-[Co(L-ser)$_2$(β-ala)]	4.75		4.98	
fac-⊿-[Co(D-ser) (β-ala)$_2$]	4.34	1.005	3.50	1.189
fac-⊿-[Co(L-ser) (β-ala)$_2$]	3.36		4.16	
fac-⊿-[Co(D-ser) (β-ala)$_2$]	6.30	1.090	6.08	1.060
fac-⊿-[Co(L-ser) (β-ala)$_2$]	5.78		5.73	
fac-⊿-[Co(β-ala)$_3$]	4.80	1.062	4.80	1.060
fac-⊿-[Co(β-ala)$_3$]	5.09		5.03	
fac-⊿-[Co(gly)$_2$(β-ala)$_2$]	5.46	1.022	—	—
fac-⊿-[Co(gly)$_2$(β-ala)$_2$]	5.58		—	
fac-⊿-[Co(gly) (β-ala)$_2$]	5.24	1.047	—	—
fac-⊿-[Co(gly) (β-ala)$_2$]	5.49		—	

are shown in Table III and Fig. 4a. As can be seen in Fig. 4a, the separation factor of the enantiomeric pair is largest with fac-[Co(β-ala)$_3$], which contains three six-membered chelate rings, and decreases regularly with increasing number of five-membered chelate rings (gly$^-$ or ser$^-$). It should be noted that the separation factors for the pair ⊿-fac-[Co(D-ser) (β-ala)$_2$] and ⊿-fac-[Co(L-ser) (β-ala)$_2$] and the pair ⊿-fac-[Co(L-ser) (β-ala)$_2$] and ⊿-fac-[Co(D-ser) (β-ala)$_2$] are different from each other, the former being exceedingly high and the latter exceedingly low. However, the average is close to the value for fac-[Co(gly) (β-ala)$_2$].

For the mechanism of the optical resolution of fac-[Co(β-ala)$_3$], the face-to-face close contact model was proposed, in which the *d*-tart^{2-} anion approaches, along the three-fold axis of the complex, the triangular facet formed by the three NH_2 groups of the complex. Here, three N–H bonds served for hydrogen bonding with three oxygen atoms of *d*-tart^{2-} to strengthen the association. The situation will change upon replacement of β-ala$^-$ with gly$^-$ or ser$^-$. As is well known, α-aminoacidato ligands form a five-membered chelate ring which is almost a plane including a central metal ion[7]. Thus, two N–H bonds are directed upwards and downwards from this plane with identical angles. Therefore, looking at the complex fac-[Co(L-ser)$_3$], for example, along its three-fold axis, the two N–H bonds of a chelate ring are directed like a letter V. There is no difference between the two N–H bonds as there is with the axial and equatorial bonds in the β-ala$^-$ chelate ring[8]. Consequently, the oxygen atoms of the *d*-tart^{2-} anion have two possibilities for forming hydrogen bonds with either of these two N–H hydrogen atoms of the complex. This situation may work unfavourably for the *d*-tart^{2-} anion in discriminating ⊿ from ⊿. The smallest separation factor of the tris-(serinato) complex can thus be understood.

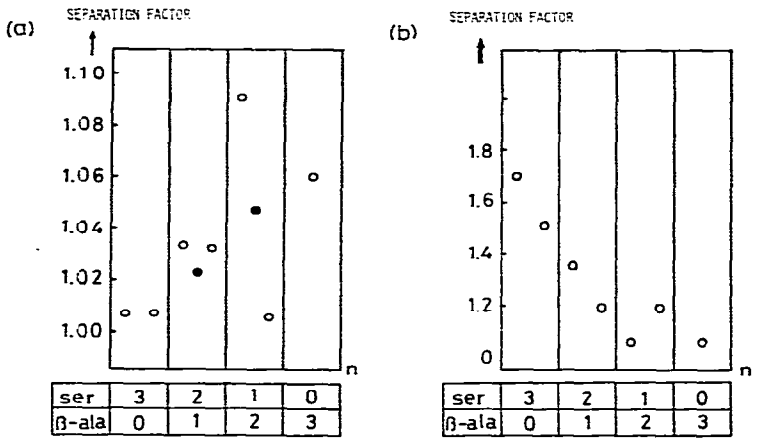

Fig. 4. Separation factors of fac-trisaminoacidatocobalt(III) complex. Eluent: (a) 0.1 *M* Na_2*d*-tart solution; (b) 0.1 *M* $Na_2[Sb_2(d\text{-tart})_2]$ solution. ○, Serine complex; ●, glycine complex.

Trend of the separation factors of enantiomeric pairs eluted with $Na_2[Sb_2(d\text{-tart})_2]$

The separation of each diastereomeric pair was also complete when 0.1 *M* $Na_2[Sb_2(d\text{-tart})_2]$ solution was used (see Fig. 2). The procedure for obtaining the separation factors of each enantiomeric pair was the same as that for *d*-$tart^{2-}$. The results are shown in Fig. 4b and Table III. As can be seen in the figure, the separation factor decreases with increasing number of β-ala^- groups. This trend is the opposite of that with *d*-$tart^{2-}$. Therefore, the discrimination mechanism with $[Sb_2(d\text{-tart})_2]^{2-}$ is presumed to be different to that with *d*-$tart^{2-}$. For the mechanism of discrimination of tris-chelate complex cations by $[Sb_2(d\text{-tart})_2]^{2-}$, we proposed in Part II a key-and-lock-type association model between the $[Sb_2(d\text{-tart})_2]^{2-}$ and the L-shaped channel of the Λ1 complex. Although the explanation of the trend of the separation factors with increasing number of β-ala^- groups in the series of $[Co(ser)_{3-n}(\beta\text{-ala})_n]$ is not clear, this model predicts that the Λ1 isomer will be eluted first, which agrees with the experimental observations.

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REFERENCES

1 H. Yoneda and T. Yoshizawa, *Chem. Lett.*, (1976) 707.
2 Y. Yoshikawa and K. Yamasaki, *Inorg. Nucl. Chem. Lett.*, 6 (1976) 523.
3 Y. Kushi, M. Kuramoto and H. Yoneda, *Chem. Lett.*, (1976) 135.
4 Y. Kushi, M. Kuramoto and H. Yoneda, *Chem. Lett.*, (1976) 339.
5 H. Yoneda and T. Taura, *Chem. Lett.*, (1977) 63.
6 R. G. Denning and T. S. Piper, *Inorg. Chem.*, 5 (1966) 1056.
7 H. C. Freeman, *Advan. Protein Chem.*, 22 (1967) 257.
8 H. Soling, *Acta Chem. Scand.*, A32 (1978) 361.