BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 45, 802—806(1972)

Stereoselective Formation of Isomers of the *Bis*(L-aspartato)-L-(or D-)alaninatocobaltate(III) Complex[1]

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(Received July 22, 1971)

The reaction of racemic α-alanine, in the presence of active charcoal, on a reaction mixture of the $[Co(CO_3)_3]^{3-}$ complex with L-aspartate led to the stereoselective formation of the $[Co(\text{L-asp})_n(\text{ala})_{3-n}]^{n-}$ (n=0—3) complexes. By means of ion-exchange chromatography and a recrystallization procedure, three stereoisomers of the *bis*(L-aspartato)alaninato complex have been obtained; *mer*$_1$-Λ-$[Co(\text{L-aspH})_2(\text{L-ala})]\cdot H_2O$, *mer*$_2$-$\Lambda$-$[Co(\text{L-aspH})_2(\text{L-ala})]\cdot 2H_2O$, and *fac*-$\Delta$-$[Co(\text{L-aspH})_2(\text{D-ala})]\cdot 4H_2O$ (where the *mer*$_1$ and *mer*$_2$ isomers have different geometries). These isomers have been characterized by comparing their CD and PMR spectra with those of the corresponding isomers obtained using L- or D-alanine in place of racemic alanine. The *fac*/*mer* ratio has been estimated to be *ca.* 64/36. The Δ/Λ ratio in the *fac* isomer has been estimated to be *ca.* 95/5, while the Δ/Λ ratio in both the *mer*$_1$ and *mer*$_2$ isomers has been estimated as *ca.* 25/75.

Previously, we reported that, when a large excess of racemic α-alanine was reacted with a reaction mixture of the tricarbonatocobaltate(III) complex with L-aspartate, a water-insoluble *fac*-*tris*(alaninato)cobalt(III) complex was precipitated, and that the precipitates consisted mainly of the *fac*-Δ(−)-$[Co(\text{D-ala})_2(\text{L-ala})]$ isomer (the (−) sign refers to the sign of the main CD peak in the first absorption band region).[2] In those experiments, the yield of the insoluble material was usually less than 50%. This fact was an indication of the existence of water-soluble complex species in such a reaction mixture; therefore, we wanted to isolate some of those complexes.

On the other hand, we have recently reported the pra-

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[1] Presented at the 24th Annual Meeting of the Chemical Society of Japan, Osaka, April, 1971.

[2] M. Shibata, H. Nishikawa, and K. Hosaka, This Bulletin, **41**, 130 (1968).

paration of a series of glycinato-L-aspartato complexes, $[Co(gly)_{3-n}(L\text{-}asp)_n]^{n-}$ ($n=1,2$), and discussed the stereoselective formations of the complexes.[3] In connection with the earlier work, the present paper will report the isolation and characterization of *bis*(L-aspartato)alaninato complexes, and will discuss the stereoselectivity in the complex formations.

Experimental

Preparation. To a green solution of tricarbonatocobaltate(III) ($CoCl_2 \cdot 6H_2O$ 6.0 g scale; 0.025 mol) prepared by the usual method,[4] 6.7 g (0.050 mol) of L-aspartic acid were added; the mixture was then stirred at 60°C for 2 hr. After adding 4.5 g (0.050 mol) of racemic α-alanine and 1.0 g of active charcoal, the solution was stirred at *ca.* 50°C for 3 hr; the color of the solution then changed from violet to red-violet, and a small amount of a water-insoluble material was precipitated. After the removal of this precipitate and the active charcoal by filtration, the filtrate was adjusted to pH *ca.* 7 with 6N acetic acid. This solution was charged on an anion-exchange column containing 100—200 mesh Dowex 1×8 resin in the chloride form (diameter, 7.0 cm; resin height, 30 cm). When water was passed through the column, a non-charged *tris*(alaninato) complex was eluted out. Then a 0.03M aqueous solution of calcium chloride was poured into the column at the rate of *ca.* 0.5 m*l*/min. After prolonged elution, seven bands were completely separated; the first band consisted of the univalently-charged *bis*(alaninato)-L-aspartato complex including isomers of both the *mer* and *fac* forms. The second and third bands were of the bivalently-charged *bis*(L-aspartato)alaninato complex belonging to the *mer* form. The fourth and fifth bands were of the same complex belonging to the *fac* form. The last two bands were the *mer* and *fac* isomers of the *tris*(L-aspartato) complex. An attempt at crystallization was actually made for the complex species of the 2—5 bands, because the first band gave a mixture of the stereo isomers of the *bis*(alaninato)-L-aspartato complex, and because the isomers of the complex corresponding to the last two bands, the isomers of the *tris*-(L-aspartato)cobaltate(III), had already been isolated.[5]

Four bands (2—5) were collected in fractions; they were conveniently labelled *mer*$_1$, *mer*$_2$, *fac*$_1$, and *fac*$_2$ according to the elution order. Each fraction was then concentrated to a small volume under reduced pressure at temperatures below 40°C. When ethanol was added to the resulting concentrate, the desired complex was obtained as calcium salt. This was then dissolved in a minimum amount of water, and the solution was acidified with 6N hydrochloric acid to precipitate the complex as hydrogen compound. This treatment was also employed for the calcium salts precipitated from the *mer*$_1$, *mer*$_2$, and *fac*$_1$ fractions. The calcium salt precipitated from the *fac*$_2$ fraction was dissolved in a minimum amount of water, and the solution was added to a small column containing Dowex 50W×8 resin in the hydrogen form. The effluence was concentrated, and ethanol was added to the concentrate to precipitate the hydrogen compound of the complex. The recrystallization of these hydrogen compounds was performed by dissolving each of them in slightly alkaline water and by then acidifying the solution with diluted hydrochloric acid. This procedure was repeated until the main CD peak of the compound showed a constant intensity. Although the exact yields were not obtained because of the repeated crystallization, the compounds were obtained in yields of roughly 8, 50, and 50 mg for the *mer*$_1$, *mer*$_2$, and *fac*$_2$ fractions respectively. The *fac*$_1$ fraction gave a trace of the solid.

A similar experiment was carried out by using L- or D-alanine in place of racemic alanine; the amount of L-(or D-)-alanine used was 2.3 g (0.025 mol). The procedures used in the chromatographic separation and in the isolation of the isomers were those described above.

The results of the elemental analyses of the isolated compounds will be shown below.

Formation Ratios. The formation ratios were compared among the *bis*(L-aspartato)alaninato complex species and actually determined spectrophotometrically using the $\varepsilon_{\max}$ value of the first absorption band and the $\Delta\varepsilon$ value at the main CD peak in the same absorption band region. However, in the determination we assumed that the Λ and Δ isomers of a complex have the same $\varepsilon_{\max}$ and $|\Delta\varepsilon|$ values, because only the Λ or Δ isomer could be obtained as a crystal from each of the fractions.

Measurements. The electronic absorption spectra were measured with a Hitachi Perkin-Elmer Model 139 UV-VIS spectrophotometer. The circular dichroism spectra were recorded on a JASCO Model ORD/UV-5 spectropolarimeter. The proton magnetic resonance spectra were recorded on a JEOL Model C-60H spectrometer (60 MHz) at about 25°C, using deuterium oxide containing an equivalent mole of Na_2CO_3 as the solvent. The values of the chemical shifts were measured in relation to sodium 3-(trimethylsilyl)-1-propanesulfonate (Na-TPS) used as an internal reference.

Results and Discussion

Characterization of the Complexes. Since the experiments on the isolation of the isomers of the desired complex were done using racemic (DL-), L-, and D-alanine separately, the isomers obtained from these experiments may conveniently be labelled as (DL), (L), and (D); for example, a compound obtained from the *mer*$_1$ fraction in the experiment using DL-alanine will be labelled *mer*$_1$ (DL).

The results of the elemental analyses and the numerical data on the absorption and CD spectra are summarized in Table 1. There is a lack of data for the *fac*$_1$-Λ because of an extremely poor formation of the species, while the lack of data for the *mer*$_1$-Λ(D) arises from a difficulty in crystallization. The geometrical form (*mer* or *fac*) could easily be determined on the basis of the absorption spectral data.[6,7] The absolute configuration (Λ or Δ) was also assigned on the basis of the sign of the main CD peak of each isomer.[8,9] The compounds obtained from the *mer*$_1$, *mer*$_2$, and *fac*$_1$ fractions were identified as the Λ isomers, while the compound obtained from the *fac*$_2$ fraction was identified as the Δ isomer. The CD and absorption spectra for the *mer*$_2$-Λ and *fac*$_2$-Δ are shown in Figs. 1 and 2.

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[3] K. Kawasaki, J. Yoshii, and M. Shibata, *ibid.*, **43**, 3819 (1970).

[4] M. Shibata, *Nippon Kagaku Zasshi*, **87**, 771 (1966).

[5] M. Shibata, H. Nishikawa, and K. Hosaka, This Bulletin, **40**, 236 (1967).

[6] F. Basalo, J. Bjerrum, and C. J. Ballhausen, *Acta Chem. Scand.*, **9**, 810 (1955).

[7] Y. Shimura and R. Tsuchida, This Bulletin, **29**, 311 (1956).

[8] M. G. B. Drew, J. H. Dunlop, R. D. Gillard, and D. Rogers, *Chem. Commn.*, **1966**, 42.

[9] R. G. Denning and T. S. Piper, *Inorg. Chem.*, **5**, 1056 (1966).

TABLE 1. ANALYSES, ELECTRONIC ABSORPTION AND CD SPECTRA

Complex	Anal. %			Absorption		CD	
	C	H	N	$\nu^{b)}$	ε_{max}	$\nu^{b)}$	$\Delta\varepsilon$
[Co(L-aspH)$_2$(ala)]·H$_2$O							
mer$_1$-*Δ*(DL)	30.21 (30.77)$^{a)}$	4.94 (4.70)	9.46 (9.79)	56.1 80.4	112 162	56.7 82.4	+3.96 −0.57
mer$_1$-*Δ*(L)	30.95	5.07	9.27	56.1 80.4	112 162	56.7 82.4	+3.96 −0.56
[Co(L-aspH)$_2$(ala)]·2H$_2$O							
mer$_2$-*Δ*(DL)	29.63 (29.53)	5.01 (4.96)	9.46 (9.39)	56.1 80.4	123 183	56.8 82.4	+3.98 −0.64
mer$_2$-*Δ*(L)	29.83	5.14	9.62	56.1 80.4	124 183	56.8 82.4	+3.96 −0.60
mer$_2$-*Δ*(D)	29.39	4.62	9.39	56.1 80.4	123 183	56.8 82.4	+4.50 −0.89
[Co(L-aspH)$_2$(ala)]·4H$_2$O							
fac$_2$-*Δ*(DL)	27.82 (27.33)	5.89 (5.43)	8.52 (8.69)	57.9 80.0	183 144	56.1 74.5 82.0 90.5	−1.69 −0.18 +0.57 −0.06
[Co(L-aspH)$_2$(ala)]·2H$_2$O							
fac$_2$-*Δ*(L)	29.70 (29.53)	4.86 (4.96)	9.09 (9.39)	57.9 80.0	183 144	56.2 74.5 82.0 90.5	−1.85 −0.19 +0.53 −0.06
fac$_2$-*Δ*(D)	29.63	5.28	9.40	57.9 80.0	184 144	56.1 74.5 82.0 90.5	−1.70 −0.18 +0.58 −0.06

a) Calcd; b) 10^{13} sec^{-1}

The stereochemistry of a *tris*-type complex containing two different aminoacidato ligands provides the existence of three geometrical isomers belonging to the *mer* form and one of the *fac* form (Fig. 3), but in the present work one *fac* isomer and two *mer* isomers labelled as *mer*$_1$ and *mer*$_2$, were obtained. The PMR spectra for the *fac*$_2$-*Δ*(L), *mer*$_1$-*Δ*(L), and *mer*$_2$-*Δ*(L) are shown in Fig. 4. The two *mer* isomers showed different spectra in the methylene proton resonance of the chelated aspartates.

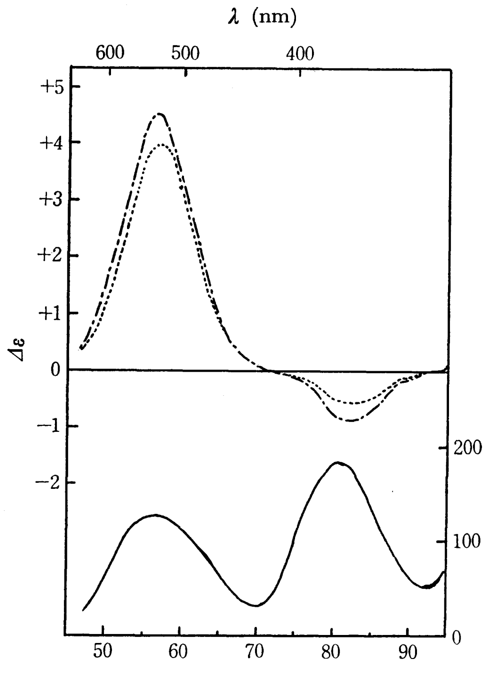

Fig. 1. Absorption and CD spectra for *mer*$_2$-*Δ* isomers in 60% HClO$_4$.
——Absorption spectra
------CD spectra for *mer*$_2$-*Δ*-(L)
–·–·CD spectra for *mer*$_2$-*Δ*-(D)

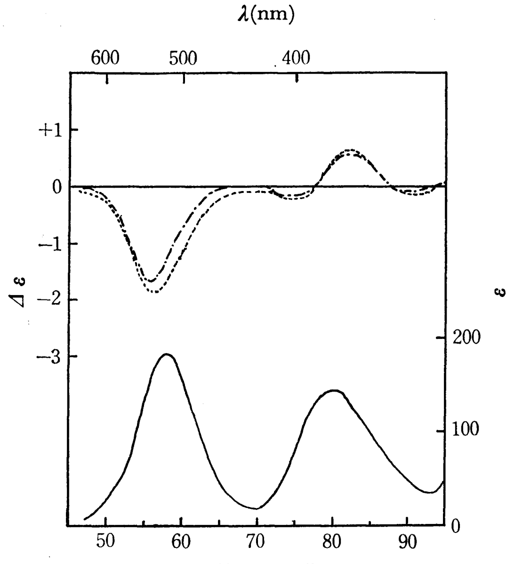

Fig. 2. Absorption and CD spectra for *fac*$_2$-*Δ* isomers in 60% HClO$_4$.
——Absorption spectra
------CD spectra for *fac*$_2$-*Δ*-(L)
–·–·CD spectra for *fac*$_2$-*Δ*-(D)

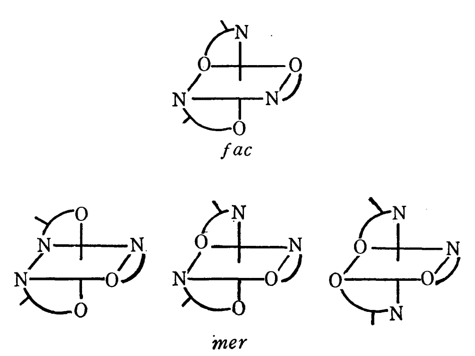

Fig. 3. The possible geometrical isomers of [Co(L-aspH)$_2$-(ala)].

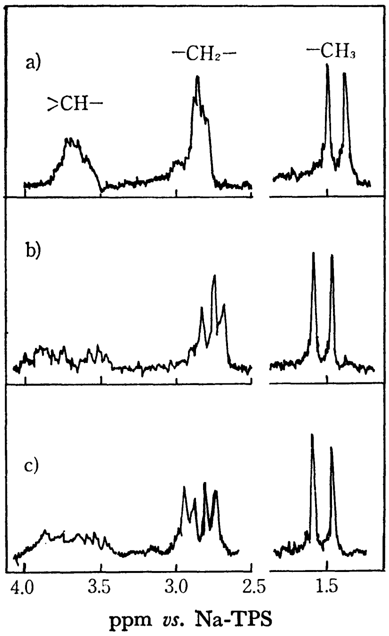

Fig. 4. PMR spectra in D$_2$O.
a) *fac*$_2$-Δ-(L)
b) *mer*$_1$-Λ-(L)
c) *mer*$_2$-Λ-(L)

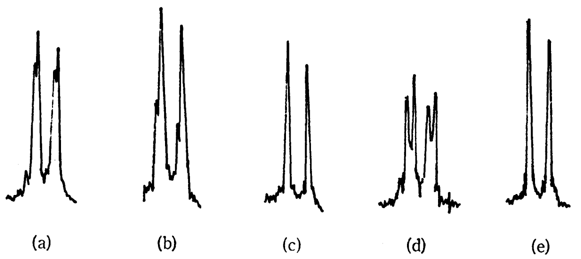

Fig. 5. Methyl proton resonance on;
a) mixed sample of *mer*$_2$-Λ-(L) and *mer*$_2$-Λ-(D),
b) crude sample of *mer*$_2$-Λ-(DL)
c) pure sample of *mer*$_2$-Λ-(DL),
d) crude sample of *fac*$_2$-Δ-(DL)
e) pure sample of *fac*$_2$-Δ-(DL),

This fact suggests that the isomers have different geometries.

The methyl proton resonance spectra of the chelated alanine are shown in Fig. 5. The spectrum for the *mer*$_2$-Λ(L) gives a single doublet at 1.55 ppm, while the spectrum for the *mer*$_2$-Λ(D) gives a similar doublet at 1.57 ppm. When a mixed sample of the two *mer*$_2$ compounds in the same amounts was measured, the spectrum revealed two closed doublets with a slight separation of *ca.* 1.5 Hz. On the other hand, when a compound from the *mer*$_2$-Λ(DL) fraction, which had been taken in the course of the recrystallization procedure, was measured, the spectrum showed a pattern similar to that of the mixed sample. The complex on which the recrystallization procedure had been completed showed only one doublet, at 1.54 ppm. Similarly, the *fac*$_2$-Δ(L) and *fac*$_2$-Δ(D) compounds showed a single doublet due to the methyl protons at 1.43 and 1.48 ppm respectively. The *fac*$_2$-Δ(DL) compound gave a doublet at 1.48 ppm, whereas a crude material of the same compound gave two pairs of doublets with a separation of *ca.* 2.5 cps. These results indicate that the crude material of an isomer, in an experiment using racemic alanine, includes both D- and L-alanine as ligands, and that the completely recrystallized material includes either L- or D-alanine as a ligand.

In order to identify the active form (D or L) of the coordinated alanine in the completely recrystallized material obtained by the use of racemic alanine, the Δε value of the isomer was compared with that of the corresponding isomer obtained by the use of L- or D-alanine. As may be seen in Table 1, the value of the *mer*$_2$-Λ(DL) is almost identical with that of the *mer*$_2$-Λ(L) and considerably different from that of the *mer*$_2$-Λ(D) isomer. When a similar comparison is made among the *fac*$_2$-isomers, a better agreement of the value of the (DL) isomer is found in that of the (D) isomer. These comparisons indicate that the *mer*$_2$-Λ(DL) isomer contains L-alanine and that the *fac*$_2$-Δ(DL) isomer contains D-alanine. Additional support is obtained from the previously-mentioned PMR data. Since the CD data for the *mer*$_1$-Λ(DL) and *mer*$_1$-Λ(L) isomers are almost identical, the former perhaps contains L-alanine.

Stereoselectivity. The percent compositions of the *mer* and *fac* isomers, and also the percent compositions of the Λ (or Δ) isomer predominantly formed for each geometrical isomer, are given in Tables 2 and 3. Simi-

TABLE 2. PERCENT COMPOSITIONS OF THE *mer* AND *fac* ISOMERS

Amino acids used	Isomers		
	mer (%)	(*mer*$_1$/*mer*$_2$)	*fac* (%)
DL-ala	36	(1/3)	64
L-ala	34	(1/3)	66
D-ala	39	(2/7)	61
gly[3]	42	—	58

TABLE 3. PERCENT COMPOSITIONS OF Δ OR Λ ISOMERS

Amino acid used	Isomers		
	mer$_1$-Λ (%)	*mer*$_2$-Λ (%)	*fac*$_2$-Δ (%)
DL-ala	78	76	95
L-ala	76	78	97
D-ala	73	78	93
gly[3]	73		95

lar data for the glycinato(L-aspartato) complex[3] are also cited in the Tables for the sake of comparison. The figures themselves are not very exact because of the assumed values of ε and $\Delta\varepsilon$ for non-isolated isomers, that is, since the vicinal contribution of the optically active ligands are disregarded, but they do seem to be useful for the estimation of the stereoselectivity. The composition of the *fac*$_2$-Δ (DL, D, or L) isomers was estimated to be *ca.* 95%, and a marked stereoselectivity was found in the Δ configuration. The compositions of all the *mer*-isomers ranged from 70 to 80%, and an appreciable selectivity was found in the Λ configuration. These results are similar to those for the *bis*(L-aspartato)glycinato complex (Table 3). The compositions of the *mer* and *fac* isomers could be estimated to be around 35 and 65%, not very different from those in the corresponding glycinato complex (Table 2). From this fact, we can consider that the orientation of the methyl group of the coordinated alanine scarcely affects the stereoselectivity in the complex concerned, and that the β-carboxylate groups of the coordinated L-aspartates affect the selectivity. In a previous paper,[3] we have assumed an enhanced stability through hydrogen-bonding between the β-carboxylate group in the L-aspartato ligand and the amino group in the adjacent ligand. In the present case, this assumption also gives an explanation of the stereoselectivity.

The fact that the *mer*$_2$-Λ(DL) and *fac*$_2$-Δ(DL) contain L- and D-alanine respectively may be caused by the difference in solubility between the diastereoisomers, rather than by the preferential coordination of one of the active forms of alanine. This is confirmed by the above-mentioned PMR study.