Stereoselectivity in the Formation of Oxalato-L-aspartato-ethylenediaminecobalt(III) Complex[1]

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(Received March 25, 1974)

The complex [Co(ox)(L-aspH)(en)] has been prepared by the reaction of the dioxalatoethylenediaminecobalt(III) complex with L-aspartic acid and separated into four diastereoisomers by means of ion-exchange chromatography: *mer*-*Δ*-, *mer*-*Λ*- and *fac*-*Δ*-[Coox(L-aspH)en]·H_2O, and *fac*-*Λ*-[Coox(L-aspH)en]·2.5H_2O. They were characterized by their absorption, CD and PMR spectra. Stereoselectivity has been found in the *mer*-*Δ* and *fac*-*Λ* isomers with degrees exceeding 80%. Related isomeric complexes containing L-glutamate or L-leucinate ion in place of L-aspartate have also been investigated, much less selectivity (~60%) being observed.

In order to study stereoselectivity we examined the mixed L-aspartato complexes with glycine,[2] L-alanine,[3] and L-proline.[4] It was found that the stereoselectivity observed can be explained in terms of the interaction between the uncoordinated *β*-carboxylate group in the chelated aspartate ion and the adjacent ligand.

In the present work, a mixed complex consisting of L-aspartate ion (L-asp), oxalate ion and ethylenediamine was chosen in order to obtain proof for the selectivity. The *mer* isomers of the mixed aminoacidato complexes used in the previous works consisted of three geometrical isomers. The present complex gives only one form for either *mer* or *fac* arrangement with respect to donor N (or O) atoms. Thus, the study on stereoselectivity could be simplified. For the sake of comparison the corresponding L-glutamato and L-leucinato complexes were also studied.

Experimental

Preparation. Preparation and chromatographic separation of the [Coox(L-asp)en]$^-$ complex were carried out as follows: The complex Na[Co(ox)$_2$en]·H_2O[5] (6.7 g, 0.02 mol), L-aspartic acid (2.7 g, 0.02 mol) and NaOH (0.8 g, 0.02 mol) were dissolved in water (250 ml). After the pH of the solution had been adjusted to *ca.* 9.0 with a NaOH solution, activated charcoal (1.0 g) was added and the mixture was allowed to stand for a week at room temperature (~15 °C) with occasional stirring, whereupon the solution turned red-violet. After the removal of a small amount of the precipitate and charcoal by filtration, a portion (50 ml) of the filtrate was charged on a cation-exchange column containing 100—200 mesh Dowex 50 W×8 resin in H-form (7.0×27 cm). Chromatographic separation was carried out by passing water at a rate of *ca.* 0.5 ml/min. The foremost band was of unreacted [Co(ox)$_2$en]$^-$ species. After a while two closely spaced but completely separated violet bands descended the column, followed by other two red bands with a narrow distance. These were collected in fractions and labeled from A-1 to A-4. This separation procedure was repeated several times in order to store up the same fractions. Each stored fraction was concentrated to a small volume with a rotary evaporator at 30 °C. The concentrated solution thus obtained was kept in a refrigerator for a few days in order to precipitate the desired isomer as its hydrogen compound. Each recrystallization was performed from water.

Such complexes as [Coox(en)$_2$]$^+$, [Co(L-asp)(en)$_2$]$^+$[6,7] and [Co(L-asp)$_2$(en)]$^-$[7] were also produced, but they remained at the top of the column during the separation of the desired isomers.

Preparation and chromatographic separation of the [Coox(L-glu)en]$^-$ complex were carried out as in the case of the aspartato complex; L-glutamic acid (2.9 g, 0.02 mol) was used.

Chromatographic separation gave two bands, violet and red. They were collected in fractions and labeled G-1 and G-2. A pair of diastereoisomers could be isolated by fractional crystallization from G-1. Another pair of diastereoisomers was obtained from G-2 by the following procedure. The solid material (100 mg) obtained by evaporating the fraction was dissolved in a minimum amount of water slightly alkalinized with aqueous NaOH, the pH of the solution being made *ca.* 7.0. The solution was poured into a column containing G-15 Sephadex (2×100 cm), and washing with water was continued at a rate of *ca.* 0.07 ml/min. The red band spread with the elapse of time. After several hours, there appeared a broadened band having different tones of color in the first and second halves. The band was collected in a number of fractions and subjected to CD measurement. The first half showed a (−) sign and the second a (+) sign at the main CD peak. The chromatographic separation was repeated several times in order to store up the fractions with the same sign. The same fractions collected were concentrated and rechromatographed. From the final two fractions thus obtained a pair of diastereoisomers was obtained by evaporating and acidifying with 6 M HCl.

For preparation and separation of the [Coox(L-leu)en] complex, L-leucine (2.6 g, 0.02 mol) and Dowex 50 W×8 resin in Na-form were used. The procedure was the same as in the case of the L-aspartato complex. The chromatographic separation gave three bands violet, red and red in turn. They were collected in fractions labeled L-1, L-2 and L-3. From L-1 a pair of diastereoisomers was obtained by fractional crystallization. Each of the other diastereoisomers was obtained from L-2 and L-3 each.

For all diastereoisomeric complexes, recrystallization was carried out until their main CD peaks became constant.

Formation Ratios. The formation ratios between the bands separated chromatographically were evaluated from the spectral data of the isolated complexes.

Measurements. The absorption spectra were measured with a Hitachi Model 323 Recording 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 JNM-PS-100 NMR spectrometer at *ca.* 22 °C, with deuterium oxide as a solvent. Sodium carbonate and 20% deuterium chloride in D_2O were used for pD adjustment. The values of the chemical shifts were referred to internal sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) or sodium D_4-trimethylsilylpropionate (TMSP). The pD of the PMR sample was measured with a Beckman Model G pH meter.

Results and Discussion

Characterization of Isomers. Elemental analyses, absorption spectra and CD spectra are summarized in Table 1. The geometrical form (*mer* or *fac*) of each isolated compound could easily be identified by its absorption spectrum. The absolute configuration (*Δ* or *Λ*) of a given geometrical isomer was determined from the sign of main CD peak. The CD spectra of the isolated compounds are shown in Figs. 1 and 2. The four possible isomers of the [Coox(L-aminoacidate)-en]-type complex are given in Fig. 3.

The chromatographic separation of the L-aspartato complex into the four isomers was carried out with the cation-exchange resin in H-form. In the beginning, an anion-exchange resin in Cl-form was used for the separation of anionic species $[Coox(L\text{-}asp)en]^-$. An extremely long column was necessary since washing procedure gave a diffuse band on the column and successive elution with an aqueous solution of electrolyte made each band spread considerably. In contrast, complete separation of the four isomers was achieved by the use of the cation-exchanger in a short period of elution. For example, elution for two weeks on an anion-exchanger column could be reduced to two days on a cation-exchange column. This improved procedure was used for the separation of the L-glutamato complex species.

In the case of the L-aspartato complex, four isomers were completely separated in the order *mer-Δ-mer-Λ-*

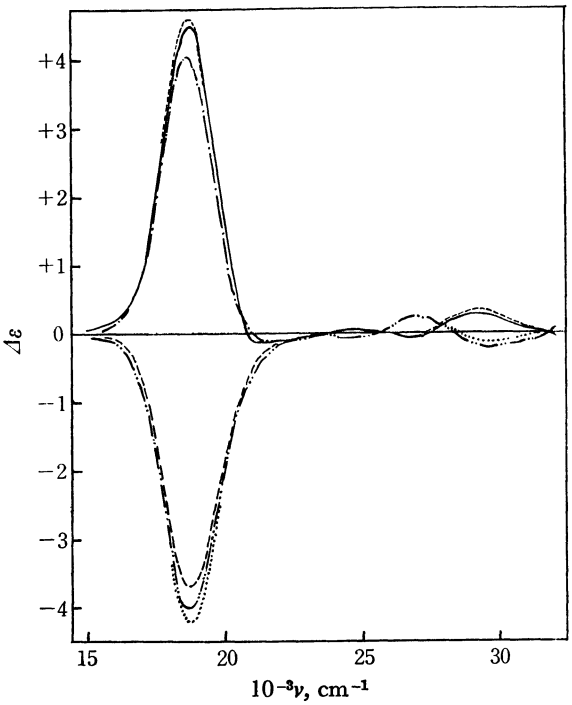

Fig. 1. CD spectra of *fac*-[Coox(L-am)en]-type complexes.
—— *fac-Λ* (L-am=L-aspH)
—·— *fac-Λ* (L-gluH) ------ *fac-Λ* (L-leu)
—··— *fac-Δ* (L-aspH) —— *fac-Δ* (L-gluH)
···· *fac-Δ* (L-leu)

Table 1. Elemental analyses, absorption and CD spectral data

Label	Complex	Elemental anal., %[a] C	H	N	Band I $10^{-3}\bar{\nu}_{max}$ cm^{-1}	ε_{max}	Band II $10^{-3}\bar{\nu}_{max}$ cm^{-1}	ε_{max}	CD $10^{-3}\bar{\nu}_{max}$ cm^{-1}	$\Delta\varepsilon_{max}$
A-1	*mer-Δ*-[Coox(L-aspH)en]·H_2O	26.73	4.34	11.79	*ca.* 19.3	96	26.95	175	17.7	−2.03
		(26.90)	(4.52)	(11.77)					20.9	−1.43
A-2	*mer-Λ*-[Coox(L-aspH)en]·H_2O	26.91	4.71	11.91	*ca.* 19.3	96	26.95	180	17.8	+1.95
		(26.90)	(4.52)	(11.77)					20.4sh	+1.24
A-3	*fac-Λ*-[Coox(L-aspH)en]·2.5H_2O	25.02	5.28	10.98	19.2	173	27.25	193	18.7	+4.51
		(25.01)	(4.99)	(10.94)					21.4	−0.14
A-4	*fac-Δ*-[Coox(L-aspH)en·]H_2O	27.18	4.80	11.65	19.2	157	27.25	170	18.8	−4.03
		(26.90)	(4.52)	(11.77)						
G-1	*mer-Δ*-[Coox(L-gluH)en]·0.5H_2O	29.47	5.10	11.33	*ca.* 19.3	95	26.95	180	17.5	−2.42
		(29.74)	(5.07)	(11.56)					20.7	−1.52
	mer-Λ-[Coox(L-gluH)en]·1.5H_2O	28.52	5.36	11.07	*ca.* 19.3	104	26.95	197	17.8	+2.07
		(28.34)	(5.36)	(11.02)					20.5	+1.41
G-2	*fac-Λ*-[Coox(L-gluH)en]·H_2O	29.06	5.02	11.17	19.2	149	27.25	170	18.6	+4.06
		(29.02)	(5.21)	(11.28)					21.4	−0.11
	fac-Δ-[Coox(L-gluH)en]·H_2O	29.33	5.30	11.29	19.2	140	27.25	155	18.7	−3.70
		(29.02)	(5.21)	(11.28)						
L-1	*mer-Δ*-[Coox(L-leu)en]·1.5H_2O	33.24	6.78	11.31	*ca.* 19.3	98	26.95	184	17.5	−2.46
		(32.98)	(6.37)	(11.54)					20.8	−1.45
	mer-Λ-[Coox(L-leu)en]·1.5H_2O	32.68	6.63	11.67	*ca.* 19.3	101	26.95	195	17.8	+1.81
		(32.98)	(6.37)	(11.54)					20.5	+1.27
L-2	*fac-Δ*-[Coox(L-leu)en]·2.5H_2O	31.76	6.76	11.14	19.2	155	27.25	173	18.7	−4.20
		(31.42)	(6.59)	(10.99)						
L-3	*fac-Λ*-[Coox(L-leu)en]·2.5H_2O	31.41	6.32	11.14	19.2	161	27.25	187	18.6	+4.60
		(31.42)	(6.59)	(10.99)					21.4	−0.08

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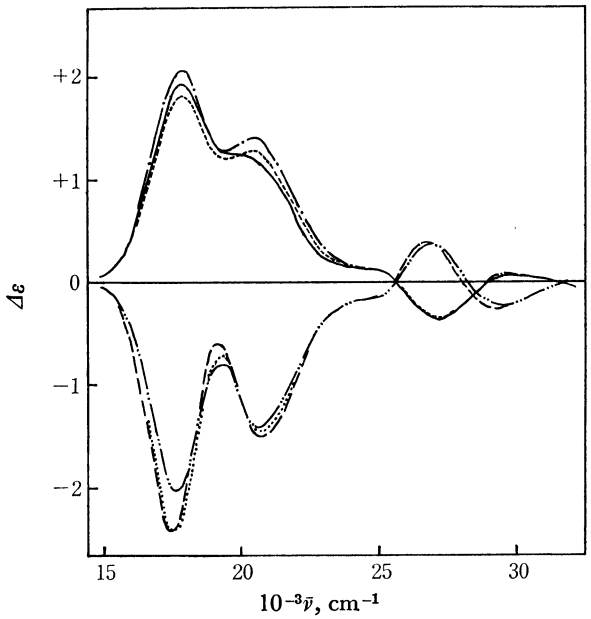

Fig. 2. CD spectra of *mer*-[Coox(L-am)en]-type complexes.

—— *mer*-Λ (L-am=L-aspH)
—·— *mer*-Λ (L-gluH) ------ *mer*-Δ (L-leu)
—··— *mer*-Δ (L-aspH) ----- *mer*-Δ (L-gluH)
···· *mer*-Δ (L-leu)

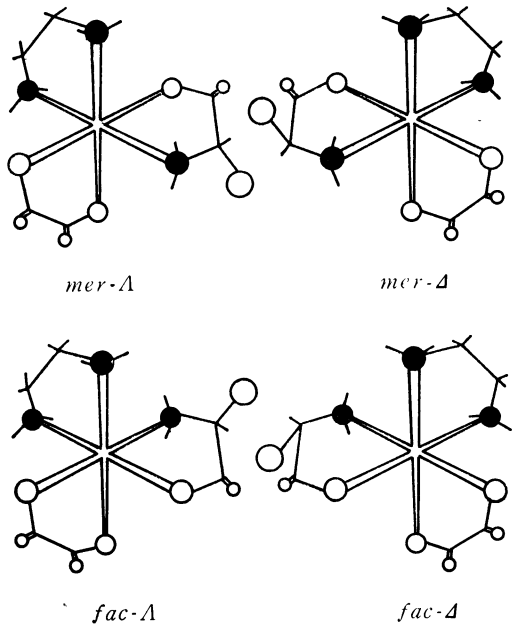

Fig. 3. Possible four isomers of [Coox(L-am)en]-type complex.

fac-Λ-*fac*-Δ, while for the L-glutamato complex only two fractions corresponding to *mer*- and *fac*-forms were obtained. This might be due to the different uncoordinated carboxylate groups (β- and γ-COO^-).

The CD spectra of the Δ and Λ diastereoisomers of *fac*-form exhibit a sharp peak in the first absorption band region (Fig. 1), similar to those of the tris-type complexes of L-amino acids.[4,8,9] On the other hand, the spectra of the Δ and Λ diastereoisomers of *mer*-form exhibit two peaks with the same signs in the first absorption band region (Fig. 2). The *mer*-Δ isomers of the $[Co(L\text{-pro})_3]$[8] and $[Co(L\text{-pro})_2(L\text{-asp})]^-$[4] complexes reveal a small shoulder at shorter-wave-length-side in the first absorption band region.

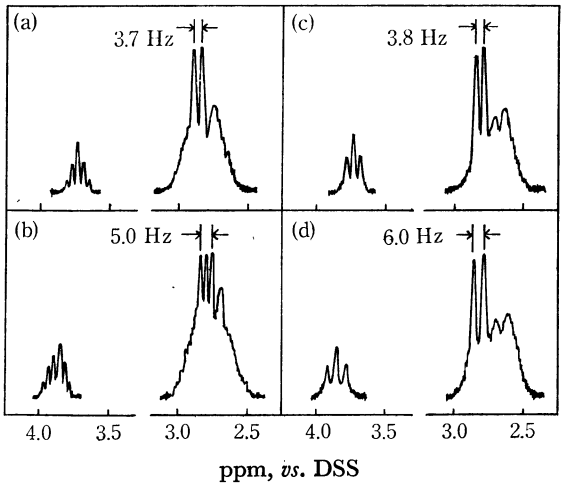

Fig. 4. PMR spectra of [Coox(L-aspH)en] in D_2O (pD 4.3—5.3).
(a) *mer*-Δ isomer (b) *mer*-Λ isomer
(c) *fac*-Λ isomer (d) *fac*-Δ isomer

Figure 4 shows the PMR spectra of the four isomers of the L-aspartato complex. It can be seen that the two sharp lines appearing in the vicinity of 2.8 ppm and multiple lines in the vicinity of 3.8 ppm are due to the CH_2 and CH groups, respectively, of the coordinated aspartate ion.[6] The isomers can be classified into those of narrow width (*mer*-Δ and *fac*-Λ) and those of broad width (*mer*-Λ and *fac*-Δ). The difference in line-width may be related to the different orientation of aspartate side-chains in the two types. A similar discussion was given previously.[4]

The isomers of the L-glutamato complex exhibited too complicated spectra for obtaining any information.

The PMR spectra, partly of CH_3 proton resonance, of the four isomers of L-leucinato complex were measured. The results show two well-separated doublets indicating different chemical environments of two CH_3 groups of the isobutyl group for the isomers.

Table 2. Percentage compositions of reaction mixtures

	mer-Δ	*mer*-Λ	*fac*-Λ	*fac*-Δ
[Coox(L-aspH)en]	72	11	14	3
[Coox(L-gluH)en]	48	32	11	9
[Coox(L-leu)en]	49	35	8	8
[Coox(gly)en][a]	84		16	

a) Percentage composition was evaluated from spectral data.[10]

Stereoselectivity. The percentage compositions of all isomers in the reaction mixture are given in Table 2. For comparison, the values for the [Coox(gly)en] are also included. The formation of the *mer* isomer (Λ+Δ) in each complex is more abundant than that of the *fac* isomer (Λ+Δ). The amount of formation of the isomers for the L-glutamato and L-leucinato complexes increases in the order *fac*-Δ≃*fac*-Λ<*mer*-Λ<

TABLE 3. PERCENTAGE COMPOSITIONS OF DIASTEREOISOMERS

	[Coox(L-aspH)en]	[Coox(L-gluH)en]	[Coox(L-leu)en]	$[Co(gly)_2(L\text{-asp})]^-$ [2]	$[Co(gly)_2(L\text{-glu})]^-$ [9]
mer-Δ	87	60	58	39	50
fac-Δ	82	55	51	11	40

mer-Δ, and that for the L-aspartato complex in the order *fac-Δ*<*mer-Λ*≃*fac-Λ*<*mer-Δ*. The increase of *fac-Λ* can be understood in terms of favorable interaction.

The stereoselectivity in a given complex is expressed in percentage composition of a diastereoisomeric pair. The results are given in Table 3, together with the data of the L-aspartato[2] and L-glutamato[9] complexes. As was expected, a marked stereoselectivity of over 80% was found in both the *mer-Δ* and *fac-Λ* isomers for the L-aspartato complex. This can be understood on the basis of stereo models; a favorable interaction through a hydrogen bond between the β-carboxylate group of the coordinated L-aspartate ion and an amino group of the chelated ethylenediamine is possible for the *mer-Δ* and *fac-Λ* isomers; on the other hand, an electrostatic repulsive interaction between the β-carboxylate group and an oxygen atom of the chelated oxalate ion is possible for the *mer-Λ* and *fac-Δ* isomers.

The stereoselectivity in the $[Co(gly)_2(L\text{-glu})]^-$ complex is much less than that in the corresponding L-aspartato complex.[9] This may be due to the lower ability of the γ-carboxylate group to interact with the adjacent group. The *mer-Δ* and *fac-Λ* isomers for the present L-glutamato complex also showed ~60% selectivity. The L-leucinato complex having an isobutyl group as the side-chain of the amino-acidate ion also showed a comparable stereoselectivity. This suggests that such a low selectivity can not be discussed in a similar way to that for the L-aspartato complex.

In the $[Co(gly)_2(L\text{-asp})]^-$ complex, the percentage compositions of the *fac-Δ* and *mer-Δ* isomers were reported to be 89 and 61%, respectively.[2] The lower selectivity of the *mer-Δ* isomer is due to the fact that a geometrical isomer for the *mer-Δ* diastereoisomer, *trans*-(N)*cis*(O), is favorable for the interaction of the β-carboxylate group. The present work showed selectivity in the same order as between the *mer-Δ* and *fac-Λ* isomers of the L-aspartato complex. It has been confirmed that the stereoselectivity in a complex containing L-aspartate ion as a bidentate ligand is closely related to a favorable interaction of the β-carboxylate group of the aspartate ion with the adjacent ligand.

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