

Short Communication

Rapid optical resolution of electrically neutral cobalt(III) chelate complexes by gel permeation chromatography

Milan Strašák^{*} and Pavol Novomeský^{*}

Department of Analytical Chemistry and Laboratory of Structural Biocoordination Chemistry, Faculty of Pharmacy, Comenius University, Odbojárov 10, 832 32 Bratislava (Czechoslovakia)

Slavomír Bystrický

Institute of Chemistry, Slovak Academy of Science, 842 38 Bratislava (Czechoslovakia)

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ABSTRACT

Three geometrical isomers of electrically neutral [Co(EBAA)(aa)] [EBAA = ethylenebis(aminoacidate); aa = an amino acidate] complexes have been synthesized and characterized. They have been resolved into the enantiomers, except for symmetrical *cis-mer*-[Co(EBAA)(aa)] isomers, by gel permeation chromatography on a Sephadex G-10 column with water as the eluent, and their absolute configurations have been determined based on their circular dichroism spectra. It has been revealed that, except for [Co(EBG)(β -ala)] [EBG = ethylenebis(glycinate)], the Δ enantiomers have a substantially greater optical purity.

INTRODUCTION

Linear tetradentate ligands provide a variety of stereochemical permutations, and linear EBAA ligands [EBAA = ethylenebis(amino acidate)], $^-OOCCH(R)NR'CH_2CH_2NR'CH(R)COO^-$, are of particular interest for studies of Co(III) and Cr(III) [1]. The interest is not only in their stereochemistry, but also their use as probes of the structure–function relationships of proteins [2]. The complexes can be used for this purpose as they are inert to substitution.

For the neutral [Co(EBAA)(aa)] system (aa = amino acidate) three geometrical isomers are pos-

sible (Fig. 1) and there are also the optical Δ and Λ isomers for the *cis- α* and both *cis- β* forms.

The separation and isolation of these geometrical isomers have been carried out by column chromatography on an anion-exchange resin (Dowex 1-X8, Cl⁻) and on a cation-exchange column (SP Sephadex C-25, Na⁺) [3].

Neutral complexes so far completely resolved into enantiomers are limited to tris-chelates. Nakazawa *et al.* [3] have reported that when the bis (μ -*d*-tartrato)diantimonate(III) anion, [Sb₂(*d*-tart)₂]²⁻, form of QAE-Sephadex is used with water as the eluent, some geometrical isomers of [Co(EBG)(aa)], where EBG is ethylenebis(glycinate) and aa = glycinate (gly) and β -alaninate (β -ala), have been completely resolved into the enantiomers. In the course of studies of metal complexes of amino acid

^{*} Author deceased.

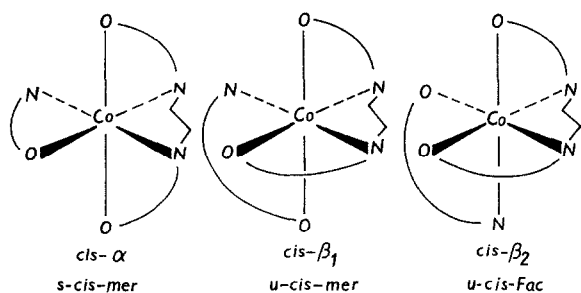


Fig. 1. Three geometrical isomers for a $[\text{Co}(\text{EBAA}) (\text{aa})]$ complex (both notation are used). *s* = Symmetrical; *u* = unsymmetrical.

derivatives, it has been reported that the partial optical resolution of neutral cobalt(III) metal complexes is more difficult than the resolution of cationic or anionic complexes because they do not form diastereoisomers with an optically active resolving agent. However, the optical resolution of such neutral complexes can be obtained chromatographically [7]. Some neutral complexes have been reported to be partially resolved into the enantiomers using a quartz powder column [8] and *mer*- and *fac*- $[\text{Co}(\text{gly})_3]$ and *cis-α*- $[\text{Co}(\text{EBG})(\text{gly})]$ by using a potato starch column [9]. The first complete resolution of neutral complexes was reported by Yoneda and Yoshizawa [10] for *fac*- $[\text{Co}(\beta\text{-ala})_3]$ on the Na^+ form of a CM-Sephadex cation exchanger with an ethanol-water solution of sodium *d*-tartrate, $\text{Na}_2(d\text{-tart})$, as the eluent. Thereafter, the complete resolution of a series of enantiomeric pairs of *fac*- $[\text{Co}(\text{D/L-ser})_3 - n(\beta\text{-ala})_n]$ ($n = 0$ to 3; ser = serinate) was achieved on the Na^+ form of the TSK-211 cation exchanger with an $\text{Na}_2[\text{Sb}_2(d\text{-tart})_2]$ aqueous solution as the eluent [11].

Anion-exchange columns have also been used to resolve neutrals complexes into enantiomers [12–14]. Neutral complexes, other than tris(aa) complexes, have also been partially resolved using a *d*-lactose column [15], a quartz column [16] or a Δ - $[\text{Ni}(\text{phen})_3]$ -montmorillonite (phen = 1,10-phenanthroline) column [17]. Recently, $[\text{Co}(\text{acac})_3]$ and $[\text{Cr}(\text{acac})_3]$ (acac = acetate) were completely resolved by high-performance liquid chromatography on a (+)-poly(triphenylmethyl methacrylate) column [18]. No resolution of the optical isomers of neutral metal complexes on molecular sieves has yet been described. This method was used here to resolve $[\text{Co}(\text{EBAA}) (\text{aa})]$ complexes.

The degree of resolution which can be achieved on one passage through chelate cationic [4] and anionic [5] complexes by gel permeation chromatography (GPC) was reported. The object of this study is the rapid optical resolution of neutral cobalt(III) complexes by GPC on a column of the molecular sieve Sephadex G-10.

EXPERIMENTAL

The complexes were prepared by a method analogous to that previously reported [3] for $[\text{Co}(\text{EBG})(\text{aa})]$. All the new compounds gave satisfactory elemental analyses. The geometrical isomers were assigned to *cis-α* and *cis-β*, respectively, according to their absorption spectra and ^1H and ^{13}C NMR spectra [6].

Optical resolution

The racemic neutral complex (20–40 mg) dissolved in an appropriate amount of water (2–5 ml) was loaded onto the column (60 cm × 1 cm I.D.) of Sephadex G-10. The elution was performed with water at a flow-rate of 0.1–0.2 ml/min. During elution the neutral complex was partially separated into enantiomers. The eluates obtained were subjected to absorption (AB) and circular dichroism (CD) measurements.

CD spectra were recorded on a Jobin-Yvon Dichrograph III. The concentrations of the solutions (10^{-4} – 10^{-2} mol/l) were determined from their absorption spectra recorded on a Specord M-40 spectrophotometer or by atomic absorption spectrometry (AAS).

RESULTS AND DISCUSSION

The optical resolution of electrically neutral cobalt(III) complexes achieved on one passage through the column is given in Table I. Figs. 2 and 3 show the typical AB and CD spectra. The ligand field symmetry of the *u-cis-fac* isomer is higher than that of the *u-cis-mer* isomer. The decrease in symmetry from facial to meridional is expected to cause a splitting, or at least a broadening, of the lowest energy absorption band in the visible spectra. In both $[\text{Co}(\text{EBA})(\text{gly})]$ and $[\text{Co}(\text{EBV})(\text{gly})]$ [EBA = ethylenebis(α -alaninate); EBV = ethylenebis(valinate)], broadening is seen clearly in the low-energy

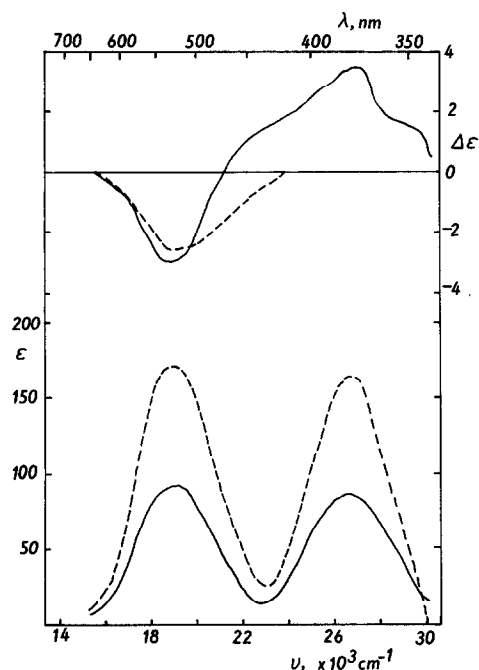


Fig. 2. Absorption spectra (lower) and CD spectra (upper) of Δ -[Co(EBA)(gly)]; (—) *u-cis-mer*; (---) *u-cis-fac*.

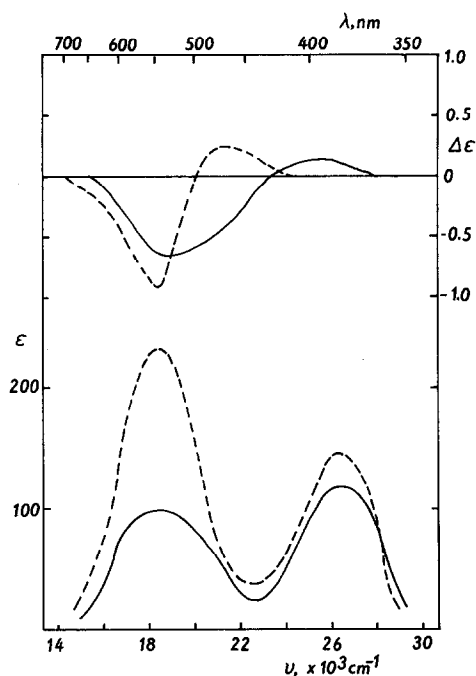


Fig. 3. Absorption spectra (lower) and CD spectra (upper) of Δ -[Co(EBV)(gly)]; (—) *u-cis-mer*; (---) *u-cis-fac*.

absorption band for the first eluted isomer. In [Co(EBG)(β -ala)], a marginal shoulder is observed for the first eluted isomer. In contrast, the last eluted isomer exhibits a relatively sharp absorption band. Therefore, the first and second eluted isomers can be assigned to the *u-cis-mer* and *u-cis-fac* isomers, respectively.

The assignment is consistent with the chromatographic behaviour. Although both isomers are electrically neutral, the *fac* isomer has a greater dipole moment than the *mer* isomer. Thus the former is considered to interact more effectively with the functional group of the SP Sephadex C-25 cation exchanger than the latter. Therefore, the *fac* isomer is expected to be eluted later than the *mer* isomer. This has been observed experimentally.

Partial optical resolution of the complexes has been attained and their absolute configuration has been established based on the fact that the sign of the dominant peak in the first d-d transition region of the CD spectrum can generally be related to the net chirality of the complex [19,20]. A Δ configuration of chelate rings is expected to produce a negative dominant peak in the d-d transition region whereas a Λ configuration produces a positive dominant peak [19]. The CD spectra shown in Figs. 2 and 3 resemble those of [Co(EBG)(β -ala)]. The $\Delta\epsilon$ values obtained for the optical isomers of [Co(EBG)(β -ala)] (Table I) can be compared with those of the pure isomers [3]. Remarkable differences can be seen. On the [Sb₂(*d*-tart)₂]²⁻ form of QAE Sephadex the *s-cis-mer* and *u-cis-mer* isomers have been completely resolved into the enantiomers, whereas GPC on Sephadex G-10 does not resolve the *s-cis-mer*.

Some other observations can be made on the resolution of these complexes on the Sephadex G-10. It is interesting that, with all the cationic [4] and anionic [5] complexes studies, the Δ enantiomers have a substantially greater optical purity; this is also true for neutral complexes, except [Co(EBG)(β -ala)]. The relationship between the absolute configurations and the order of elution is given in Table II. An examination of the CD spectra of the eluates shows that the first eluted enantiomers have a Δ configuration, except for [Co(EBG)(β -ala)]. In spite of the results from the optical resolution of the cationic [CoN₄O₂]⁺-type complexes [4], where the enantiomers with the Λ configuration are eluted

TABLE I

ABSORPTION (AB) AND CIRCULAR DICHROISM (CD) DATA FOR THE FIRST ELUTED ENANTIOMER OF COBALT (III) COMPLEXES

Complex	AB		CD	
	λ (nm)	ϵ (l mol ⁻¹ cm ⁻¹)	λ (nm)	$\Delta\epsilon$ (l mol ⁻¹ cm ⁻¹)
<i>s-cis-mer</i> -[Co(EBG)] (β -ala)	562	sh ^a	Unresolved	
	504	114	Unresolved	
	373	127	Unresolved	
<i>u-cis-mer</i> [Co(EBG)] (β -ala)	586	sh	554	(+) ^b
	508	80	477	(-)
	373	92		
<i>u-cis-fac</i> -[Co(EBG)] (β -ala)	524	248	560	+ 1.42
	374	153	480	- 1.35
<i>s-cis-mer</i> -[Co(EBA)] (gly)	552	108	Unresolved	
	520	sh	Unresolved	
	374	110	Unresolved	
<i>u-cis-mer</i> -[Co(EBA)] (gly)	529	92	538	- 2.91
	374	86	372	+ 3.52
<i>u-cis-fac</i> -[Co(EBA)](gly)	531	172	535	- 2.61
	373	164	Unresolved	
<i>s-cis-mer</i> -[Co(EBV)](gly)	543	111	Unresolved	
	510	sh	Unresolved	
	373	141	Unresolved	
<i>u-cis-mer</i> -[Co(EBV)](gly)	513	100	537	- 0.62
	374	117	390	+ 0.14
<i>u-cis-fac</i> -[Co(EBV)](gly)	529	232	541	- 0.93
	373	144	460	+ 0.24

^a sh = Shoulder.^b Partially resolved.

TABLE II

CORRELATION BETWEEN THE SIGN OF THE DOMINANT CD PEAK OF THE FIRST ELUTED ENANTIOMERS AND THEIR ABSOLUTE CONFIGURATIONS

Isomer	Sign of the longer wavelength CD peak	Absolute configuration	Reference ^a
<i>s-cis-mer</i> -[Co(EBG)](gly)	+	<i>A</i>	3
<i>u-cis-mer</i> -[Co(EBG)](gly)	+	<i>A</i>	3
<i>u-cis-fac</i> -[Co(EBG)](gly)	+	<i>A</i>	3
<i>s-cis-mer</i> -[Co(EBG)](β -ala)	+	<i>A</i>	3
<i>u-cis-mer</i> -[Co(EBG)] (β -ala)	+	<i>A</i>	3
<i>u-cis-fac</i> -[Co(EBG)](β -ala)	+	<i>A</i>	3
<i>u-cis-mer</i> -[OC(EBG)](β -ala)	+	<i>A</i>	This work
<i>u-cis-fac</i> -[Co(EBG)](β -ala)	+	<i>A</i>	This work
<i>u-cis-mer</i> -[Co(EBA)](gly)	-	<i>A</i>	This work
<i>u-cis-fac</i> -[Co(EBA)](gly)	-	<i>A</i>	This work
<i>u-cis-mer</i> -[Co(EBV)](gly)	-	<i>A</i>	This work
<i>u-cis-fac</i> -[Co(EBV)](gly)	-	<i>A</i>	This work

^a Chromatographic conditions: [Sb₂(D-tart)₂]²⁻ form of QAE Sephadex anion exchanger with water for Ref. 3; on Sephadex G-10 with water for this work.

TABLE III
ADJUSTED RETENTION VOLUMES AND SEPARATION
FACTORS OBTAINED ON ELUTION WITH WATER

Complex	Retention volume (ml)	Separation factor
<i>A-u-cis-mer</i> -[Co(EBG)(β -ala)] Δ Isomer	—	^a
<i>A-u-cis-fac</i> -[Co(EBG)(β -ala)] Δ Isomer	4.65 4.93	1.060
<i>A-u-cis-mer</i> -[Co(EBA)(gly)] Δ Isomer	5.02 4.85	1.035
<i>A-u-cis-fac</i> -[Co(EBA)(gly)] Δ Isomer	4.74 4.65	1.019
<i>A-u-cis-mer</i> -[Co(EBV)(gly)] Δ Isomer	3.84 3.69	1.041
<i>A-u-cis-fac</i> -[Co(EBV)(gly)] Δ Isomer	4.06 3.95	1.028

^a Partially resolved.

first, it is difficult to find a simple relationship between the order of elution and the absolute configuration of the anionic [CoN₂O₄]⁻ [5] and neutral [CoN₃O₃] complexes.

Each elution curve consists of two peaks, a large first peak and a smaller second peak. By repeating the elution procedure several times, fractions of the first and second peaks were collected and used for measurements of the visible and CD spectra. Thus two series of retention volumes of the enantiomeric pairs, *A* and Δ , were obtained. The separation factor for each enantiomeric pair was obtained from the retention volumes of two peaks in each elution curve because these complexes originally exist as an enantiomeric pair. The results are shown in Table III. It is obvious that the separation factor of the enantiomeric pair is largest with *fac*-[Co(EBG)(β -ala)], which contains a six-membered chelate ring, and decreases with increasing number of five-membered chelate rings. A similar trend had been observed during the separation of neutral cobalt(III) aa complexes on the Na⁺ form of the TSK-211 cation exchanger with Na₂[Sb₂(*d*-tart)₂] as the eluent [11].

From a comparison of the data in Table I, the degree of optical resolution decreases in the order

cis- β > *cis*- α . This is because both *cis*- β isomers have lower symmetrical chromophore (C₁) than the *cis*- α (C₂) isomer. As the optical resolution determined by column chromatography of chiral (or prochiral) complexes is based on the interactions between a chiral adsorbent and a chiral complex, the symmetry-dependent chiral discriminations (chirodiastaltic interactions) are greater for complexes with low symmetry.

This chromatographic method can be used to obtain the partial resolution of racemic amino acids and related compounds in the form of cobalt(III) complexes. The separation of optical isomers on the molecular sieve Sephadex G-10 is convenient and appears to be generally applicable for the optical resolution of non-labile metal chelate compounds. The method is rapid and simple and the molecular sieve in the column is stable for several years.

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