

**SYNTHESIS AND CHARACTERIZATION
OF THE FOUR TRIS[(S)-ASPARAGINATO-O,N(-1)]COBALT(III) ISOMERS.
A CIRCULAR DICHROISM STUDY OF ISOMER FORMATION***

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The four isomers of tris[(S)-asparaginato-O,N(-1)]cobalt(III) have been synthesized and characterized by ¹H-NMR and circular dichroism spectroscopies. The four isomers have been prepared by the reaction of (S)-(+)-asparagine with cobaltic hydroxide at elevated temperature and were separated through a combination of solubility characteristics and chromatographic procedures. The *fac* and *mer* isomers were distinguished by their electronic absorption spectra. The absolute configuration of the individual isomers has been deduced from circular dichroism and ¹H-NMR spectra. The Λ -*mer* isomer is preferred thermodynamically. This conclusion is based on increasing reaction times and through the use of activated charcoal. In the latter case only Λ -*mer* isomer is formed. This isomer has a stereochemistry ideally suited for extensive hydrogen bonding which enhances the solubility differences between isomers and account for its exclusive production.

Metal complexes with Co(N₃O)₃ chromophore can, in principle, exist in facial and meridional stereochemistry. When analogous bidentate ligands coordinate, the complexes have Λ -*mer*, Δ -*mer*, Λ -*fac* and Δ -*fac* topology. Results¹ show that in the case of the (S)-monoaminomonocarboxylic acids a mixture of all of these isomers is synthesized due to the nearly planar chelate rings. A higher yield of Λ isomers can be expected when the α -carbon atom of the amino acid contains a sterically bulky nonpolar side chain or has a side chain capable of hydrogen bond formation. This varying Λ : Δ isomer distribution reflects subtle interrelations of structure and stereochemistry.

Owing to the above mentioned facts, we have studied the synthesis of tris[(S)-asparaginato-O,N(-1)]cobalt(III) isomers in more detail utilizing the circular dichroism technique. We report here the circular dichroism together with the characterization of all four possible isomers.

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EXPERIMENTAL

(*S*)-(+)-Asparagine ($[\alpha]_D +31^\circ$ in 5*M*-HCl) and (*R*)-(–)-asparagine ($[\alpha]_D -29^\circ$ in 3*M*-HCl) were purchased from Fluka. All other chemicals were reagent grade. Electronic absorption spectra were measured on a Specord UV VIS spectrophotometer (C. Zeiss, Jena, G.D.R.). Circular dichroism curves were obtained using Cary 60 spectropolarimeter fitted with a CD accessory. A Varian XL-100 (100 MHz) spectrometer was used to measure proton magnetic resonance spectra.

Preparation of Isomers

0.025 mol of freshly prepared $\text{Co}(\text{OH})_3$ was suspended in a solution of 0.06 mol of (*S*)-(+)-asparagine in 70 ml of water. The mixture was stirred and heated for 5 h at 70°C . After this time unreacted $\text{Co}(\text{OH})_3$ together with Λ -isomers were filtered off giving solid S_1 and filtrate F_1 . S_1 was extracted with warm water until colorless washings were obtained. Cooling this extract yield Λ -mer isomer which was filtered off, washed with water, ethanol and dried in air. For $\text{C}_{12}\text{H}_{21}\cdot\text{N}_6\text{O}_9\text{Co}\cdot\text{H}_2\text{O}$ (470.3) calculated: 30.64% C, 4.93% H, 17.87% N; found: 30.67% C, 5.05% H, 17.79% N.

Solid S_1 after dissolution in concentrated HCl and dilution with water afforded red Λ -fac isomer which was washed with water to neutrality, further with ethanol and dried in air. For $\text{C}_{12}\text{H}_{21}\text{N}_6\text{O}_9\text{Co}\cdot 3\text{H}_2\text{O}$ (506.4) calculated: 28.74% C, 5.37% H, 16.61% N; found: 28.71% C, 5.28% H, 16.63% N.

Filtrate F_1 was passed through a column of a cation exchanger (Dowex 50WX8, 50–100 mesh, in H^+ cycle). Reduction of the eluate volume *in vacuo* produced red crystals of Δ -fac isomer which were filtered off, washed with water, ethanol and dried in air. For $\text{C}_{12}\text{H}_{21}\text{N}_6\text{O}_9\cdot 2\text{H}_2\text{O}$ (488.2) calculated: 29.50% C, 5.16% H, 17.22% N; found: 29.57% C, 5.18% H, 17.28% N.

Filtrate after Δ -fac isomer was evaporated with a small portion of alumina to dryness and then applied on the top of alumina column (2.5×30 cm). Elution with 20% 1-propanol gave two bands. First one after evaporation of the eluate to dryness yielded Δ -mer isomer. For $\text{C}_{12}\text{H}_{21}\text{N}_6\cdot\text{O}_9\text{Co}\cdot 4\text{H}_2\text{O}$ (524.4) calculated: 27.48% C, 5.57% H, 16.04% N; found: 27.52% C, 5.61% H, 15.98% N. Further elution of the remaining red band and evaporation of the eluate to dryness afforded second crop of Δ -fac isomer.

Preparation of Δ -mer-Tris[(*R*)-asparaginato]cobalt(III)

This isomer was prepared according to ref.², starting from (*R*)-(–)-asparagine. For $\text{C}_{12}\text{H}_{21}\text{N}_6\cdot\text{O}_9\text{Co}\cdot\text{H}_2\text{O}$ (470.3) found: 30.61% C, 5.00% H, 17.90% N.

Product Analysis

500 ml of a solution containing 0.005 mol of $[\text{Co}(\text{NH}_3)_6](\text{NO}_3)_3$, 0.015 mol of (*S*)-(+)-asparagine and 1 g of activated charcoal (Norrit A) was heated under reflux and at a proper time interval 100 ml of reaction mixture were removed and filtered giving solid S_1 and filtrate F_1 . S_1 was dissolved in 10 ml of 1 : 1 HCl, filtered and filtrate F_2 obtained was immediately added to F_1 and volume was completed to 250 ml. Circular dichroism spectrum of this solution was recorded. Another 100 ml of F_1 was sorbed on Dowex 50WX8 cation exchanger (50–100 mesh, H^+ cycle), eluate F_3 obtained was mixed with 10 ml of 1 : 1 HCl extract of S_1 and volume of this solution was completed to 250 ml and the circular dichroism spectrum was scanned.

20 μ l of F_3 was chromatographed on an alumina layer (without binder) using 20% 1-propanol as solvent. Simultaneously Δ -*fac* isomer as a marker was running. Detection which was made spraying the chromatogram with a solution of Na_2S revealed two compounds. One at the start line (Λ -*mer*) and second one which corresponded to the Δ -*fac* isomer.

RESULTS AND DISCUSSION

SYNTHESIS AND IDENTIFICATION OF ISOMERS

Several methods are used for the synthesis of cobalt(III) amino acid complexes. However, only the reaction of $Co(OH)_3$ with (S)-(+)-asparagine afforded all expected isomers, but in the reaction mixture Λ -*mer* isomer predominates (probably due to the equilibration catalyzed by Co(II) ions). At the same time its amounts increases with increasing reaction time. On the other hand charcoal catalyzed reaction produces Λ -*mer* isomer as a main product.

Other methods utilizing $[Co(NH_3)_5(H_2O)]Cl_3$, $[Co(NH_3)_4CO_3]NO_3$ or $K_3.[Co(CO_3)_3]$ as a Co(III) precursor, or starting from Co(II) gives the mixture of Λ -*mer* and Δ -*fac* isomers of different ratios, but yield of Λ -*mer* isomer was always higher than that of Δ -*fac* one.

TABLE I

Electronic Absorption Spectra and CD Extrema of the Isomers
Spectra were taken in 15% hydrochloric acid, λ_{max} in $10^{-3} cm^{-1}$.

Isomer	pseudo $^1A_{1g} \rightarrow ^1T_{1g}$		pseudo $^1A_{1g} \rightarrow ^1T_{2g}$		CD	
	$\lambda_{max} \bar{\nu}$	ϵ	$\lambda_{max} \bar{\nu}$	ϵ	$\lambda_{max} \bar{\nu}$	$\Delta\epsilon$
Λ - <i>mer</i>	18.7	110	26.8	180	18.9	+2.85
					27.2	-0.4
Δ - <i>mer</i>	18.8	112	26.8	183	18.9	-1.6
					26.9	+0.4
Δ - <i>mer</i> ^a	18.8	110	26.7	181	18.8	-2.85
					27.0	+0.41
Λ - <i>fac</i>	19.1	214	26.3	205	18.3	+2.4
					27.2	-0.4
Δ - <i>fac</i>	19.0	210	26.3	208	19.0	-2.0
					26.9	+0.4

^a $Co((R)\text{-Asn})_3$.

Cobalt(III) amino acid complexes which exist as *fac* (C_{3v} symmetry) and *mer* isomers (C_{2v}) differ from each other in their symmetry. Yamatera³ showed that the degenerate ${}^1T_{1g}$ octahedral level is split into A_2 and E_a components for isomers with C_{3v} symmetry. On the other hand, isomers with C_{2v} symmetry have A_2 , B_2 and B_1 components, which suggests a greater splitting of the ${}^1A_{1g} \rightarrow {}^1T_{1g}$ transition. Electronic absorption spectra which have been obtained (Table I) are consistent with the expected splitting.

The absolute configuration of the isomers has been deduced either from the circular dichroism spectra (Figs 1–3 and Table I) and from the ${}^1\text{H-NMR}$ spectra (Table II).

Configurational effects are manifested in the circular dichroism spectra of complexes belonging to the D_3 group by two bands with negative and positive sign. At the same time the major circular dichroism band is due the E_a transition⁴. Because in cobalt(III) amino acid chelates (symmetry C_3 and C_1) the octahedral ${}^1T_{1g}$ state splits down into components ${}^1A + {}^1E$ or ${}^1A + {}^1A + {}^1A$ respectively⁵, which correspond (in the case of isomer with axial symmetry) to the A_2 and E_a dihedral states, it is possible to relate major positive circular dichroism absorption for the ${}^1T_{1g}$ transition

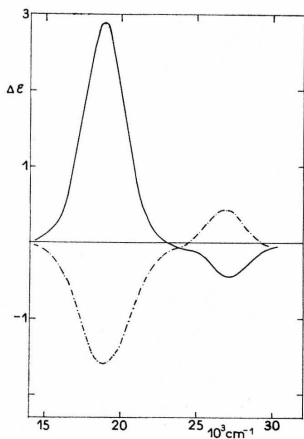


FIG. 1

The Circular Dichroism Spectrum of Δ -mer (—) and Δ -mer (---) Isomers

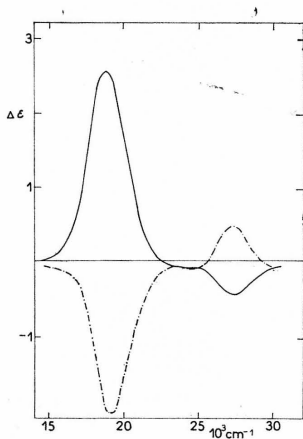


FIG. 2

The Circular Dichroism Spectrum of Δ -fac (—) and Δ -fac (---) Isomers

with the (+)-[Co(en)₃]³⁺ (en means ethylenediamine), the absolute configuration of which is Λ (ref.⁶). Similarly the major negative band observed in the ¹T_{1g} spectral region can be compared with the (-)-[Co(en)₃]³⁺ ion, which has Δ absolute configuration.

Isomers were also identified using ¹H-NMR spectra. As a result of the different symmetry of the *fac* and *mer* isomers, their spectra are quite different. Resonances of individual protons, which are given in Table II, were assigned both on the basis of their signal integrated intensity and the temperature dependence of the chemical shift of —NH₂ protons. In the case of Λ -*mer* isomer, the NH resonances partly overlapped giving peak ratios of 2 : 1 : 1 : 2, while for symmetrical *fac* isomers a ratio of 2 : 2 was observed. From the temperature dependence of the chemical shift it follows that both the —CONH₂ groups and the axial (Λ -isomer) or equatorial (Δ -isomer) hydrogen atoms of the —NH₂ group are hydrogen bonded with the

TABLE II
Proton Chemical Shifts^a (ppm) of the Ligands in Co((S)-Asn)₃ Isomers

—CONH ₂	NH _{eq} (<i>trans</i>)	NH _{eq} (<i>cis</i>)	NH _{ax} (<i>cis</i>)	NH _{ax} (<i>trans</i>)	α -CH	β -CH ₂
<i>Λ-mer</i>						
7.23 ^b	6.57	6.09	5.76 ^b	5.42	4.36 ^c	3.32
<i>Δ-mer</i>						
7.22	6.50	5.58 ^d	5.18 ^b		4.46 ^c	3.40
—CONH ₂	NH _{eq}		NH _{ax}		α -CH	β -CH ₂
<i>Λ-fac</i>						
7.22 ^b	6.38		5.81		4.08	3.34
<i>Δ-fac</i>						
7.16 ^b	5.62		5.22		4.26	3.42

^a Trifluoroacetic acid (sodium 2,2-dimethyl-2-silapentane-5-sulfonate as external standard); ^b protons undergoing upfield shift on heating; ^c peaks with intensity ratio 2 : 1, ^d common peak together with NH_{ax(trans)}. For assignments of NH_{ax} and NH_{eq} see ref.¹³.

solvent. Further, the steric situation at the α -CH site which is different for Λ and Δ isomers allows in the case of *mer* isomers to determine their absolute configuration. Isomers in which methine protons are sterically compressed resonate at a lower field in comparison with those in which α -CH protons are not shielded⁷. Even though this was originally applied to the bis(amino-acidato)cobalt(III) complexes, it is valid also for tris-bidentate amino acid complexes because the critical sites are mutually in *cis* positions. Thus, the ¹H-NMR spectra which have been obtained confirm the absolute configuration of the isomers as inferred from their circular dichroism.

QUESTION OF STEREOSELECTIVITY

Recently it was found² that the substitution of ammonia in the $[\text{Co}(\text{NH}_3)_6]^{3+}$ ion by (*S*)-(+)-asparagine (Asn) or (*S*)-(+)-glutamine (Gln) in the presence of charcoal leads to the formation of Λ -*mer*- $[\text{Co}((\text{S})-(+)-\text{Asn})_3]$ and Λ -*fac*- $[\text{Co}((\text{S})-(+)-\text{Gln})_3]$, respectively. At the same time, the above substitution in the absence of charcoal but in alkaline medium did not afford these simple reaction products.

Substitution reactions of the $[\text{Co}(\text{NH}_3)_6]^{3+}$ ion with amino acids which proceed through a series of intermediates of the $[\text{Co}(\text{NH}_3)_4(\text{AB})]^{2+}$ and $[\text{Co}(\text{NH}_3)_2(\text{AB})_2]^+$ types should give, in the absence of any stereoselective contributions, a statistical *mer* : *fac* 3 : 1 ratio of isomers. Besides this statistical factor, the ratio is affected by other factors, such as differences in solubilities between the *fac* and *mer* isomers. The solubilities are influenced by the masking of the $-\text{NH}_2$ groups by the amino acid side chains. The degree of this masking depends both on the conformation and the steric volume of these chains. The masking can change both the solvent-solute interactions and the intermolecular interactions which hinder and cause precipitation, respectively. In this study, the solvent-solute effects appear to predominate. Furthermore, the prevention of *mer* \rightarrow *fac* isomerization when amino acid with a sterically bulky side chain is coordinated also must be taken into account. Also, in the absence of any intramolecular interactions (bonding or nonbonding) the synthesis should give a 1 : 1 mixture of the Λ and Δ diastereoisomers. However, the higher thermodynamic stability of Λ -isomers with an equatorial disposition of the alkyl side chains needs to be considered, even though this difference is small (assumed⁸ to be about 1 kcal mol⁻¹). Actually, when the substitution was studied with the (*S*)-amino acid containing the same number of carbon atoms as in asparagine (*i.e.* with (*S*)- α -aminobutanoic acid) to determine the factors influencing distribution of geometrical isomers⁹, the same geometrical isomer was obtained as in case of asparagine. However, the product was racemic at the cobalt centre, thus unequivocally demonstrating the influence of the $-\text{CONH}_2$ groups on the diastereoisomer distribution.

To understand the factors contributing to the observed preferred formation of the Λ -*mer* isomer for tris((*S*)-Asn)cobalt(III), the circular dichroism spectra of synthetic

mixtures were taken. From the CD spectra (Fig. 4) it can be concluded that the optical activity of reaction mixture results from two contributions: from the optical activity of the $\text{Co}((S)\text{-Asn})_3$ isomers and from the reaction intermediates (curves 1, 2). The concentration of the latter decrease with increasing reaction time. In Fig. 4, the CD spectra of reaction mixtures obtained after using ion exchange chromatography to remove charged intermediates are also given (curves 3 and 4). The CD patterns of the eluates strongly resemble the CD spectrum of $\Lambda\text{-mer-}[\text{Co}((S)\text{-Asn})_3]$ (Fig. 1). From the values of the ellipticity it follows that either the Δ -isomer is present in the reaction mixture or the yield of $\Lambda\text{-mer}$ isomer must be low. From the cobalt content in the eluate obtained after 24 h of reaction, a $\Delta\epsilon = +1.41$ was calculated. This value, when compared with that obtained for pure $\Lambda\text{-mer}$ isomer ($\Delta\epsilon = +2.85$), is somewhat low. This difference suggests that the $\Lambda\text{-mer}$ isomer is not the sole product. From the chromatography it follows that at least one of the Δ -isomers is also present in the reaction mixture. Both Λ isomers have higher $\Delta\epsilon$ values (Table I).

The above mentioned results undoubtedly indicate that the $-\text{CONH}_2$ groups have some importance on isomer discrimination and this influence can be understood

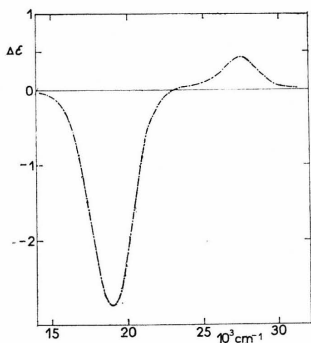


FIG. 3

The Circular Dichroism Spectrum of $\Delta\text{-mer-Co}((R)\text{-Asn})_3$

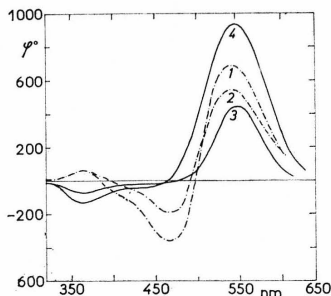


FIG. 4

The Circular Dichroism Spectrum of $[\text{Co}(\text{NH}_3)_6]^{3+} + 3(S)\text{-Asn}$

Reaction mixture after: 1 30 min, 2 120 min, 3 120 min, ion exchange chromatography (eluate), 4 24 h ion exchange chromatography (eluate contains 12.37 mg Co/250 ml). Conditions: $[\text{Co}(\text{NH}_3)_6]^{3+}$ 0.001 mol, (S)-Asn 0.003 mol.

in terms of the conformation of the amino acid side chain. A study of cobalt(III) complexes of (*S*)-leucine, (*S,S*)-isoleucine and (*S*)-norleucine (amino acids differing from each other only in the type of their side alkyl chain) revealed⁹ that the isomer ratio depends on the steric volume of the alkyl side chain protruding from the α -carbon atom of the amino acid proper and on the conformation of this chain. The free rotation of the alkyl groups reduces the differences in the solvation of the $-\text{NH}_2$ group between isomers. On the other hand, if the conformation is fixed (for example, by the formation of hydrogen bonds), the differences in solvation (and hence, solubility) between the isomers will be enhanced.

Amino acids form only slightly puckered chelate ring¹⁰, and thus, differences in thermodynamic stability between diastereoisomers are small. Therefore, preferred formation (if any) of one of the four expected isomers may reflect solubility differences between diastereoisomers in a given solvent. The solubility of uncharged chelates in water depends on the degree which the ligand nucleophilic groups are masked. In the case of Asn these are the $-\text{NH}_2$ and $-\text{CONH}_2$ groups. If both $-\text{NH}_2$ and $-\text{CONH}_2$ groups are involved in bonding interactions for one of the four possible stereoisomers, the solvation is prevented. The solubility of that isomer will be low and it will be preferentially precipitated from solution. In other words, if a set of isomers have differing abilities toward hydrogen bonding, the one isomer in which the most extensive interchelate hydrogen bonding occurs will precipitate. Inspection of Dreiding models reveals the following sites of interaction in the tris(*S*-Asn) cobalt(III) complexes:

1) between two *cis* $-\text{CONH}_2$ groups, 2) between a $-\text{CONH}_2$ group and an equatorial hydrogen atom of the $-\text{NH}_2$ group of the neighbouring chelate ring.

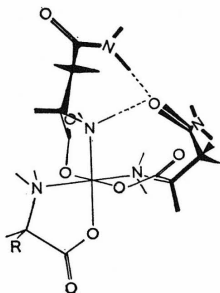


FIG. 5
Possible Conformation of Side Chains
in Λ -mer Isomer from the Viewpoint of
Hydrogen Bond Formation

Considering these possible sites of hydrogen bonding together with the optimum bond angle and bonding distances required for the effective hydrogen bonding between $-\text{CONH}_2$ groups, the differences in formation of individual isomers becomes more apparent. Only in Λ -*mer*-[Co((S)-Asn)₃] (or in Δ -*mer*-[Co((R)-Asn)₃], which is formed in the substitution reaction starting from (R)-asparagine and shows that the configuration around cobalt center depends on the absolute configuration of the ligand used) are all of the necessary factors¹¹ realized along with the fact that the multiple hydrogen bonds between the two $-\text{CONH}_2$ groups and the equatorial hydrogen atom of $-\text{NH}_2$ group (Fig. 5) with a bond angle of 60° and a bond distance of 2.85 Å (ref.¹¹) can be constructed only for this isomer. This interaction, which prevents the formation of hydrogen bonds between the solvent (water) and both H atoms of the $-\text{CONH}_2$ and $-\text{NH}_2$ groups, leads to the precipitation of Λ -*mer* isomer. ¹H-NMR spectra of these groups in deuterated trifluoroacetic acid show evidence of hydrogen bonds. In the remaining three isomers, the optimum bond angle and distances necessary for strong hydrogen bonds can not be realized due to the steric restrictions.

The differences account for the exclusive production of Λ -*mer* isomer inasmuch as the charcoal allows the system to continually approach an equilibrium situation. The natural 3 : 1 *mer* to *fac* ratio causes initial precipitation of some of the Λ -*mer* isomer (independent equilibration experiments of individual isomers in the presence of charcoal showed the exclusive precipitation of the Λ -*mer* isomer occurs before saturation is reached for the Δ -*fac* isomer, which has the second lowest water solubility). The precipitation of some of the Λ -*mer* isomer will necessitate a shift toward more of the Λ -*mer* isomer in solution at the expense of the remaining Λ and Δ isomers. Hence, only the Λ -*mer* isomer is isolated synthetically. Other cobalt(III) amino acid systems with coordinated amino acids possessing polar side chains Λ : Δ ratios other than 1 : 1 (ref.¹²) should be considered from this point of view.

The elemental analyses were carried out at the Analytical Department of this Institute (Head Dr L. Helešić).

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