in the Experimental Section, these rate plots were based on an assumed R_∞, namely, on the assumption that at infinite time the complex would have essentially the same isotopic composition as the solvent, which was present in vast excess. All oxygen atoms in the dimer must therefore be kinetically equivalent and presumably structurally equivalent as well.[23]

Activation energies and enthalpies, calculated from the data of Tables II and IV, are, for the water-exchange reaction, E_a = 23.4 kcal/mole, $\Delta S^{\pm}$ = +0.5 eu, and for the cleavage reaction, E_a = 21.3 kcal/mole, $\Delta S^{\pm}$ = −11 eu.

(23) Earnshaw and Lewis (*J. Chem. Soc.*, 396 (1961)) assert that the dimer "definitely" falls into the class having two bridging groups, but no reason is given for this assertion. The compound which they prepared, by a method attributed to Dwyer, but never published, was formulated as $[(phen)_2Cr(OH)_2Cr(phen)_2]I_4 \cdot 4H_2O$, so that the distinction between a single oxo and two hydroxo bridges could not be made on the basis of stoichiometry. Probably the most compelling argument against the oxo bridge is that the magnetic susceptibility the compound, measured by Earnshaw and Lewis, is very nearly that expected for three unpaired electrons per chromium atom, whereas considerable interaction between the two chromium atoms would be expected if a single oxo bridge connected them.

CONTRIBUTION FROM THE DEPARTMENT OF INDUSTRIAL CHEMISTRY, FACULTY OF ENGINEERING, UNIVERSITY OF TOKYO, TOKYO, JAPAN

Ferric Complexes with L-Cysteine at Low Temperature[1a]

BY AKIRA TOMITA,[1b] HIDEFUMI HIRAI, AND SHOJI MAKISHIMA

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Reactions between ferric ion and L-cysteine in ethanolic solution give labile blue, red, and violet ferric complexes, which become inert when chilled to −78°. The violet complex was isolated and characterized for the first time. A green complex was obtained only at low temperatures. The absorption, optical rotatory dispersion, and circular dichroism spectra of these complexes were measured and their structures were assigned as follows. The blue, red, and violet complexes are S,O-coordinated complexes, and the mole ratios of cysteine to iron are 1:1, 1:2, and 1:3, respectively. The new green complex is an S,N-coordinated tris complex. The violet and green complexes are thermally interconvertible in ethanolic solution. The mechanism of the catalytic oxidation of cysteine is discussed briefly.

Introduction

The study of ferric complexes with cysteine is of great interest from biological points of view. One of them is that ferric ion catalyzes the oxidation of cysteine which may occur in living cells. Ferric cysteinate complex is an intermediate compound in this catalytic reaction. The other point is that this complex is a simple model compound for some nonheme iron proteins having iron–sulfur bindings, such as ferredoxin, and this kind of study would give some information about the structures of these proteins. Many studies on this problem have been made, but they leave many questions still unexplored, probably because these complexes are very labile. Harris[2] stated that the violet coloration obtained by adding ammonia to a cysteine solution is due to the presence of ferric ion. Schubert[3] reported that the oxidation of bis(cysteinato)iron(II) gives a violet ferric complex in alkaline media. The blue complex in acidic solution and the red complex in neutral or alkaline solution were also reported,[4,5] and the absorption spectra were determined.[5] Tanaka, Kolthoff, and Stricks[6] studied the catalytic oxidation of cysteine by ferric ion and reported that the violet complex has an absorption maximum at about 580 mμ. Many studies[7,8] on iron thioglycolate complexes have also been made.

In our previous work,[9] we found that these labile complexes become inert in ethanolic solution when chilled to −78°. The present paper deals mainly with an isolated violet complex and a new green complex. In addition, the composition of these colored complexes and the relation among them will be discussed by means of the absorption spectra.

Experimental Section

Materials.—Ferric chloride, ferric ammonium sulfate, L-cysteine, and L-cysteine hydrochloride monohydrate were standard reagent grade chemicals. The purity of amino acids was assayed to be more than 95% by ferricyanide oxidation; they were used without further purification. Ethanol was refluxed over Drierite and twice distilled.

Spectra.—Absorption spectra were recorded on a Hitachi EPS-2 double-beam spectrophotometer. Optical rotatory dispersion (ORD) and circular dichroism (CD) curves were obtained with a Japan Spectroscopic ORD/UV-5 spectropolarimeter with a CD attachment. The cell used for the measurements was a vacuum-jacketed low-temperature cell[10] which was constructed to fit into the cell compartments of the above instruments. The change in temperature of the sample was determined with a copper–constantan thermocouple inserted into the contents of a

(1) (a) A. Tomita, H. Hirai, and S. Makishima, 10th International Conference on Coordination Chemistry, Nikko, Japan, 1967. (b) To whom correspondence should be directed: Chemical Research Institute of Non-Aqueous Solutions, Tohoku University, Sendai, Japan.

(2) L. J. Harris, *Biochem. J.*, **16**, 739 (1922).

(3) M. Schubert, *J. Am. Chem. Soc.*, **54**, 4077 (1932).

(4) L. Michaelis and E. S. G. Barron, *J. Biol. Chem.*, **83**, 191 (1928).

(5) F. M. Page, *Trans. Faraday Soc.*, 919 (1955).

(6) N. Tanaka, I. M. Kolthoff, and W. Stricks, *J. Am. Chem. Soc.*, **77**, 1996 (1955).

(7) D. L. Leussing and I. M. Kolthoff, *ibid.*, **75**, 3904 (1953).

(8) C. Michou-Saucet and J.-C. Merlin, *Bull. Soc. Chim. France*, 1905 (1965).

(9) A. Tomita, H. Hirai, and S. Makishima, *Inorg. Chem.*, **6**, 1746 (1967).

(10) W. B. Rose, J. W. Nebgen, and F. I. Metz, *Rev. Sci. Instr.*, **36**, 1319 (1965).

cell, and we found that a constant temperature of −75° could be obtained during spectral measurements, using liquid nitrogen as a coolant. The temperature accuracy is estimated to be ±1° at this temperature.

Blue Complex.—This complex was obtained from freshly prepared 0.02 *M* ferric chloride and 0.02 *M* cysteine hydrochloride monohydrate in 90% ethanol, 0.5 *M* ammonium chloride and 0.05 *M* hydrogen chloride being added to adjust the pH to 2.40.

Red and Violet Complex in Water-Free Ethanol.—These complexes were obtained from freshly prepared 0.005 *M* ferric chloride in water-free ethanol and 0.005 *M* cysteine dissolved in water-free basic ethanol. Ethanol was made basic by bubbling through it gaseous ammonia (for 20 min) which was previously dried over potassium hydroxide pellets.

Violet and Green Complex in 90% Ethanol.—The violet complex was prepared with ferric hydroxide, cysteine-free base, and ammonia as starting materials. Ferric hydroxide gel was prepared from ferric ammonium sulfate (0.964 g, 0.002 mole) and aqueous ammonia, and the resulting precipitate was separated from the solution by centrifugation and decantation. This process was repeated if the supernatant had any remaining sulfate ions. Freshly prepared gel was added with vigorous stirring to cysteine (0.765 g, 0.006 mole) in 200 ml of ammoniacal 90% ethanol at room temperature. Immediately upon addition of the gel, an intense violet color developed, and it turned to green when chilled to −78°. Removal of unchanged ferric hydroxide gel by twice filtering left a green solution, which gave green precipitate on standing at −78°. This precipitate was filtered, washed with ammoniacal ethanol at −78°, and dried at room temperature giving a violet mass, tris(cysteinato)iron(III) dihydrate; two filtrations lower the yield (32%) of the desired complex. *Anal.* Calcd for $FeC_9H_{22}N_3O_8S_3$: Fe, 12.35; C, 23.90; H, 4.90; N, 9.29. Found: Fe, 12.69; C, 23.99; H, 4.81; N, 9.68. These agreements are quite reasonable, in view of the impossibility of any purification procedures. The infrared spectrum (KBr disk) of this complex shows COO^- stretching bands at 1618 and 1385 cm^{-1}, and NH_3^+ stretching bands at about 3000, 1600, and 1491 cm^{-1}.

If this complex was prepared in 90% ethanol from ferric chloride, cysteine hydrochloride monohydrate, and potassium hydroxide, it contained potassium chloride as impurity.

In order to isolate the green complex, the green precipitate was separated from solution by centrifugation at −78°. It was washed with ammoniacal ethanol and ammoniacal ether, and dried *in vacuo* at −78° for 20 days. This green solid, however, changed irreversibly into the violet one immediately upon raising the temperature.

Results

Acidic Range.—The blue complex could not be prepared in water-free ethanol or in water-free acetone, but a blue color developed when small amounts of water were added to these solutions. The spectral data for this complex are listed in Table II. Figure 1 shows a continuous variation plot at the wavelength 620 mμ corresponding to the absorption maximum of the blue complex. No intensely colored species is present under this condition. This figure indicates that the blue complex is of a 1:1 mole ratio. The absorption band at 620 mμ was optically inactive.

Alkaline Range in Water-Free Ethanol.—Two complexes, red and violet, were obtained in ammoniacal water-free ethanol (pH ~11). The absorption spectra at different mole ratios are shown in Figure 2, and the ORD and CD spectra of the red and violet complexes are shown in Figure 3. The red complex shows the absorption maximum at 525 mμ. The absorption and ORD curves of the violet complex are similar to those obtained in aqueous borate buffer solution,[9] and the absorption maxima are at 590 and 443 mμ. The formation of these complexes depends on both the mole ratio of cysteine to iron and the concentration of the system as shown in Table I.

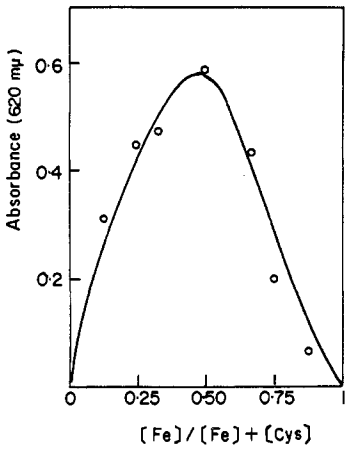


Figure 1.—Continuous variation plot for the ferric cysteinate complex in 90% ethanol in the presence of NH_4Cl–HCl as buffer (pH 2.40).

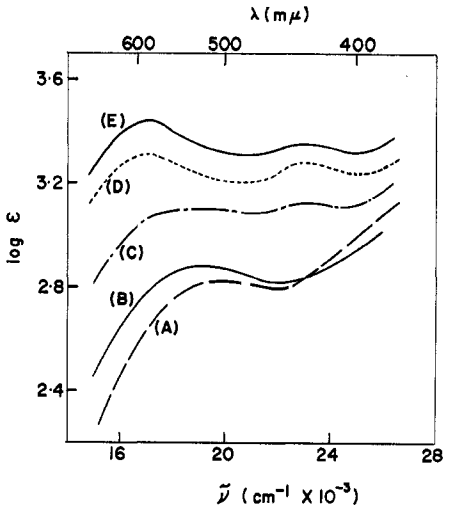


Figure 2.—Absorption spectra of the ferric cysteinate complex in ammoniacal water-free ethanol at −75°. [Fe^{3+}], 0.5 m*M*; mole ratios (Fe^{3+}:Cys) are: (A) 1:1, (B) 1:2, (C) 1:3, (D) 1:5, (E) 1:9.

TABLE I

EFFECTS OF MOLE RATIO AND CONCENTRATION ON THE FORMATION OF RED (R) AND VIOLET (V) COMPLEXES

	Cysteine:iron				
Concn, m*M*	1	2	3	5	9
0.1	R	R	R	R + V	V
0.5	R	R	R + V	V	V
2.0	R	R + V	V	V	V

When the coolant is removed after the measurement of the spectrum of the violet complex, the band at 525 mμ due to the red complex appears within 1 min, and then the red color fades rapidly. The decomposition at −78° is much slower. On the other hand, the isolated violet complex seems to be considerably stable at room temperatures.

TABLE II

SUMMARY OF SPECTRAL DATA FOR FERRIC COMPLEXES WITH THIOL ACIDS

Color	Ligand	Solvent (pH)	Absorption max, mμ	Extinction coeff	Ref
Blue	Cysteine	H_2O (1.80)	622	150	*a*
		90% C_2H_5OH (2.40)	620	590	*b*
	TGA[c]	90% C_2H_5OH (acidic)	620	*Ca.* 500[d]	*b*
Red	Cysteine	H_2O (7.40)	480	3000	*a*
		C_2H_5OH (~11)	525	770	*b*
	TGA	C_2H_5OH (~11)	490	1130	*b*
Violet	Cysteine	H_2O (9.50)	570, 440	3410, 2410	*c*
		90% C_6H_5OH[f]	590, 440	*Ca.* 3000, 2000[g]	*b*
		C_2H_5OH (~11)	590, 443	2830, 2190	*b*
	TGA	C_2H_5OH (~11)	535, 410	2900, 2080	*b*
Green	Cysteine	90% C_2H_5OH (~11)	584	640[g]	*b*

[a] Reference 5. [b] Present study. [c] Thioglycolic acid, $HSCH_2COOH$. [d] This complex is rather labile even at −75°. [e] Referenece 9. [f] $FeCl_3$:$HSCH_2CHNH_2COOH \cdot HCl$:KOH = 1:3:6 (see text). [g] Trace of precipitate was observed.

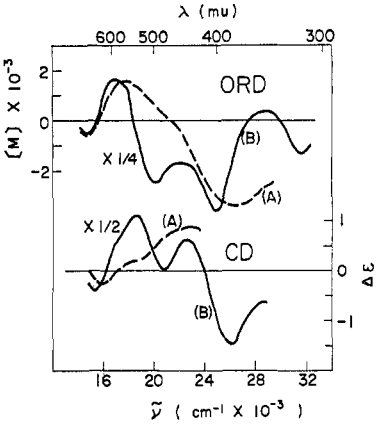
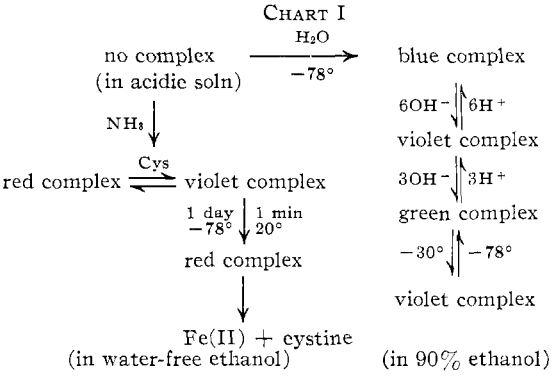


Figure 3.—Rotatory dispersion and circular dichroism spectra of the red (A) and violet (B) complexes in ammoniacal water-free ethanol at −75°.

CHART I



Alkaline Range in 90% Ethanol.—The behavior of complex formation in 90% ethanol is quite different from that in water-free ethanol. The most remarkable difference is that a green complex can be prepared easily in 90% ethanol. The blue complex prepared from ferric chloride and cysteine hydrochloride monohydrate (1:3) in 90% ethanol at −78° changes into the violet complex upon addition of 6 equiv of potassium hydroxide and further into the green complex by an additional 3 equiv of potassium hydroxide. This green complex goes back into the violet complex and then into the blue complex by lowering pH. The green complex can also be obtained by chilling the violet complex prepared at room temperature in the presence of excess ammonia. This green complex is thermally interconvertible with the violet complex in solution. Raising the temperature of the green solution to about −30° resulted in rapid formation of the violet solution. This change is, however, irreversible in the solid state. The relations among these four complexes are shown in Chart I. The left-hand side corresponds to the results in water-free ethanol, and the right-hand side to those in 90% ethanol.

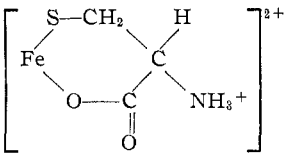
Thioglycolate Complex.—We reexamined the absorption spectra of the ferric thioglycolate complexes which will show a very close analogy with S,O-coordinated cysteine complexes. The blue complex was obtained in acidic 90% ethanol. The red and violet complexes were obtained in ammoniacal water-free ethanol, and the formation of these complexes depends on the mole ratio of thioglycolic acid to ferric ion. This tendency is quite similar to that of cysteine complexes. On the other hand, it is noteworthy that the green thioglycolate complex cannot be prepared under the same condition as that for cysteine complex.

The spectral data for the ferric complexes with cysteine and thioglycolic acid are summarized in Table II. The absorption band of the blue complex, which is assigned to be a d–d transition,[5] is affected slightly by the structure of the ligand, whereas the bands of the red and violet complexes are much more sensitive to the ligand: the band of the thioglycolate complex is located at shorter wavelength than that of the corresponding cysteine complex.

Discussion

Blue Complex.—Table II indicates that the blue complex obtained in 90% ethanol is the same one as obtained in aqueous solution,[5] although their intensities are somewhat different. Page[5] determined the extinction coefficient of this very labile complex at room temperature. It is felt, therefore, that he might underestimate this value. The coordination to iron is considered to occur by means of the sulfhydryl and carboxyl groups of cysteine, since the absorption spectrum of this complex is very similar to that of the blue ferric thioglycolate complex as shown in Table II.

This similarity, coupled with the result of Figure 1, suggests formula I for this complex. Page[5] also concluded that the blue complex in aqueous solution is of 1:1 mole ratio. No information is available concerning the coordination number and the other coordinating ligands, but it is obvious that some water molecules coordinate to iron because of the necessity of water for complex formation. As the band at 620 mμ is optically inactive, the bindings between iron and cysteine are rather weak, as suggested by Page[5] from his spectral data.



I

Red and Violet Complexes.—The violet complex was obtained in three different ways as shown in Chart I: (1) at higher mole ratio of cysteine to iron in ammoniacal water-free ethanol, (2) at relatively lower pH in 90% ethanol, and (3) at higher pH and at higher temperatures in 90% ethanol. These three complexes and that obtained in aqueous solution[6,9] are spectrophotometrically identical (see Table II). The elemental analysis of the isolated violet complex shows the mole ratio of this complex to be 1:3. The infrared spectrum indicates the presence of a chelated carboxylate and a protonated amino group. The antisymmetric stretching band of free carboxylate anion appears at 1600 cm^{-1}, and the symmetric one at 1400 cm^{-1}. The shift of +18 cm^{-1} for the former band and that of −15 cm^{-1} for the latter band are characteristic of the chelated carboxyl anion. Hence the oxygen does coordinate to ferric ion, and the nitrogen does not. As the sulfhydryl group has a great affinity with ferric ion, we conclude that this violet complex is an S,O-coordinated tris complex (II).

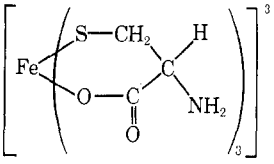
Tanaka, Kolthoff, and Stricks[6] postulated an equilibrium comprising a 1:2 and a 1:3 species in ammoniacal aqueous solution, the pH of which is similar to our system

$$[Fe(OH)(Cys)_2]^{2-} + Cys^{2-} \rightleftharpoons [Fe(Cys)_3]^{3-} + OH^-$$

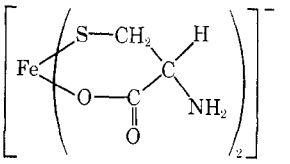
$[Fe(Cys)_3]^{3-}$ corresponds to the violet complex as mentioned above. We may safely assume that $[Fe(OH)(Cys)_2]^{2-}$ corresponds to our red complex (III), since Figure 2 and Table I show that the red complex is the only species except the violet complex in that pH region.

In studies of the ferric thioglycolate complex, the presence of $[Fe(OH)(TGA)_2]^{2-}$ [7] and $[Fe(TGA)_3]^{3-}$ [8] was reported, but no reasonable assignment of their absorption spectra was given.[11] We determined the absorption spectra of the red and violet complexes (see Table II). They reasonably correspond to those of cysteine complexes, in spite of some hypsochromic shifts. In the cases of thioglycolate complexes, we also concluded that $[Fe(OH)(TGA)_2]^{2-}$ is red and $[Fe(TGA)_3]^{3-}$ is violet. This coincidence strongly suggests S,O coordination in the red and violet cysteine complexes.

The ORD and CD curves of these two complexes (Figure 3) seem to support this conclusion that they have similar orientation of ligands around ferric ion, because both have three CD bands in the visible region and they have the same signs, that is, −, +, +, in order of increasing frequency. ORD curves in this region are also analogous in shape.

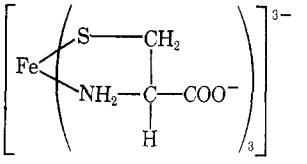


II



III

Green Complex.—The pure violet tris complex was obtained after a complete washing of the green precipitate at −78°. This fact suggests that the green complex must also be a tris complex. In other words, if it were a bis complex (including the possibility of an S,N,O-coordinated bis complex) or polymeric species, no tris complex could have been obtained because of a lack of the third cysteine ligand. This complex is proposed to be an S,N-coordinated tris complex (IV) for the following reason. There is no such green complex in the case of thioglycolate complex: the violet thioglycolate complex in ammoniacal 90% ethanol does not change even if chilled to −78°. Therefore the green and violet complexes could not be geometrical isomers. This assignment is consistent with the fact that the green complex was obtained by increasing the pH of the violet solution at −78°. This increase would result in the removal of a proton from the amino cation, and then make it possible for the amino group to coordinate to the ferric ion. Certainly this proposed structure must not be unique, but it seems most reasonable at this moment.



IV

Octahedral complexes with three ligands having dissimilar chelating groups (II and IV in this case) can exist in two stereoisomeric forms (*cis,cis* and *cis,trans* isomers). However, from the present evidence it is impossible to tell whether the complex is a single isomer, and if so, which isomer.

A pair of II and IV is a new example of linkage isomers which are reversibly interconvertible. An analogous example was reported by Neville and Gorin[12]

(11) Leussing and Kolthoff[7] showed that $[Fe(OH)(TGA)_2]^{2-}$ has an absorption maximum at 530 mμ (ε 3730). However the absence of a 1:3 species is quite questionable under their experimental condition that thioglycolic acid is in large excess. We believe that the band at 530 mμ is due to a 1:3 species (see also text and Table II).

(12) R. G. Neville and G. Gorin, *J. Am. Chem. Soc.*, **78**, 4893 (1956).

with respect to tris(cysteinato)cobalt(III). The red complex is S,O coordinated, and the green one is S,N coordinated. The isomerization between these two complexes is irreversible: treatment of the red isomer with hot sodium cysteinate at pH 11–12 results in rapid formation of the green isomer.

Oxidation Mechanism.—There is an equilibrium between the 1:1 and 1:2 complexes, and the latter complex is a main species in the presence of excess cysteine. This violet tris complex decomposes into the red complex rapidly at room temperature. In view of these facts, the catalytic oxidation of cysteine may be explained reasonably in the following manner. The red-violet color observed in the presence of excess cysteine is not due to a 1:2 complex as postulated by Martell and Calvin[13] but to a 1:3 complex. This tris complex decomposes into a bis complex and then into ferrous ion and cystine as suggested by many workers. This scheme is outlined in Chart I.

Acknowledgment.—The authors are indebted to Dr. S. Okuzawa for many helpful discussions.

(13) A. E. Martell and M. Calvin, "Chemistry of the Metal Chelate Compounds," Prentice-Hall, Inc., New York, N. Y., 1952, p 384.

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Stereochemistry of Complexes of Multidentate Ligands. II. Geometrical and Optical Isomers of Bis(2,3-diaminopropionato)cobalt(III) Ion

BY WADE A. FREEMAN AND CHUI FAN LIU

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Reaction of DL-2,3-diaminopropionic acid with tris(carbonato)cobaltate(III) ion gives the expected five geometrical isomers of bis(2,3-diaminopropionato)cobalt(III) ion. The five are separated by ion-exchange chromatography. Formation of the complex with either enantiomeric 2,3-diaminopropionic acid gives three optically active geometrical isomers of specific absolute configuration. Uv, ORD, and CD data for these isomers are used in determining the geometry of the several complex ions.

Introduction

Only a few complexes of cobalt(III) and tridentate ligands have been prepared and studied. The bis-cobalt(III) complex of 1,2,3-triaminopropane has been prepared.[1] Also, the 2:1 diethylenetriamine:cobalt(III) and the 2:1 imidodiacetate:cobalt(III) complexes are known.[2,3] Recently, Legg and Cooke[4] prepared the first cobalt(III) complexes containing two different tridentate ligands and investigated the stereochemistry of the complex ions.

In this paper we report the preparation and separation of all of the possible geometrical and optical isomers of the bis-cobalt(III) complex of L-2,3-diaminopropionic acid, of D-2,3-diaminopropionic acid, and of racemic 2,3-diaminopropionic acid. In both of the first two cases, formation of three optically active geometrical isomers is expected. In the third case, five geometrical isomers are expected to form because of the possibility of having the two ligands in the complex possess opposite absolute configurations. In all cases separation of the geometrical isomers was effected by ion-exchange chromatography.

2,3-Diaminopropionic acid, like 1,2,3-triaminopropane, is constrained to occupy an octahedral face about the cobalt atom when coordinated. Unlike 1,2,3-triaminopropane, 2,3-diaminopropionic acid contains an asymmetric carbon atom. For octahedral complexes of this ligand, in a given geometrical isomer, the absolute configuration of the ligands (if both ligands have the same absolute configuration) specifies the absolute configuration of the complex ion. The geometries and configurations of the complexes are assigned on the basis of visible–uv, ORD, and CD spectra and less soluble diastereomers data.

(1) F. G. Mann and W. J. Pope, *Proc. Roy. Soc.* (London), **A107**, 80 (1925).
(2) F. G. Mann, *J. Chem. Soc.*, 461 (1934).
(3) M. Mori, M. Shibata, E. Kyuno, and F. Maryama, *Bull. Chem. Soc. Japan*, **35**, 75 (1962).
(4) J. I. Legg and D. W. Cooke, *Inorg. Chem.*, **5**, 594 (1966).

Experimental Section

Reagents.—Commercial reagent grade chemicals were used throughout, except where otherwise indicated.

Ligands.—L-2,3-Diaminopropionic acid hydrochloride was purchased from the California Corp. for Biochemical Research, Los Angeles, Calif. *Anal.* Calcd for $C_3H_9N_2O_2Cl$: C, 25.63; H, 6.45; N, 19.92. Found: C, 25.82; H, 6.5; N, 19.9; $[\alpha]_D$ +25.6°.

D-2,3-Diaminopropionic acid hydrochloride was prepared from D-asparagine (Nutritional Biochemicals Corp.) by means of a Hoffmann degradation of N-acetyl-D-asparagine followed by acid hydrolysis of the glyoxalidonecarboxylic acid, according to the method of Karrer and Schlosser.[5] *Anal.* Found: C, 25.32; H, 6.37; N, 20.02; $[\alpha]_D$ −24.0°.

DL-2,3-Diaminopropionic acid hydrobromide was prepared by the amination under pressure of 2,3-dibromopropionic acid, according to the literature method.[6] *Anal.* Calcd for C_3H_9-

(5) P. Karrer and A. Schlosser, *Helv. Chim. Acta*, **6**, 411 (1923).
(6) K. Poduska, J. Rudinger, and F. Sorm, *Collection Czech. Chem. Commun.*, **20**, 1174 (1955).