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Complexes of Cobalt(III) with the Two Isomeric Diethylenetriamineacetic Acids

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The two isomeric tetradentate ligands 1- and 4-diethylenetriamineacetic acids (DTMA and *i*-DTMA) and the following complexes of cobalt(III) have been prepared and characterized: $Co(DTMA)(NO_2)_2$, $Co(i\text{-}DTMA)(NO_2)_2$, $Co(DTMA)Cl_2$, $Co(i\text{-}DTMA)Cl_2$, $Co(DTMA)(NCS)_2$, $Co(DTMA)CO_3$, $Co(i\text{-}DTMA)CO_3$, and the corresponding amino acid chelates with glycine, alanine, and phenylalanine. Provisional assignments have been made for the stereochemistry of these complexes on the basis of their visible absorption spectra. The diaquo cobalt(III) complexes of DTMA and *i*-DTMA are less acidic than the analogous tetramine complexes.

Introduction

Cationic chelates of cobalt(III) such as *cis*-β-$Co(trien)(OH)(H_2O)^{2+}$, *cis*-$Co(tren)(OH)(H_2O)^{2+}$, or $Co(en)_2(OH)(H_2O)^{2+}$[2-6] have been found to effect hydrolysis of amino acid esters and amides and to remove selectively the N-terminal amino acid groups from simple peptides. The amino acid residue resulting from hydrolysis becomes coordinated to the metal ion forming $Co(A_4)(\text{amino acid})^{2+}$ chelates, where A_4 signifies coordination of four of the octahedral positions by the amine nitrogens of en, trien, or tren.

The potential of these reagents for the selective degradation of peptides and the relevance of these reactions to the general subject of the chemistry of coordinated ligands prompted us to investigate other tetradentate ligands such as the two hitherto unreported diethylenetriamineacetic acids $NH_2CH_2CH_2NHCH_2CH_2NHCH_2COOH$ (DTMA) and $(NH_2CH_2CH_2)_2NCH_2COOH$ (*i*-DTMA).[7]

These ligands were selected for three reasons. In the light of our earlier investigations of such peptide cleavage reactions it appeared desirable to modify the tetradentate ligand such that the acidity of the coordinated water molecules in *cis*-diaquocobalt(III) complexes is decreased. Preliminary kinetic studies of the reaction of glycine esters with *cis*-β-$Co(trien)(H_2O)_2^{3+}$ indicated that the "free base form" of the glycine ester and the aquohydroxo form of the cobalt(III) complex are the kinetically reactive species.[4] The rate of disappearance of the cobalt(III) complex, given by k[cobalt(III) complex][glyOR], passes through a maximum at pH 6.4–6.6 which is determined by the pK values of the glycine ester (7–8) and those of the coordinated water molecules in the complex (5.3 and 7.6). Cobalt(III) complexes of DTMA and *i*-DTMA can be compared to those of trien and tren by having one of the primary amino groups replaced by the carboxylate oxygen. Since the acidity of coordinated water molecules is reduced by substituting negative for neutral ligands, the concentration of aquohydroxo complex will be maximized at higher pH values where more of the amino acid derivative is in the "free-base form."

In analogy to the trien and tren system the use of the tetradentate ligands DTMA and *i*-DTMA should prevent side reactions and the formation of products derived from ligand interchange. The reaction of $Co(en)_2(H_2O)_2^{3+}$ with glycine and its derivatives was complicated by a variety of products formed, including $Co(en)_2gly^{2+}$, $Co(en)(gly)_2^{+}$, and $Co(en)_3^{3+}$.

Two isomeric amino acid complexes, designated as β_1 and β_2 (Figure 1), can arise from the reaction of amino acids and their esters with the *cis*-β-$Co(trien)(OH)(H_2O)^{2+}$ ion. They have been distinguished by infrared, visible, and nmr spectroscopy.[8]

Starting with one isomer (Figure 1) of *cis*-$(H_2O)(OH)$(DTMA or *i*-DTMA)Co^{3+} ions, two isomeric amino acid complexes can be envisioned: one having the two carboxylate groups (derived from the tetradentate ligand and the amino acid ligand, respectively) in mutually *cis* positions and the other having the carboxylate groups *trans* to one another, corresponding to the β_1 and β_2 forms in the trien system. It was thought that isomeric amino acid complexes of this type could be distinguished because of the pronounced difference in the visible absorption spectra of *cis*- and *trans*-dicarboxylatecobalt(III)–tetramine complexes.[9]

Herein we report the preparation and some properties of the novel ligands DTMA and *i*-DTMA and their cobalt(III) complexes. These compounds complete the series of tetradentate ligands containing amino and carboxyl groups: tren and trien with four amino groups, DTMA and *i*-DTMA having three amino

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(2) The following abbreviations are used in this article: DTMA, 1-diethylenetriamineacetic acid; *i*-DTMA, 4-diethylenetriamineacetic acid; cyclen, 1,4,7,10-tetraazacyclododecane; en, ethylenediamine; trien, triethylenetetramine; tren, 4-(2-aminoethyl)diethylenetriamine; dien, diethylenetriamine; gly, ala, and phe for glycine, alanine, and phenylalanine, respectively; tetramine and triamine for any four or three nitrogen atoms attached to cobalt(III).

(3) J. P. Collman and D. A. Buckingham, *J. Am. Chem. Soc.*, **85**, 3039 (1963).

(4) D. A. Buckingham, J. P. Collman, D. A. R. Happer, and L. G. Marzilli, *ibid.*, **89**, 1082 (1967).

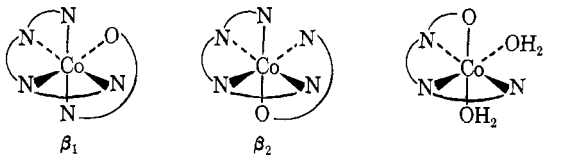
(5) S. A. Young, Ph.D. Thesis, University of North Carolina, Chapel Hill, N. C., 1967.

(6) D. A. Buckingham and J. P. Collman, *Inorg. Chem.*, **6**, 1803 (1967).

(7) The abbreviations DTMA and *i*-DTMA follow those of related compounds; DTPA is used for diethylenetriaminepentaacetic acid: C. K. Jørgensen, "Inorganic Complexes," Academic Press, New York, N. Y., 1963, p 118.

(8) L. G. Marzilli, honors paper in chemistry, Brown University, Providence, R. I., 1965; L. G. Marzilli and D. A. Buckingham, *Inorg. Chem.*, **6**, 1042 (1967).

(9) M. Linhard and M. Weigel, *Z. Anorg. Allgem. Chem.*, **264**, 321 (1951).



β_1 β_2

Co(trien)(amino acid)$^{2+}$ β-*mer*-Co(DTMA)$(H_2O)_2^{2+}$

$\overset{\frown}{N}\overset{\frown}{N}$ = >NCH_2CH_2N<; $\overset{\frown}{N}\overset{\frown}{O}$ = >NCHRCOO$^-$

Figure 1.

groups and one carboxyl group, N,N- and N,N′-ethylenediaminediacetic acid having two amino and two carboxyl groups, and nitrilotriacetic acid with one amino and three carboxyl groups.

Results and Discussion

Unlike the hydrochlorides of chelate-forming polyamines such as tren[5] or cyclen[10] which can be purified readily by recrystallization, the hydrochlorides, hydroperchlorates, and hydrosulfates of DTMA and *i*-DTMA are very hygroscopic. Attempts to isolate salts of these ligands in purely crystalline form were unsuccessful. This difficulty was circumvented in one instance by preparing an aqueous solution containing the pure ligand and in another instance by preparing cobalt(III) complexes from a mixture containing the desired ligand and other chelating compounds and then separating the required complex by taking advantage of differences in solubility.

i-DTMA was prepared from a dien derivative in which both primary amines were selectively protected by phthaloyl groups. Condensation of dien with phthalic anhydride or a modification of Mann's procedure[11] afforded the 1,7-diphthaloyl dien which was alkylated with ethyl bromoacetate to give the diphthaloyl ethyl ester of *i*-DTMA. Its hydrolysis with hydrochloric acid and subsequent removal of the phthalic acid by filtration gave a solution containing practically pure *i*-DTMA. The cobalt(III) complexes of *i*-DTMA were prepared following general methods for the preparation and interconversion of cobalt(III)–amine complexes (Figure 2).[12]

Since no convenient path for the specific synthesis of DTMA was apparent, this ligand was prepared from "unprotected" dien in aqueous solution by alkylation with chloroacetic acid. Owing to the similar reactivities of primary and secondary amino groups toward alkyl halides,[13] this reaction should lead to the formation of DTMA as well as *i*-DTMA and other dien derivatives which have two or more carboxymethylene groups. DTMA was not separated as the free ligand from this mixture. However, pure cobalt(III)–DTMA complexes could be isolated as follows.

Like other neutral cobalt(III) complexes,[14,15] Co(DTMA)$(NO_2)_2$ and Co(*i*-DTMA)$(NO_2)_2$ are only

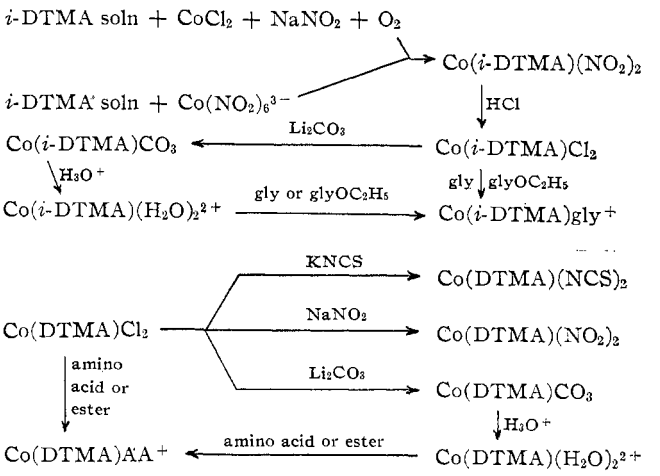


Figure 2.

sparingly soluble in water. They probably constitute the major portion of the nitro complexes which precipitate on air oxidation of a solution containing the alkylation mixture, cobalt(II), and nitrite ions. Although its components were not identified, it seems feasible that those dien derivatives with three or more carboxymethylene groups were eliminated at this stage. The presence of "unreacted" dien whose trinitro complex is almost insoluble[14] was minimized by using a slight excess of chloroacetic acid in the alkylation step. The dien di- and triacetic acids and their cobalt(III) complexes have not been reported. It is uncertain whether their nitro complexes will form under the above oxidation conditions or whether the steric requirements of these ligands are too severe to allow coordination of all carboxylate groups.[16] No evidence for the formation of such cobalt(III) complexes was obtained.

In our studies of the (*i*-DTMA)Co^{III} complexes, it was found that Co(*i*-DTMA)Cl_2 is formed from the dinitro complex by treatment with hydrochloric acid. However, the dichloro complex is very soluble in hydrochloric acid and can only be obtained in a crystalline state by addition of alcohol to a saturated solution in hydrochloric acid or by evaporation to dryness.[17] On the other hand, Co(DTMA)Cl_2 is insoluble in hydrochloric acid. It dissolves in hot water whereby one or both of the coordinated chlorides undergo aquation. The pure dichloride can be recovered as fine, metallic-violet crystals by the addition of hydrochloric acid. All of the (DTMA)Co^{III} complexes were prepared conveniently from this dichloro compound.

The geometric isomers of the (DTMA)- and (*i*-DTMA)Co^{III} complexes containing identical groups X in the fifth and sixth coordination positions are shown in Figure 3. Apart from the *trans*-Co(DTMA)X_2, the stereochemistry of the *cis* complexes may best be described with reference to the steric arrangement of the nitrogen atoms of the tetradentate ligand, *e.g.*, the meridional, *mer*(N), and facial, *fac*(N), series.[18,19]

(10) J. P. Collman and P. W. Schneider, *Inorg. Chem.*, **5**, 1380 (1966).

(11) F. G. Mann, *J. Chem. Soc.*, 463 (1934).

(12) See, *e.g.*, G. H. Searle, Ph.D. Thesis, Australian National University, Canberra, A. C. T., Australia, 1963.

(13) See, *e.g.*, D. J. Cram and G. S. Hammond, "Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1959, p 52.

(14) P. H. Crayton and J. A. Mattern, *J. Inorg. Nucl. Chem.*, **13**, 248 (1960).

(15) N. Matsuoka, J. Hidaka, and Y. Shimura, *Bull. Chem. Soc. Japan*, **39**, 1257 (1966).

(16) Hydrolysis of the nitro complexes with hydrochloric acid may result in charged chloro complexes with a "dangling" carboxylate group.

(17) Co(*i*-DTMA)Cl_2 dissolves in water with aquation as evident by the color change from bluish violet to violet.

(18) The terms *meridional* and *facial* were used to designate the arrangement of dien in mixed cobalt(III) complexes with aminodicarboxylic acids[19] and of the nitrogen atoms of en and gly in Co(en)glyCO_3.[15]

TABLE I

ELECTRONIC SPECTRA OF DTMA, *i*-DTMA, AND RELATED COBALT(III) COMPLEXES

Complex	λ, mμ (ε)	Complex	λ, mμ (ε)
Co(DTMA)CO_3	540 (149), 380 (166)	β-Co(gly)$_3$ (*fac*(N))	520 (155), 372 (138)
Co(*i*-DTMA)CO_3	513 (139), 365 (133)	Co(DTMA)$(NO_2)_2$	460 (225), 328 (4100)
mer(N)-Co(en)glyCO_3	529 (117), 370 (148)	Co(*i*-DTMA)$(NO_2)_2$	455 (185), 333 (4250)
fac(N)-Co(en)glyCO_3	529 (148), 373 (135)	Co(en)gly$(NO_2)_2$	442(185), 326 (3160)
α-Co(gly)$_3$ (*mer*(N))	550 (95), 374 (139)	Co(DTMA)$(NCS)_2$	520 (244), 310 (2710)

mer(N) series, amino groups of tetradentate ligand span an edge

fac (N) series, amino groups of tetradentate ligand span a face

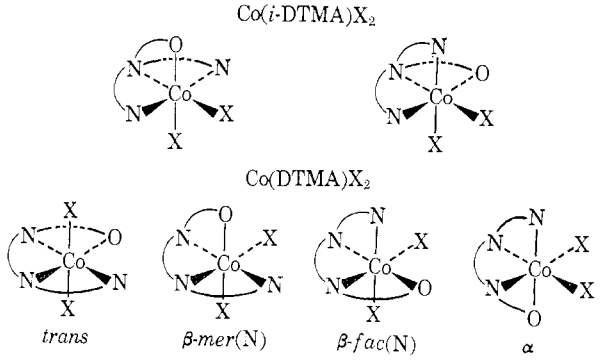


Figure 3.—Geometric isomers of (DTMA)- and (*i*-DTMA)Co^{III} complexes.

Since three isomeric DTMA complexes with the X groups in *cis* positions are possible, further differentiation has to be made. The prefix α or β[20] distinguishes the *cis*-Co(DTMA)X_2 complexes. In the amino acid complexes, the subscripts 1 and 2[3] specify the relative positions of the carboxylate groups of the tetradentate ligand and of the amino acid (Figure 4).

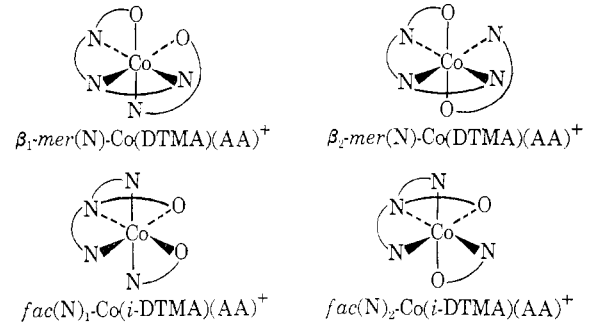


Figure 4.—Isomeric amino acid (AA) chelates in the (DTMA)- and (*i*-DTMA)Co^{III} series.

In Figures 5 and 6 and Table I visible and ultraviolet spectral data are given and compared with those of related cobalt(III) complexes.[15] Since the dichloro complexes hydrolyze readily, their spectra are of little value and are omitted. The spectral difference between *cis*- and *trans*-dinitro complexes is not clear-cut. However, the absorption maxima of Co(DTMA)$(NO_2)_2$ and Co(*i*-DTMA)$(NO_2)_2$ at 328 and 333 mμ compare to the position of the nitro-specific band[21] which is located at 335–320 mμ for *cis*-cobalt(III) complexes.

The absorption spectra of the DTMA and *i*-DTMA carbonato complexes (Figure 6, Table I) resemble those

(19) J. I. Legg and D. W. Cooke, *Inorg. Chem.*, **5**, 594 (1966).
(20) This is similar to cobalt(III)–trien complexes.[4,8,12]
(21) Y. Shimura, *J. Am. Chem. Soc.*, **73**, 5079 (1951).

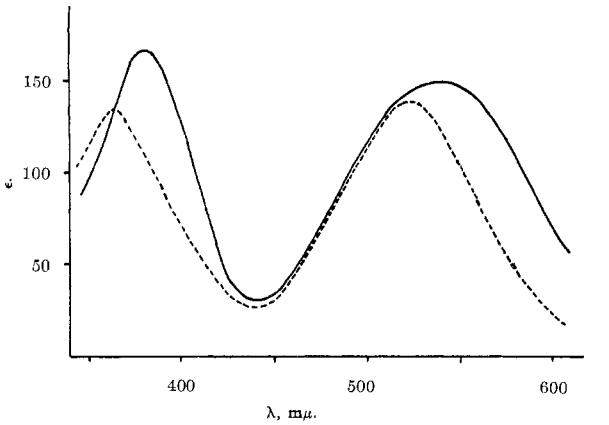


Figure 5.—Visible absorption spectra of Co(DTMA)CO_3 (———) and Co(*i*-DTMA)CO_3 (--------).

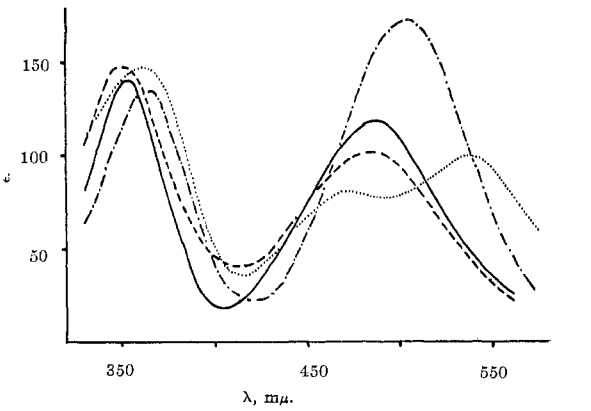


Figure 6.—Visible absorption spectra of β$_1$-*mer*(N)-Co(DTMA)gly$^+$ (-·-·-·-), β$_2$-*mer*(N)-Co(DTMA)gly$^+$ (·····), Co(*i*-DTMA)gly$^+$ (I) (--------), and Co(*i*-DTMA)gly$^+$ (II) (———).

of the isomeric α and β forms of Co(gly)$_3$ or Co(ala)$_3$.[22] Both bands in the spectrum of Co(*i*-DTMA)CO_3 are narrow and of about equal intensities. In contrast, the band at 540 mμ is considerably broadened for the DTMA isomer. A similar trend was observed in the spectra of the *mer*(N) and *fac*(N) isomers of Co(en)glyCO_3.[15] Although the spectrum of α-Co(gly)$_3$ exhibits a clear shoulder, the broad band of Co(DTMA)CO_3 suggests a similar ligand field about the central cobalt ion. Accordingly, the ε values of the two absorption bands at 540 and 380 mμ differ less for the carbonato DTMA complex than for Co(gly)$_3$.

Diaquo complexes of DTMA and *i*-DTMA were obtained from the carbonato complexes by hydrolysis with perchloric acid. Each complex was eluted from a cation-exchange column as a broad band. All of the

(22) B. E. Douglas and S. Yamada, *Inorg. Chem.*, **4**, 1561 (1965).

TABLE II

ELECTRONIC SPECTRA OF AMINO ACID CHELATES OF DTMA AND *i*-DTMA COBALT(III) COMPLEXES

Complex	λ, mμ (ε)	Elemental analysis, % C Calcd	C Found	H Calcd	H Found	N Calcd	N Found
β_1-*mer*(N)-[Co(DTMA)gly]$ClO_4 \cdot H_2O$	504 (175), 362 (138), ...	23.4	23.5	4.9	4.9	13.7	13.6
β_2-*mer*(N)-[Co(DTMA)gly]I	538 (102), 470 sh (82), 360 (148)	22.9	22.8	4.3	4.3	13.2	13.3
β_2-*mer*(N)-[Co(DTMA)ala]I	540 (97), 470 sh (82), 360 (147)	24.3	24.5	4.7	4.7	12.9	12.7
β_2-*mer*(N)-[Co(DTMA)phe]$I \cdot H_2O$	540 (99), 470 sh (82), 360 (148)	34.1	34.1	5.0	5.0	10.6	10.6
[Co(*i*-DTMA)gly]ClO_4 (I)	485 (103), 353 (148), ...	24.5	24.3	4.6	4.5	14.3	14.1
[Co(*i*-DTMA)gly]ClO_4 (II)	486 (120), 355 (140), ...	24.5	24.5	4.6	4.7	14.3	14.3

TABLE III

ACID DISSOCIATION CONSTANTS OF SOME DIAQUOCOBALT(III) COMPLEXES[a]

Parent carbonato complex	pK_{a1}	pK_{a2}	Parent carbonato complex	pK_{a1}	pK_{a2}
Co(DTMA)CO_3	5.59 ± 0.05	8.41 ± 0.02	Co(cyclen)CO_3^+	5.46 ± 0.02	7.60 ± 0.04
Co(*i*-DTMA)CO_3	5.90 ± 0.03	8.22 ± 0.05	Co(tren)CO_3^+	5.39 ± 0.05	7.67 ± 0.03
Co(en)glyCO_3	5.63 ± 0.08	8.40 ± 0.02	β-Co(trien)CO_3^+	5.45 ± 0.05	7.80 ± 0.02

[a] The diaquo complexes were prepared *in situ* from the parent carbonato complexes by hydrolysis with perchloric acid.

fractions collected have identical absorption spectra. This, and the constancy of the visible absorption spectra of the parent carbonato complexes upon several recrystallizations, strongly indicates the presence of only one isomeric species for each tetradentate ligand.

Although it is difficult to discriminate between the *fac*(N) and *mer*(N) configurations solely on the basis of the absorption spectrum of only one carbonato complex isomer and not having the other one for comparison, we assign the carbonato DTMA and *i*-DTMA complexes the *mer*(N) and *fac*(N) configurations, respectively. These assignments are substantiated by the formation of a *trans*-carboxylato(amino acid) complex of DTMA, whereas only *cis*-carboxylato complexes were obtained for the *i*-DTMA ligand.

In Table II and Figure 6 visible absorption spectra of DTMA and *i*-DTMA amino acid complexes are given. These compounds were obtained from the reaction of Co(DTMA)Cl_2, Co(DTMA)$(H_2O)_2^{2+}$, and Co(*i*-DTMA)$(H_2O)_2^{2+}$ with amino acids and esters. In each reaction only one pair of isomers was formed, as demonstrated by the isolation of both isomers by fractional crystallization, ion-exchange chromatography of the reaction mixtures, or examination of the visible spectra of the filtrate after one isomer was isolated.

The visible absorption spectra of the two amino acid complexes arising from the diaquo or dichloro DTMA cobalt(III) complexes are typical for tetraminecobalt(III) complexes having two carboxylate groups in mutually *cis* and *trans* positions to one another.[9] Consequently, the *trans* complex must have the β_2-*mer*(N) configuration. The two glycinato isomers were eluted from ion-exchange columns following their expected dipole moments. In accordance with observations by Legg and Cooke[19,23] on related compounds, the β_2-*mer*(N) isomer with a low dipole moment was eluted first, followed closely by the *cis* isomer which has a more unsymmetrical charge distribution. The ala and phe complexes were not separated by ion-exchange chromatography. Their β_2-*mer*(N) isomer was isolated and spectral evidence was obtained for the presence of the *cis* isomer in the reaction mixture. We assign the *cis* complexes the β_1-*mer*(N) configuration. This tentative assignment is based on the stereochemical homogeneity of the parent carbonato complex and on the assumption that no isomerization takes place in the sequence of reactions: carbonato, diaquo, and amino acid complex.

Only small spectral differences were observed for the two Co(*i*-DTMA)gly$^+$ isomers, both spectra being typical for a *cis* arrangement of the carboxylato groups. Since only three geometric isomers are possible in the *cis*-carboxylato *i*-DTMA complexes, at least one of the obtained glycinato complexes must belong to the *fac*(N) series. Analogous to the assumptions made for the *cis*-carboxylato DTMA complex and by comparison of the relative intensities of their spectral absorption bands with those of related Co(dien)IDA$^+$ ions,[19] we tentatively assign the Co(*i*-DTMA)gly$^+$ complexes I and II the *fac*$(N)_1$ and *fac*$(N)_2$ configurations, respectively. From ion-exchange columns, first the glycinato complex I and then complex II were eluted in two slightly overlapping bands.

The acid dissociation constants of *cis*-cobalt(III) diaquo complexes are of intrinsic importance for kinetic studies of the peptide-cleavage reactions, since the rate of the initial reaction between the cobalt(III) complex and the peptide depends upon the concentration of the aquohydroxy form of the complex. In Table III the results of potentiometric titrations of a series of diaquo complexes are given. Most unexpected is the small variation of the pK_1 values in the tetramine– and the triamine–carboxylate complexes. Since the acidity of coordinated water is affected by (i) inductive and conjugate effects, (ii) the net charge of the complex, (iii) the oxidation state of the central metal ion, (iv) statistical effects, (v) geometrical effects, and (vi) hydrogen bonding, the situation is too complex to allow a detailed interpretation. The possibility that the pK values are obscured by release of the carboxylate group from the coordination sphere of the cobalt(III) ion during the acid hydrolysis of the carbonato complex was excluded by infrared spectroscopy. Only one band at 1635–1640 cm^{-1} was observed in the D_2O solutions

(23) J. I. Legg and D. W. Cooke, *Inorg. Chem.*, **4**, 1576 (1965).

of $Co(DTMA)(H_2O)_2^{2+}$, $Co(i\text{-}DTMA)(H_2O)_2^{2+}$, and $Co(en)(gly)(H_2O)_2^{2+}$. In the solid state the coordinated COO^- group in cobalt(III) tetramine–amino acid complexes absorbs at about 1635 cm^{-1}. Busch and Alexander[24] reported a pK value of 2.1 and an infrared absorption at 1735 cm^{-1} for the "dangling" COO^- group in the $Co(en)_2(gly)Cl^+$ complex.

The pK_2 values of the cobalt(III) tetramine–carboxylate complexes are considerably higher than those of the tetramine analogs. The difference between pK_1 and pK_2 (ΔpK) is 2.8 for DTMA as compared with 2.3 for the *i*-DTMA complex. ΔpK for the cobalt(III) and chromium(III) bisethylenediamine diaquo complexes are 2.1 and 2.4 for the *cis* and 3.4 for the *trans* isomers. It is tempting to rationalize the ΔpK of the DTMA and *i*-DTMA complexes in terms of inductive and conjugative effects of the COO^- groups in *cis* and *trans* positions to the ionizing water.

At present we have not attempted a kinetic and mechanistic study of the peptide-cleavage reaction using the novel tetradentate ligands. The preparative methods reported herein do not allow predictions concerning the number and stereochemistry of amino acid complexes formed under more controlled conditions. It will be interesting to see which amino acid complex will be formed and how the rate of reaction will be influenced by the decreased acidity of the diaquocobalt(III) complexes.

Experimental Section

1,7-Diphthaloyldiethylenetriamine. Method A.—This modification of the procedure of Mann gives considerably higher yields. A solution of thionyl chloride (300 g, 2.5 mol) in 250 ml of chloroform was added dropwise to a vigorously stirred solution of diethanolamine (100 g, 1 mol) in 250 ml of chloroform. After the addition was completed, the semisolid reaction mixture was heated to gentle reflux for 2.5 hr during which time all of the solid dissolved. The solution was cooled in ice and the crystals of 2,2′-dichlorodiethylamine hydrochloride were collected by filtration and washed with ice-cold chloroform to remove the off-white impurities; yield 139 g (77%).

A mixture of 2,2′-dichlorodiethylamine hydrochloride (90 g, 0.5 mol) and potassium phthalimide (330 g, 1.8 mol) in 500 ml of DMF was heated overnight to 90–110° and then, while hot, poured slowly with stirring into a mixture of 1 l. of water, 1000 g of crushed ice, and 100 g of potassium carbonate. After standing for 1 hr in ice, the precipitated, crystalline diphthaloyl compound was collected by filtration and washed with ice water and ethanol; yield 180 g (98%). The product was used without further purification in the alkylation reaction. A small sample was recrystallized from ethanol; mp 178–180°.

Method B.—A solution of dien (52 g, 0.5 mol) in 100 ml of toluene was added dropwise to phthalic anhydride (148 g, 1 mol) in 1500 ml of boiling toluene. After the addition was completed, the mixture was heated further until 18 ml of water was collected by azeotropic distillation. Then about 500 ml of toluene was distilled off and the remaining solution was decanted while hot from some viscous product which was sticking to the walls of the reaction flask. The reaction flask was rinsed several times with small portions of hot toluene which was then added to the main solution. After refrigeration for 2 days, 160 g (88%) of the white product was obtained; mp 179–180°.

Ethyl 1,7-Diphthaloyl-4-diethylenetriamineacetate.—A mixture of the diphthaloyl compound (160 g, 0.44 mol), ethyl bromoacetate (120 g, 0.72 mol), and sodium carbonate (44 g, 0.44 mol) in 2000 ml of ethanol was heated under reflux overnight. Then 500 ml of the ethanol was removed by distillation and the remaining solution was transferred while hot into a 4-l. beaker. Crushed ice followed by water was added slowly to make a total volume of 3500 ml and to keep the temperature below 5°. After standing in ice for 1 hr, precipitation was complete and the ethyl ester (180 g, 91%) was collected by filtration and recrystallized from 150 ml of ethanol and 250 ml of ethyl acetate; yield 152 g (77%); mp 100–102°. *Anal.* Calcd for $C_{24}H_{23}N_3O_6$: C, 64.2; H, 5.2; N, 9.4. Found: C, 64.2; H, 5.3; N, 9.2.

4-Diethylenetriamineacetic Acid (*i*-DTMA).—A suspension of the ethyl ester (45 g, 0.1 mol) in 200 ml of 6 *M* hydrochloric acid was heated under reflux for 6 hr. A few drops of 1-octanol were added to reduce foaming. On cooling phthalic acid precipitated and was removed by filtration. Several attempts to isolate the *i*-DTMA as its salts were unsuccessful. Therefore the filtrate was evaporated several times to near dryness in vacuum to remove as much excess hydrochloric acid as possible. The semisolid residue was diluted quantitatively with water and small aliquots were used for the preparation of the cobalt(III) complexes.

The 1,7-ditosylate of *i*-DTMA was prepared from the stock solution; mp 161–162°. *Anal.* Calcd for $C_{20}H_{27}N_3O_6S_2$: C, 51.1; H, 5.8; N, 8.9. Found: C, 51.0; H, 5.5; N, 8.7.

***cis*-Dinitro(*i*-DTMA)cobalt(III) Hydrate. Method A.**—An aliquot of the *i*-DTMA stock solution (4 mmol) was added dropwise to sodium hexanitrocobaltate (1.6 g, 4 mmol) in 5 ml of water at 50–60°. Nitric oxides were evolved and warming was continued for 30 min. On cooling the sparingly soluble yellow complex was collected by filtration, then washed with water, ethanol, and ether; yield 525 mg (40%). *Anal.* Calcd for $[Co(C_6H_{14}N_2O_2)(NO_2)_2]\cdot H_2O$: C, 21.9; H, 5.0; N, 21.3. Found: C, 21.9; H, 4.9; N, 21.2.

Method B.—A vigorous stream of air was passed through 50 ml of a solution (pH 7–7.5, adjusted with sodium hydroxide) containing *i*-DTMA (70 mmol) and cobalt(II) chloride hexahydrate (23.8 g, 100 mmol). Then, sodium nitrite (11.6 g, 120 mmol) in 20 ml of water was added in small portions and the aeration was continued for 2 hr. The yellow precipitate was collected by filtration, then washed with ice-cold 1 *N* hydrochloric acid, water, and ethanol; yield 9.6 g (42%). *Anal.* Found: C, 21.6; H, 5.0; N, 21.8.

The infrared spectra of the dinitro complexes prepared by methods A and B are identical in the range 625–4000 cm^{-1}. Elemental analytical data of products obtained by method B are always somewhat poorer than those for compounds prepared by method A. Owing to the low solubility of those dinitro complexes, recrystallization does not improve the purity; molar conductance (methods A and B) 10^{-3} ohm^{-1} cm^2.

***cis*-Dichloro(*i*-DTMA)cobalt(III).**—A suspension of *cis*-$[Co(i\text{-}DTMA)(NO_2)_2]\cdot H_2O$ (2.1 g, 9.1 mmol) in 20 ml of concentrated hydrochloric acid was heated on a steam bath for 2 hr. Nitric oxides were evolved; the color of the solid materials in the mixture gradually changed from yellow to red-brown and finally a bluish violet solution was obtained. Ethanol (50 ml) was added slowly to the hot solution and after refrigeration for 2 hr 870 mg (33%) of blue-violet crystals were obtained. *Anal.* Calcd for $Co(C_6H_{14}N_3O_2)Cl_2$: C, 24.8; H, 4.9; N, 14.5; Cl, 24.5. Found: C, 24.4; H, 5.1; N, 14.5; Cl, 24.6.

The red-brown intermediate was analyzed. *Anal.* Calcd for $[Co(i\text{-}DTMA)(NO_2)Cl]\cdot HCl$: C, 21.4; H, 4.5; N, 16.6; Cl, 21.1. Found: C, 21.6; H, 4.6; N, 16.1; Cl, 21.2. The suggested formulation as a nitrochloro complex hydrochloride is further supported by the strongly acidic reaction of its aqueous solution and by the formation of $Co(i\text{-}DTMA)(NO_2)_2$ upon heating the red-brown compound in water with sodium carbonate.

Carbonato(*i*-DTMA)cobalt(III) Hydrate.—A mixture of *cis*-$[Co(i\text{-}DTMA)(NO_2)_2]\cdot H_2O$ (3.6 g, 11 mmol) and 20 ml of concentrated hydrochloric acid was slowly evaporated to dryness on a steam bath. The blue-violet residue was taken up in 20 ml of water. Lithium carbonate (1.5 g, 20 mmol) was added and the

(24) M. D. Alexander and D. H. Busch, *Inorg. Chem.*, **5**, 1596 (1966).

mixture was stirred at 80–90° for 2 hr and then kept on a steam bath overnight. Calcium chloride (890 mg, 8 mmol) was added and the calcium carbonate was removed after 5 hr at 20° by filtration. The crude carbonato complex was precipitated with 60 ml of ethanol. Recrystallization from water–ethanol yielded 1.3 g (40%). *Anal.* Calcd for $[Co(C_6H_{14}N_3O_2)CO_3]\cdot H_2O$: C, 28.3; H, 5.4; N, 14.2. Found: C, 28.6; H, 5.3; N, 14.1; molar conductance 3.3×10^{-2} ohm^{-1} cm^2.

Dichloro(DTMA)cobalt(III). (a) **Preparation of 1-Diethylenetriamineacetic Acid.**—A mixture of chloroacetic acid (41.4 g, 0.44 mol) and sodium hydroxide (35.2 g, 0.88 mol) in 100 ml of water was added to a solution of dien (42 g, 0.4 mol) in 100 ml of water. The solution was stirred at 20° for 30 min and was finally heated to 90–95° for 2 hr. The ligand could not be isolated and this solution was used directly in the next step.

(b) **Preparation of the Nitro Complexes.**—The above solution and acetic acid (24 g, 0.4 mol) were added slowly to a vigorously aerated solution of cobalt(II) chloride hexahydrate (96 g, 0.4 mol) and sodium nitrite (69 g, 1 mol) in 100 ml of water. Yellow nitro complexes precipitated almost immediately and after 45 min of aeration the complexes were collected by filtration; yield 60 g.

(c) **Hydrolysis of the Nitro Complexes.**—A suspension of 60 g of the nitro complexes from (b) in 80 ml of 10 *N* hydrochloric acid was heated to 60–70° for 3 hr. The crude brown-violet precipitate was collected by filtration; yield 26 g. This was heated in 80 ml of water until all of the solid had dissolved. After filtration, 60 ml of concentrated hydrochloric acid was added and the mixture was heated to 70–80° for 20 min. The pure dichloro DTMA complex precipitated as small metallic-violet crystals and was collected by filtration; yield 22 g (19% based on dien in step a). *Anal.* Calcd for $Co(C_6H_{14}N_3O_2)Cl_2$: C, 24.8; H, 4.9; N, 14.5; Cl, 24.5. Found: C, 24.8; H, 4.9; N, 14.3; Cl, 24.4.

Dinitro(DTMA)cobalt(III).—Sodium nitrite (207 mg, 3 mmol) was added to a solution of $Co(DTMA)Cl_2$ (290 mg, 1 mmol) in 10 ml of water at 80°. After heating for 1 hr, 290 mg (93%) of the pure complex was obtained. *Anal.* Calcd for $Co(C_6H_{14}N_3O_2)(NO_2)_2$: C, 23.2; H, 4.5; N, 22.5. Found: C, 23.1; H, 4.5; N, 22.3; molar conductance 2.7×10^{-2} ohm^{-1} cm^2.

Dithiocyanato(DTMA)cobalt(III).—Potassium thiocyanate (292 mg, 3 mmol) was added to a solution of $Co(DTMA)Cl_2$ (290 mg, 1 mmol) in 10 ml of water and the mixture was heated to 80° for 1 hr. After cooling, the pure, wine-red product was collected by filtration; yield 300 mg (88%). *Anal.* Calcd for $Co(C_6H_{14}N_3O_2)(NCS)_2$: C, 28.7; H, 4.2; N, 20.9. Found: C, 28.5; H, 4.3; N, 20.8; molar conductance 5.1×10^{-3} ohm^{-1} cm^2.

Carbonato(DTMA)cobalt(III) Hydrate.—A suspension of lithium carbonate (18.4 g, 250 mmol) and $Co(DTMA)Cl_2$ (33 g, 115 mmol) in 100 ml of water was heated to 70–80° for 3 hr. During this time the solution became alkaline and its color changed from deep violet to red violet. Calcium chloride (2 g) was added to the cool solution and the precipitated calcium carbonate was removed by filtration after 3 hr. The filtrate was then poured into 400 ml of ethanol and the mixture was stored in a refrigerator overnight. The crude carbonato complex was collected by filtration. Recrystallization from water–alcohol yielded 17.2 g (43%) of reddish violet crystals. *Anal.* Calcd for $[Co(C_6H_{14}N_3O_2)CO_3]\cdot H_2O$: C, 28.3; H, 5.4; N, 14.2. Found: C, 28.3; H, 5.6; N, 14.5; molar conductance 1.3×10^{-2} ohm^{-1} cm^2.

Cobalt(III) DTMA and *i*-DTMA Amino Acid Complexes. (A) From the Dichloro Complexes.—The dichloro complex (1.16 g, 4 mmol), sodium bicarbonate or sodium hydroxide (5 mmol), and the respective amino acid or ester hydrochloride (5 mmol) in 10 ml of water were heated on a steam bath for 1–3 hr. Then, sodium perchlorate or iodide (8–15 mmol) was added. If the products did not crystallize spontaneously, sufficient alcohol was added to induce crystallization. After a first crop of crystals was obtained, a second fraction was precipitated by the addition of more ethanol and refrigeration. The products were recrystallized from water–alcohol mixtures containing some sodium perchlorate or iodide.

(B) **From the DTMA Carbonato Complex.**—The carbonato complex (1.49 g, 5 mmol), amino acid or ester hydrochloride (6 mmol), and perchloric acid (5 mmol) in 10 ml of water were heated on a steam bath for 1–3 hr. The product complexes were isolated as under (A) as their perchlorates. Elemental analyses are given in Table II.

The $[Co(i\text{-DTMA})gly]ClO_4$ complexes I and II were prepared by method A with gly and $glyOC_2H_5\cdot HCl$. The former isomer crystallized first. Only two isomeric gly complexes were detected by ion-exchange chromatography.

The β_1-*mer*(N)- and β_2-*mer*(N)-Co(DTMA)gly perchlorates were prepared by methods A and B. The former isomer was isolated as a perchlorate salt and the latter was detected by ion-exchange chromatography in the reaction mixture. The β_2-*mer*(N) complexes of the amino acids gly, ala, and phe were isolated as their iodide salts by method A. The presence of the β_1-*mer*(N) isomer was demonstrated by the visible absorption spectra of the reaction mixture and, in the case of the gly complex, was confirmed by ion-exchange chromatography.

Acid Dissociation Constants.—An aliquot (0.4 mmol) of the respective carbonato complex was hydrolyzed with 2.5 ml of 1 *N* perchloric acid at 30–40°. Carbon dioxide was removed in a stream of nitrogen. After the hydrolysis was complete (15–30 min), 10 ml of 0.1 *N* sodium hydroxide was added and the volume of the solution was brought to 95 ml with carbon dioxide free water. The ionic strength was adjusted to 0.1 with sodium perchlorate. The results of the titrations with 0.1 *N* sodium hydroxide are given in Table III. The pK values were calculated by an iterative process using the equation

$$a = \frac{2[H]^2 + K_1[H]}{[H]^2 + K_1[H] + K_1K_2}$$

where a is the degree of protonation of the complex.

Solution infrared spectra of the diaquo complexes in D_2O were obtained by dissolving the carbonato complexes in D_2O followed by acidification with one drop of 65% perchloric acid. Teflon standard taper sleeves which are transparent from 2000 to 1300 cm^{-1} were used as infrared cells in these measurements. Only one single absorption band was observed (1640 cm^{-1}) in the region of coordinated and dangling carboxylate groups for the diaquo complexes of DTMA and *i*-DTMA.

Ion-Exchange Chromatography.—The method according to Legg and Cooke,[19] using 50 ml of Dowex 50W-X8 resin, was followed. Elution was accomplished with the following reagents: 0.5 *M* sodium perchlorate for amino acid complexes; 0.5 *M* sodium perchlorate and 0.1 *M* perchloric acid for the diaquo complexes, 0.5 ml/min. The β_1-*mer*(N) and β_2-*mer*(N) isomers of $Co(DTMA)gly^+$ were almost cleanly separated. The isomers of $Co(i\text{-DTMA})gly^+$ were eluted as slightly overlapping bands. Only one single broad band was obtained for the diaquo complexes.

Visible and ultraviolet absorption spectra were measured with a Perkin-Elmer 202 spectrophotometer; infrared spectra were measured with a Perkin-Elmer 237B grating spectrophotometer, using the KBr-disk technique; conductivity measurements were made upon aqueous solutions of the complexes, 0.001 *M* at room temperature, using an Industrial Instruments conductivity bridge; measurements of pH and titrations were made using a Fisher Accumet 310 expanded-scale pH meter, Fisher glass and calomel electrodes, and a Metrohm micro piston buret. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

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