the formation of $Fe(OH)^+$ by the close approximation $\bar{n}_o = Q/h$, where Q is the equilibrium quotient for reaction 1 and unit weights were employed. In Table I the maximum $\bar{n}_o$ attained was 0.006 which is in good agreement with the observation of Hedstrom[3] but somewhat less than the value reported by Bolzan and Arvia[6] (0.012).

The value of log Q obtained from the data of Table I is -9.58 with an estimated error of 0.08. This estimate includes random scatter of the least-squares fit and estimated systematic error due to errors in the original acidity. A small correction for activity coefficients was made using the Debye–Hückel expression to obtain the infinite dilution value or log K of -9.49 ± 0.08. This is in agreement with the relatively uncertain value of Sweeton and Baes (-9.3 ± 0.5). We would expect the value of log Q in 1 M $NaClO_4$ to be somewhat lower than log K.

The log Q for $Fe^{III}(OH)^{2+}$ (0.01 M $NaClO_4$)[7] is -2.4 or 7 log units more stable than $Fe^{II}(OH)^+$. This relatively low stability for $Fe^{II}(OH)^+$ compared with $Fe^{III}(OH)^{2+}$ must be attributed chiefly to the charge and radius of the cation since ligand field effects are small, particularly for ligands like OH^- displacing H_2O.

Also, the difference in stabilities predicted for the 1:1 fluoride complexes for Fe^{2+} and Fe^{3+} is only 4 log units based on the relationship between (Z_+^2/r_+) for the cation and the stability of fluoride complexes.[8] Not only are the hydroxide complexes several orders of magnitude more stable than the corresponding fluoride complexes but the difference between ferric and ferrous complexes are greater for the hydroxides.

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An Ion-Exchange Study of the Ethylenediamine-*N*,*N*′-diacetatodiaquochromium(III) System

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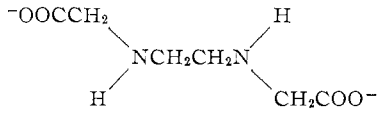
Studies concerning the geometry of coordinated ethylenediamine-*N*,*N*′-diacetate ion (EDDA)



have recently been reported in the literature for the metal ions Co(III),[1] Pt(II),[2] Pt(IV),[2] Ni(II),[3] and Cr(III).[4,5] In these studies it has been found that EDDA is capable of functioning as a bidentate, tridentate, or tetradentate ligand. This note is concerned with those cases where EDDA functions as a tetradentate ligand. For the octahedral cases the diaquo parent complexes have been reported for Co(III) and Ni(II). Although a number of mixed ligand–EDDA complexes with chromium(III) have been reported, studies pertaining to the parent diaquo complexes have not yet appeared in the literature. We wish to report here the results of our studies on the parent 1:1 (metal ion:ligand) complexes formed between Cr(III) and EDDA.

Experimental Section

Chemicals.—Reagent grade $CrCl_3 \cdot 6H_2O$ or $Cr(NO_3)_3 \cdot 9H_2O$ was used as the source of Cr(III) ion. Ethylenediamine-*N*,*N*′-diacetic acid, obtained from Pfaltz and Bauer, Inc., was recrystallized once from hot water before being used. All other chemicals were reagent grade. Deionized, distilled water was used in all experiments.

Preparation of the $Cr(EDDA)(OH_2)_2^+$ Reaction Mixture.—To 25 ml of an aqueous solution containing 1.36 g (7.7 mmol) of H_2EDDA and 0.52 g (3.8 mmol) of K_2CO_3 was added 10 ml of an aqueous solution containing 1.86 g (7.0 mmol) of $CrCl_3 \cdot 6H_2O$. The resulting solution was heated on a steam bath for 1.5 hr with the final volume being approximately 25 ml. Preparation of the mixture using KOH instead of K_2CO_3 and/or $Cr(NO_3)_3 \cdot 9H_2O$ instead of $CrCl_3 \cdot 6H_2O$ gave identical results in terms of ratios of the desired products. The complexes could not be obtained in solid form; hence separation and characterization studies were carried out in solution.

Separation of the $Cr(EDDA)(OH_2)_2^+$ Isomers Using Cation-Exchange Chromatography.—The reaction mixture was introduced into a 3.5 × 30 cm jacketed column containing Dowex 50W-X8 (100–200 mesh) cation-exchange resin in the H^+ form. The column was cooled to 2° during the charging and elution procedures. After washing the column with H_2O, elution of the positively charged complexes was begun with 0.20 F HNO_3. Upon elution two red bands with 1+ charge characteristics were obtained with a dark blue band of $Cr(OH_2)_6^{3+}$ remaining unmoved at the top of the resin. The relative sizes of the two red bands were quite different, the first (eluted) or less polar red band being present to a much greater extent than the second or more polar red band. Upon elution from the column each band was collected in a container cooled to 0° and immediately thereafter the visible spectrum of each species was recorded. When using $CrCl_3 \cdot 6H_2O$ as starting material a small band of a residual chloro-containing complex was noted upon elution. The red complexes were analyzed in solution for chromium and nitrogen in order to obtain Cr:EDDA ratios.

Spectral Measurements.—All visible absorption spectral measurements were made on a Coleman 46 uv–visible spectrophotometer with the cell compartment cooled to approximately 8°. During the time (approximately 15 min) required to define the visible spectrum, no decomposition of the desired species was observed.

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When measuring molar absorptivities of the complexes, only the middle third of each band was used.

Method of Analysis.—The chromium analyses were performed as previously described.[6] Nitrogen was determined by the Kjeldahl method. Hydrochloric acid was used as eluent when preparing samples for nitrogen analysis.

Results and Discussion

There are three possible geometrical isomers for the $Cr(EDDA)(OH_2)_2^+$ system and these are shown in Figure 1. To date the trans configuration for EDDA

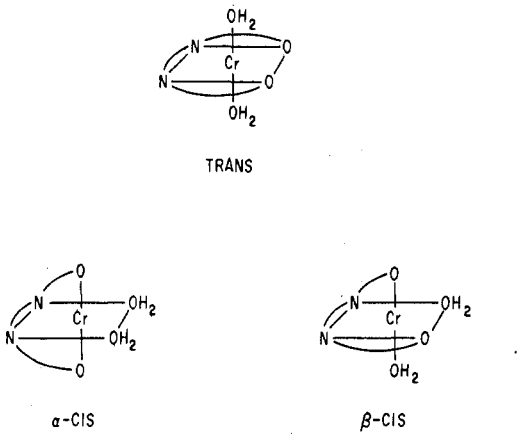

Figure 1.—The three possible geometrical isomers in the ethylenediamine-*N*,*N*′-diacetatodiaquochromium(III) system.

has been found only in the Pt systems.[2] The relative lack of abundance of the trans configuration can be rationalized in terms of bond angle strain when EDDA is coordinated in a strictly planar fashion. Similarly, considering C–N–C bond angle strain, it would appear the the β-cis configuration would be more favorable than the trans, with the α-cis configuration being the most favorable. Considering the parent EDDA–diaquometal ion complexes reported to date, this reasoning appears to be borne out by experiment. In the $Ni(EDDA)(OH_2)_2$ system only the α-cis isomer has been found,[3] while in the $Co(EDDA)(OH_2)_2^+$ system only the α-cis and β-cis isomers are present with the β-cis form isomerizing to the more stable α-cis configuration upon standing.[7] Extrapolating these results to the $Cr(EDDA)(OH_2)_2^+$ system it would appear that the α-cis and β-cis isomers would be the most likely products in a 1:1 mole ratio mixture of Cr(III) and EDDA.

In all cases studied to date containing Co(III) and EDDA it has been found that the α-cis isomer has been eluted from an ion-exchange column before the β-cis isomer. Because of the symmetry of the trans configuration one might expect the trans isomer to be eluted before the α-cis isomer or at least exhibit similar elution characteristics. Hence, the first (eluted) red band could be assigned either the trans or the α-cis configuration. Analysis of the red bands for the relative amount of +1 charged Cr(III) species present indicated that the first band contained 94 ± 2% of the total 1+ charged material while the second band contained the remainder, 6 ± 2%. Considering both the large amount of band 1 formed and the absence of the trans isomer in the Co(III) and Ni(II) systems we conclude that the trans isomer is not present in the $Cr(EDDA)(OH_2)_2^+$ system. On the other hand, both the elution order and the relative amount of each isomer obtained are consistent with the assignment of the α-cis configuration to the first band and β-cis to the second. The relative elution rates under the conditions described above were 2.3 for the first band *vs.* 1.0 for the second. Isomer purity within a given band was indicated by the reproduction of spectra for fractions within that band.

The ion-exchange columns were cooled to 2° to inhibit as much as possible isomerization reactions for the separated complexes. After allowing solutions initially pure in each isomer to stand at room temperature for approximately 1 week and then reintroducing each into a cation-exchange column it was found that isomerization had occurred in each system back to the initial equilibrium distribution. Some $Cr(OH_2)_6^{3+}$ was also present in each solution.

The visible absorption spectra of the isomers is also in agreement with the above tentative assignment of geometrical configuration. It would be expected that the molar absorptivities in the visible region would be somewhat higher for the β-cis configuration as compared to the α-cis configuration because of the lower degree of symmetry of the β-cis geometry. To date in all cases where quantitative spectral data have been obtained for the α-cis and β-cis isomers of EDDA complexes coupled with the assignment of geometrical structure based on an independent measurement, it has been found that the β-cis isomer always exhibits larger molar absorptivities than does the corresponding α-cis isomer.[1,8] The visible spectra of the $Cr(EDDA)(OH_2)_2^+$ species are recorded in Table I. The spectral curves for both complexes were symmetrical about the maxima with no noticeable splitting being observed.

TABLE I
VISIBLE ABSORPTION SPECTRAL DATA OBTAINED FOR THE $Cr(EDDA)(OH_2)_2^+$ SYSTEM

First band eluted (α-cis)	λ_1[a] (ϵ)[b,c]	529 (77)
	λ_2 (ϵ)	401 (47)
Second band eluted (β-cis)	λ_1 (ϵ)	527 (119)
	λ_2 (ϵ)	392 (72)

[a] Peak maxima in nanometers. [b] Molar absorptivity. [c] Standard deviation in ϵ less than 1 in all cases based on three determinations.

Analysis of each band for chromium and nitrogen was carried out as described in the Experimental Section. For band 1 (α-cis isomer) the result was Cr:EDDA = 1.00:0.99. The analysis of band 2 is complicated by the fact that free protonated ligand present in an equilibrium solution preparation apparently has the same elution characteristics as the complex com-

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prising band 2. Chromium:ligand ratios for band 2 were low denoting the presence of excess ligand. Free ligand presence is expected due to the presence of a sizable amount of unreacted Cr(III) on the cation column. Because of column conditions excess ligand could easily be eluted as H_3EDDA^+. To determine if this was in fact the reason for the high ligand to chromium ratio, excess Cr(III) was added to various reaction mixtures and the resin column was lengthened in expectation of approaching the correct ratio. The experimental conditions and analytical results for band 2 were as follows: (1) 10% excess EDDA present, Cr:EDDA = 1.00:2.47; (2) 40% excess Cr(III) present, Cr:EDDA = 1.00:1.64; (3) 100% excess Cr(III) present and 50% resin increase over (1) and (2), Cr:EDDA = 1.00:1.40. It can be seen that as the utilization of EDDA becomes more complete, the correct chromium:ligand ratio is approached. It should also be noted that the presence of only a small amount of unreacted EDDA could cause the above problem because of the very small percentage, totally, of band 2 formed.

The following evidence also supports the fact that the complex contains a Cr:EDDA mole ratio of 1:1. Specifically, (1) the complex unquestionably exhibits a 1+ charge as evidenced by its column motion in experiments using 0.10 *F* HCl as eluent. (2) The visible spectrum of the complex correlates well with other known $Cr-N_2O_4$ type complexes (cis N's) as compiled by Weyh and Hamm.[6]

Considering all evidence in the $Cr(EDDA)(OH_2)_2^+$ system we tentatively assign the α-cis and β-cis geometries to the most and least abundant isomers, respectively. Finally, there is no evidence to support the presence of the trans isomer.

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Fast Kinetics by Stopped-Flow Chlorine-35 Nuclear Magnetic Resonance. Reactions of Mercury(II)–Bovine Serum Albumin with Various Ligands

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The use of ^{35}Cl nmr line widths for the study of metal complexes of biological macromolecules is well established.[1-6] In a previous report,[7] we studied the ability of various ligands to remove a number of metal ions from their respective complexes with bovine serum albumin (BSA).

The inactivation of enzymes, particularly those containing sulfhydryl groups, through binding of heavy metals is known to be one of the crucial mechanisms of heavy-metal poisoning.[8-10] It is known that mercury(II) binds strongly to the sulfhydryl group of proteins.[1,6,7] Therefore, the present studies may serve as a model for an important step in chelation therapy.

The longitudinal relaxation time (T_1) of ^{35}Cl changes by approximately a factor of 10 depending on whether BSA is free or bound to mercury(II). For any reaction in which mercury(II) is removed from BSA, the rate of reaction can be measured by following T_1 as a function of time after mixing, provided that T_1 is short compared to the half-life of the reaction as is the case in the present studies.

As noted previously,[1-7] one of the advantages of the ^{35}Cl technique is the ability to study proteins at low concentrations, providing favorable conditions for the determination of fast kinetics by this technique. In the present work the ^{35}Cl line width is monitored by continuous-wave nmr as a function of elapsed time after mixing Hg–BSA with various chelating agents. The effects of temperature, pH, and ligand structure are explored. The half-lives of these reactions are measured, and a mechanism for the removal of mercury(II) is proposed.

Experimental Section

The nmr studies were carried out using 1.5 *M* NaCl solutions prepared with deionized water. Crystalline bovine serum albumin (Mann Research, Fraction V, twice recrystallized) was standardized spectrophotometrically.[11] Reagent grade mercuric acetate was used for preparation of Hg–BSA. All solutions were buffered with 0.05 *M* sodium acetate–acetic acid. pH titrations and other measurements were carried out with a Leeds and Northrup Model 7664 pH meter, standardized with pH 4 and 10 buffers at the temperatures corresponding to the measurements.

The ^{35}Cl spectra were obtained with a Varian HR-60 spectrometer as previously described.[7] For rate determinations the spectrometer was adjusted so that the recorder pen remained on the peak maximum. Peak heights were related to line widths from previously determined peak shapes.

The stopped-flow apparatus consisted of two pneumatically driven 5-ml syringes mounted in an aluminum casing. An all-Teflon mixing chamber was fabricated according to the design of Strittmatter.[12] This apparatus permitted addition and with-

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