Results and Discussion

The equilibrium constants and absorptivities obtained in the present study are summarized in Table II, together with the free energy of formation for Co(TPTZ)$^{2+}$ and Co(TPTZ)$_2{}^{2+}$, ΔG_{I} and ΔG_{II}, calculated from our measurements. It should be noted that the large estimated error in free energy of formation is associated with the large standard error in K_4. The low estimate of ΔG_{I} should not be associated with the high estimate of ΔG_{II} and *vice versa*.

Pagenkopf and Margerum[2,9] determined K_3 previously and demonstrated that a second step was required to explain their kinetic data for the formation of Fe(TPTZ)$_2{}^{2+}$. They obtained an estimate of K_4 based on pH and photometric measurements at $\mu = 0.1$. Our values of K_3 and K_4 are concentration constants at $\mu = 0.23$ based on H$^+$ concentrations instead of pH and

(9) D. W. Margerum, personal communication, 1969.

are somewhat different from theirs. The differences very likely can be ascribed to changes in the ionic medium and to the difficulty of determining a precise value for K_4.

All of the ΔH values are 0 within the experimental error (± 1 kcal mol^{-1}), and hence the stability of the complexes may be ascribed to large entropy increases. The bis(terpyridine)cobalt(II) complex is a low-spin system and has an overall ΔH of formation of -34 kcal mol^{-1}. We speculate that most of the large difference in entropy of formation arises from a more rigid, tightly bound structure in the latter complex as opposed to Co(TPTZ)$_2{}^{2+}$.

On the Complete Assignment of Nuclear Magnetic Resonance Spectra of Glycinate Protons in Cobalt(III)–Aminocarboxylate Chelates[1]

By JAMES L. SUDMEIER,[*2] ALAN J. SENZEL, and GARY L. BLACKMER

Complete assignments of the individual glycinate methylene protons in Co$^{\mathrm{III}}$PDTA (PDTA = dl-1,2-propylenediamine$N,N,$-N',N'-tetraacetate) and Co$^{\mathrm{III}}$EDTA (EDTA = ethylenediamine-N,N,N',N'-tetraacetate) were accomplished by 100-MHz pmr spectroscopy. "Tickling" experiments were used to establish the identities of the overlapping AB patterns in Co$^{\mathrm{III}}$PDTA. Stereospecific acid- and base-catalyzed deuterations of selected out-of-plane glycinate protons were carried out, leading to assignment of the out-of-plane glycinate proton resonances. Subsequent intramolecular scrambling of out-of-plane and in-plane glycinate groups was carried out by electrolytic reduction to the labile cobalt(II) complexes, followed by chemical reoxidation to the cobalt(III) complexes. This rapid and convenient method of scrambling enabled assignment of the in-plane glycinate protons. The magnitudes of the glycinate proton geminal coupling constants provide a valuable diagnostic for assignment of out-of-plane ($J \approx -18$ Hz) and in-plane ($J \approx -16$ Hz) AB patterns.

The use of proton magnetic resonance (pmr) in studies of the structures and reactions of aminocarboxylate chelates of cobalt(III),[3–9] rhodium(III),[9,10] palladium(II),[11,12] platinum(II),[13] and other metals has

(1) This research was supported by U. S. Public Health Service Grant No. 9-R01ES00477-04.

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(3) (a) R. J. Day and C. N. Reilley, *Anal. Chem.*, **36**, 1073 (1964); (b) *ibid.*, **37**, 1326 (1965).

(4) (a) J. I. Legg and D. W. Cooke, *Inorg. Chem.*, **4**, 1576 (1965); (b) *ibid.*, **5**, 594 (1966); (c) D. W. Cooke, *ibid.*, **5**, 1411 (1966); (d) P. F. Coleman, J. I. Legg, and J. Steele, *ibid.*, **9**, 937 (1970).

(5) J. B. Terrill and C. N. Reilley, *ibid.*, **5**, 1988 (1966).

(6) J. L. Sudmeier and G. Occupati, *ibid.*, **7**, 2524 (1968).

(7) G. L. Blackmer, R. E. Hamm, and J. I. Legg, *J. Amer. Chem. Soc.*, **91**, 6632 (1969).

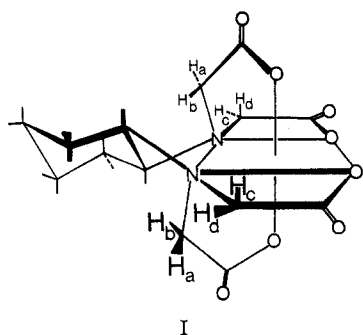(8) B. B. Smith and R. H. Betts, *ibid.*, **91**, 7749 (1969).

(9) B. B. Smith and D. T. Sawyer, *Inorg. Chem.*, **7**, 922 (1968).

(10) B. B. Smith and D. T. Sawyer, *ibid.*, **7**, 2020 (1968).

(11) (a) L. E. Erickson, J. W. McDonald, J. K. Howie, and R. P. Clow, *J. Amer. Chem. Soc.*, **90**, 6371 (1968); (b) L. E. Erickson, H. L. Fritz, R. J. May, and D. A. Wright, *ibid.*, **91**, 2513 (1969).

(12) B. B. Smith and D. T. Sawyer, *Inorg. Chem.*, **8**, 379 (1969).

(13) B. B. Smith and D. T. Sawyer, *ibid.*, **8**, 1154 (1969).

undergone steady growth. The pmr spectra of these chelates frequently include a number of overlapping AB patterns due to various pairs of nonequivalent spin-coupled glycinate protons. These complicated mixtures of AB patterns have been extremely difficult to solve completely and unambiguously. The use of H–N–C–H coupling to out-of-plane glycinate protons and the theory of anisotropic shielding has allowed a number of individual proton assignments to be made at 60 MHz for several aminocarboxylate chelates of the tetradentate and pentadentate variety. Work of this nature has recently been summarized by Legg and co-workers.[4d] There has, however, been only a single example, that of Co$^{\mathrm{III}}$CyDTA, I (CyDTA = *trans*-1,2-cyclohexanediamine-N,N,N',N'- tetraacetate), where a complete assignment of the glycinate AB patterns of a hexadentate aminocarboxylate chelate has been accomplished, leading to identification of the chemical shifts of all eight glycinate protons.

I

In the present work, the complete solution is presented for a far more difficult case, that of $Co^{III}PDTA$, II (PDTA = dl-1,2-propylenediamine-N,N,N',N'-
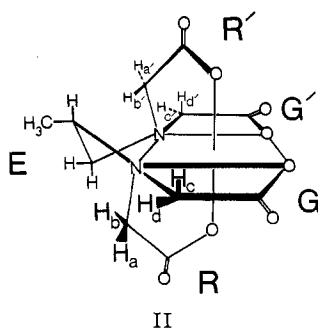
II

tetraacetate). The first complete assignment of glycinate protons in $Co^{III}EDTA$ (EDTA = ethylenediamine-N,N,N',N'-tetraacetate) was also accomplished, by the use of stereospecific base-catalyzed deuteration and 100-MHz pmr. The special techniques employed in unraveling the four overlapping AB patterns in $Co^{III}PDTA$ should prove useful in future studies of this type. These techniques include the use of "tickling," stereospecific deuteration, and reduction–oxidation (redox)-induced scrambling of in-plane and out-of-plane glycinate arms. Complete assignment of the compounds in the present study should contribute to a greater understanding of the factors affecting chemical shifts and spin–spin coupling constants in these and related compounds.

Experimental Section

Chemicals.—The purest commercially available chemicals were used without further purification. EDTA was obtained from Baker Chemical Co. and CyDTA was obtained from Aldrich Chemical Co. The method of Dwyer and Garvan[14] was used to prepare PDTA. D_2SO_4 (99.5% minimum isotopic purity, 98% in D_2O), NaOD (40% in D_2O), and D_2O (99.8% minimum isotopic purity) were obtained from Diaprep, Inc. KCl and KNO_3 were obtained from Baker Chemical Co.

Preparation of the Complexes.—The chelates $K[Co^{III}CyDTA]\cdot 3H_2O$, $K[Co^{III}PDTA]\cdot H_2O$, and $K[Co^{III}EDTA]\cdot H_2O$ were prepared by the method of Dwyer and Garvan.[14,15]

Proton Magnetic Resonance Spectra.—All spectra were recorded at 100 MHz using a Varian HA-100 nmr spectrometer at $36 \pm 1°$, the internal temperature of the probe. Chemical shifts were measured relative to sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) internal standard. All spectra were recorded at a sweep width of 500 Hz (1 mm = 1 Hz) and a scan rate of 0.5 Hz/sec. The sweep width linearity of the Varian HA-100 was checked by the usual audio-side-band method.

Acid- and Base-Catalyzed Deuterations.—The acid-catalyzed deuterations were carried out at a pD value of 0.9 obtained by the addition of D_2SO_4. The base-catalyzed deuterations were carried out at a pD value of 10.0 obtained by the addition of NaOD. All pD values were measured with a Leeds and Northrup pH meter, Model 7664, using the appropriate correction factor.[16] The samples were placed in sealed tubes in a constant-temperature water bath at 95° to increase the acid-catalyzed isotopic exchange rates.

Electrolytic Reduction of the Cobalt(III) Complexes.—All reductions were carried out at a constant potential of 0.90 V using an Analytical Instruments potentiostat. An electrolytic H cell (12-mm Pyrex glass tubing, 6 cm high and 5 cm wide) employing platinum working and auxiliary electrodes was designed and constructed conveniently to accommodate a volume of 0.5 ml, the amount required for each pmr experiment. A medium-grade frit separate the cathoded and anode compartments. An agar–KCl gel was placed on the anode side to prevent excessive bulk diffusion. A saturated aqueous solution of KNO_3 served as the electrolyte for the anode compartment. The reference electrode was a Leeds and Northrup Model 124138 silver–silver chloride microelectrode which was placed into the cobalt-(III) solution in the cathode compartment of the cell. Stirring was provided by bubbling N_2 gas saturated with D_2O through the solution in the cathode compartment. Reduction of the cobalt-(III) species was indicated by a color change from deep violet to light pink and was considered to be complete at currents less than 1.0 mA. The time required for electrolysis ranged from 10 to 30 min.

Reoxidation of the Cobalt(III) Species.—Following the electrolytic reductions, each solution was adjusted to pD 5.9 with anhydrous Na_2CO_3. One drop of 30% H_2O_2 was added and the solution was then allowed to stand for 3 days to complete the oxidation process. The pD was then adjusted to 0.9 and the spectrum was recorded.

"Tickling" Experiments.—Weak-field double irradiation was applied to the sweep coils of the HA-100 with a Hewlett-Packard Model 200AB external oscillator. The output voltage which passed from the external oscillator was adjusted by a Hewlett-Packard Model 350D attenuator. The output voltage was then fed through a 30 to 1 step-down impedance-matching transformer into the sweep coils. Optimum output voltages for successful "tickling" experiments were in the range of 100–200 mV peak to peak, as read on the HA-100 oscilloscope.

Procedure for a "tickling" experiment consisted of first measuring the resonance frequency of each peak of interest. Next, the weak-field irradiation from the external oscillator was centered in turn on each peak in the spectrum, while the entire spectrum was recorded in the frequency-sweep mode. The irradiation had to be centered to within 0.5 Hz of each peak or skewing of peaks resulted. Spinning side bands of the beat pattern were sometimes a troublesome problem, but they could be partially overcome by using high-precision nmr tubes and by changing the spinning rate to move the spinning side bands.

Results and Discussion

"Tickling."—Irradiation with fields that are weak with respect to the spin–spin coupling constants, or "tickling," at a frequency equal to a transition frequency gives rise to splitting of the individual lines of multiplets into submultiplets.[17] The degree of line splitting is directly proportional to the strength of the irradiating field. Assigning numbers 1–4 to the lines of an AB pattern in order of increasing field strength, we can show that tickling line 4 splits line 1 into a broad doublet and splits line 2 into a sharp doublet. Tickling line 3 splits line 1 into a sharp doublet and line 2 into a

(14) F. P. Dwyer and F. L. Garvan, *J. Amer. Chem. Soc.*, **81**, 2955 (1959).

(15) F. P. Dwyer and F. L. Garvan, *ibid.*, **83**, 2610 (1961).

(16) P. K. Glascoe and F. A. Long, *J. Phys. Chem.*, **64**, 188 (1960).

(17) R. Freeman and W. A. Anderson, *J. Chem. Phys.*, **37**, 2053 (1962); **39**, 806 (1963).

broad doublet. The results for tickling lines 1 and 2 are derived from the previous statements by interchanging the terms "line 4" with "line 1" and "line 3" with "line 2." In addition, changes in certain line intensities due to the general nuclear Overhauser effect[18] are usually observed such that broad doublets increase in total intensity and sharp doublets decrease in total intensity.
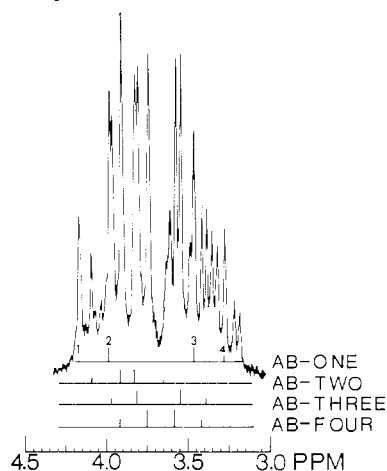

Figure 1.—Pmr spectrum of $Co^{III}PDTA$ at 100 MHz.

Figure 1 shows the unperturbed pmr spectrum of $Co^{III}PDTA$ at 100 MHz. Sixteen of the approximately twenty-four spectral lines are arranged into four AB patterns due to the eight glycinate protons and are designated by the vertical lines below the spectrum. The unlabeled lines remaining are attributed to three of the protons (two methylene and one methine) on the propylenediamine "backbone."[19] The task of determining the correct pairings of AB patterns and finding which lines were extraneous was accomplished through tickling each line and recording the resulting frequency-sweep spectra.

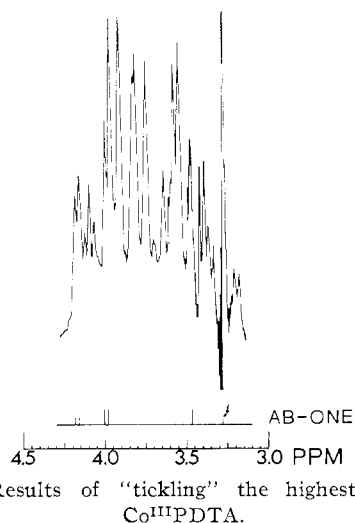Figure 2 shows the results of one of these "tickling"

Figure 2.—Results of "tickling" the highest field line of $Co^{III}PDTA$.

experiments in which the highest field line (line 4) of AB-one was tickled. Although Figure 2 lacks the high resolution of Figure 1, it is apparent that line 1 has been split into a broad doublet. Because line 2 is so close to another spectral line (on its right), it is less apparent that line 2 has been split into a sharp doublet, part of which overlaps the neighboring spectral line. The beat pattern located at the frequency of line 4 is caused by interference of the observing and irradiating magnetic fields. Peaks separated by as little as 3 Hz can be irradiated selectively. These "tickling" experiments provide direct proof of the correctness of the tentative assignments reached by earlier workers.[3b,5]

Geminal Proton Coupling Constants.—All glycinate proton geminal coupling constants which have been reported for cobalt (III)–aminocarboxylate compounds[3-9] fall into two categories[20] those in the range -16 ± 0.5 Hz[21] and those in the range -18 ± 0.5 Hz. In every case, it appears that $J \approx -18$ Hz indicates out-of-plane (R) glycinate rings and $J \approx -16$ Hz indicates in-plane (G) glycinate rings. One factor which would account for this trend is the hyperconjugative withdrawal of electrons by the π-electron system of the carbonyl group—removing electrons from the antisymmetric bonding orbital of the CH_2 group. According to the molecular orbital theory of geminal coupling,[21,22] the maximum effect occurs when the H–H internuclear axis is perpendicular to the nodal plane of the π system, producing a more negative coupling constant. Because out-of-plane glycinate rings are known to be more planar than in-plane glycinate rings,[23] the coupling constants should be more negative for the former than for the latter, which is indeed the case. In summary, it appears that the magnitude of the geminal coupling constant is a valuable diagnostic for the degree of planarity of the glycinate ring, which in turn is diagnostic of the out-of-plane or in-plane position of the ring with respect to the Co^{III} complex. Therefore, with no knowledge gained from analogous compounds and no other information except that gained in the above "tickling" experiments, it would be tentatively possible to assign AB-one and AB-two in the $Co^{III}PDTA$ spectrum to out-of-plane rings and AB-three and AB-four to in-plane rings on the basis of the geminal coupling constants.

Stereospecific Deuteration.—Previous studies have shown that acid-catalyzed[5] and base-catalyzed[6] deuterations of glycinate protons in cobalt (III)–aminocarboxylate compounds are stereospecific. That is, the

(18) R. Kaiser, *J. Chem. Phys.*, **39**, 2435 (1963); **42**, 1838 (1965).

(19) Incidentally, a first-order analysis of the four lines belonging to the highest field methylene proton indicates probable values of $J_{gem} = -13.2$ Hz and $J_{vic} = 3.4$ Hz, the latter value for a dihedral angle close to $60°$. For an explanation of analysis of other PDTA complexes, see J. L. Sudmeier and A. J. Senzel, *Anal. Chem.*, **40**, 1693 (1968); *J. Amer. Chem. Soc.*, **90**, 6860 (1968).

(20) There is one possible exception to this statement to data. Reference 3b quotes $J = 11.0$ Hz for one of the four AB patterns in Co^{III}-*meso*-BDTA, where BDTA = 2,3-butylenediaminetetraacetate. A highly overlapping spectrum, however, leads to poor substantiation of this value. If the value does prove to be correct, on the other hand, the explanation will probably lie in the fact that Co^{III}-*meso*-BDTA is the only complex of its type which has been studied containing an axial substituent on the diamine "backbone." The axial methyl group would have very strong steric interactions with one of the glycinate R rings.

(21) The geminal coupling constants in all these cobalt(III)–aminocarboxylate compounds are assumed to be negative in absolute sign by analogy with aliphatic CH_2 groups in many similar compounds. See, for example, A. A. Bothner-By, *Advan. Magn. Resonance*, **1**, 195 (1965).

(22) J. A. Pople and A. A. Bothner-By, *J. Chem. Phys.*, **42**, 1339 (1964).

(23) H. A. Weakliem and J. L. Hoard, *J. Amer. Chem. Soc.*, **81**, 549 (1959).

rates of deuteration of the out-of-plane glycinate protons which are farthest removed from the diamine "backbone," for example, H_a and $H_{a'}$ in I and II, are far more rapid than those of H_b, $H_{b'}$, or any other CH proton. Examination of the spectrum after deuteration of only H_a (or even partial deuteration of H_a) enables the immediate assignment of H_a because of the loss of intensity and of H_b because of the redistribution of intensity resulting from the 6.5 times smaller (the ratio of the proton to deuterium gyromagnetic ratios) deuterium–proton geminal coupling constants. In effect, a new peak appears to grow in at a frequency corresponding to the chemical shift of H_b.

Base-catalyzed deuteration was employed whenever possible (*e.g.*, for $Co^{III}EDTA$) because the method is more rapid and more stereospecific than acid catalysis.[6] However, for certain compounds such as $Co^{III}PDTA$ and, to some extent, $Co^{III}CyDTA$, the spectra become very broad at pD 11 and above, presumably due to formation of a paramagnetic Co(II) species. In these cases, the more tedious acid-catalyzed deuteration method was employed.

Redox-Induced Scrambling.—Because of the lability of cobalt(II)–aminocarboxylate compounds, a rapid interchange of out-of-plane (R) and in-plane (G) rings occurs in the Co(II) form. This results in scrambling or randomization of H_a with H_c and H_b with H_d (as in structure I for stereospecific ligands such as CyDTA and PDTA. Reduction and reoxidation of cobalt(III)–aminocarboxylate compounds in which H_a is deuterated lead to loss of intensity of H_c and redistribution of H_d intensity, thus enabling assignments of H_c and H_d. Concurrently, a partial renewal of the H_a intensity (gained from H_c) and a redistribution of the H_b intensity takes place.

Redox-induced scrambling is much simpler than removal of the cobalt by complexation of the metal and precipitation of the partially deuterated chelating agent—a method used by previous workers.[5] The present method is also applicable to ligands that are not readily isolated from solution.

$Co^{III}PDTA$.—In $Co^{III}PDTA$, the presence of the methyl group on the diamine "backbone" destroys the C_2 axis of symmetry found in $Co^{III}CyDTA$ and Co^{III}-EDTA Therefore, as shown in II, two nonequivalent sets of protons H_a through H_d are present, the set farthest removed from the methyl group being designated by primes ($H_{a'}$ through $H_{d'}$). If the deuteration rates had been equal for H_a and $H_{a'}$ and for H_b and $H_{b'}$, this method would not have permitted us to distinguish H_c from $H_{c'}$ or H_d from $H_{d'}$. In a previous attempt[5] to assign the latter four protons, Terrill and Reilley were able to isolate "3D-PDTA" because of their finding that the rate of acid-catalyzed deuteration of $H_{b'}$ is greater than that of H_b. Our finding that the progressive deuteration of $Co^{III}PDTA$ goes in the order $H_a >$ $H_{a'} > H_{b'} > H_b$ is in agreement with this.

In the present work, however, we were able to study first the scrambled form of the intermediate having only H_a appreciably deuterated ("1D-PDTA") and then the

scrambled form of the intermediate having both H_a and $H_{a'}$ deuterated ("2D-PDTA"). This approach leads to the first successful assignment of all eight protons, as will be shown.

In the spectrum of nondeuterated $Co^{III}PDTA$ in Figure 1, two of the four AB patterns, labeled AB-one and AB-two, are known to arise from out-of-plane (R) rings, based upon deuteration studies and geminal coupling constants of ~ -18 Hz. The assignment of AB-one to ring R in II and AB-two to ring R' was based in part on the similarity in AB chemical shift differences in R' to the analogous compounds $Co^{III}CyDTA$ and $Co^{III}EDTA$ in accordance with previous workers.[5] This assignment is further substantiated by invoking the theory of steric compressions.[24,25] Proton H_b of R is sterically compressed by the methyl group on the propylenediamine backbone which results in its resonance being shifted to lower fields (*i.e*, 4.06 ppm). Correlation of AB chemical shift differences in cobalt-(III)–aminocarboxylate compounds with various structural features such as alkyl substituents on the nitrogen atoms or carbon atoms of the ethylenediamine "backbone" has been quite successful,[4,6,25] which lends support to the assignments of AB-one and AB-two. Figure 3 shows the spectrum of $Co^{III}PDTA$ after acid-catalyzed deuteration (pD 0.9, 95°) for 65 min. The upfield portion of AB-one, assigned to H_a, has decreased by approximately 75% or greater of its initial intensity, and the upfield portion of AB-two, assigned to $H_{a'}$, has decreased by no greater than 33% of its initial intensity. This is the point of maximum difference in the extents of deuteration of H_a and $H_{a'}$. The vertical arrow below the spectrum marks the appearance of a new peak at 4.06 ppm, the chemical shift position of H_b, resulting from a redistribution of the H_b intensity.

Reduction of the species whose pmr spectrum is shown in Figure 3 to the Co(II) complex and subse-

AB-ONE
AB-TWO
AB-THREE
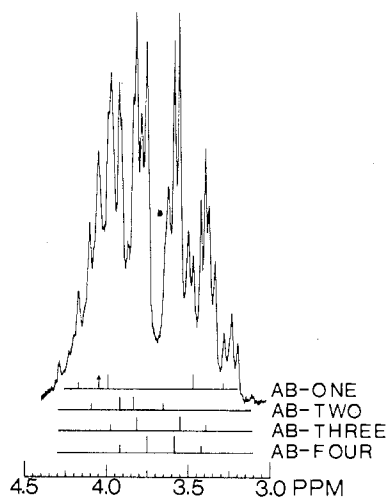AB-FOUR

4.5   4.0   3.5   3.0 PPM

Figure 3.—Pmr spectrum of Co^{III} after acid-catalyzed deuteration (pD 0.9, 95°) for 65 min.

(24) T. Schaefer, W. F. Reynolds, and Y. Yonemoto, *Can. J. Chem.*, **41**, 2069 (1963).

(25) J. C. Dabrowiak and D. W. Cooke, *J. Amer. Chem. Soc.*, **92**, 1097 (1970).

quent reoxidation yields a species whose spectrum is shown in Figure 4. As discussed above, this redox-induced scrambling procedure causes H_a (assigned to the upfield portion of AB-one) to randomize with H_c and $H_{a'}$ (assigned to the upfield portion of AB-two) to randomize with $H_{c'}$. The resultant effect upon AB-three is far greater than the effect upon AB-four, as shown in Figure 4. Since H_a was approximately 75%
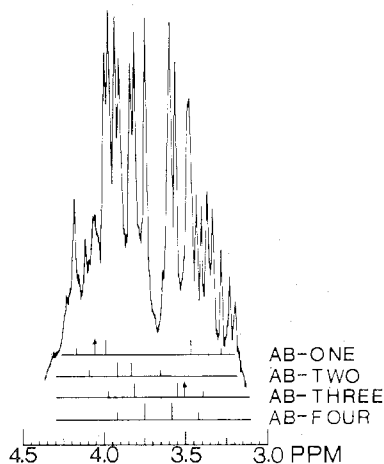

Figure 4.—Pmr spectrum of $Co^{III}PDTA$ after acid-catalyzed deuteration (pD 0.9, 95°) for 65 min followed by reduction to the Co(II) complex and subsequent reoxidation.

deuterated before redox-induced scrambling and $H_{a'}$ was only approximately 33% deuterated before scrambling, AB-three can be assigned to H_c and H_d and AB-four can be assigned to $H_{c'}$ and $H_{d'}$. By comparison with Figure 1, it is apparent in Figure 4 that the downfield portion of AB-three has decreased in intensity and the upfield portion of AB-three has redistributed its intensity, resulting in the appearance of a new peak at 3.50 ppm, indicated by the vertical arrow below the spectrum. This enables us to assign the downfield portion of AB-three to H_c and the upfield portion to H_d. Concurrently, a partial renewal of the intensity of the upfield portion of AB-one, corresponding to H_a, and a partial redistribution of the intensity of the downfield portion of AB-one, corresponding to H_b, back to its original unperturbed form (as in Figure 1) has taken place. The partial renewal of the original H_a intensity has, of course, been gained from H_c. The partial redistribution of the H_b intensity has resulted in a decrease in intensity of the peak at 4.06 ppm, the chemical shift position of H_b.

Figure 5 shows the spectrum of $Co^{III}PDTA$ after acid-catalyzed deuteration (pD 0.9, 95°) for 2 hr. The upfield portion of AB-one, assigned to H_a, has now virtually disappeared, and the upfield portion of AB-two, assigned to $H_{a'}$, has decreased to approximately 50% of its initial intensity. The vertical arrow below the spectrum marks the appearance of a new peak at 3.98 ppm, the chemical shift position of $H_{b'}$, resulting from a redistribution of the $H_{b'}$ intensity.

Reduction of the species whose pmr spectrum is shown in Figure 5 to the Co(II) complex and subse-

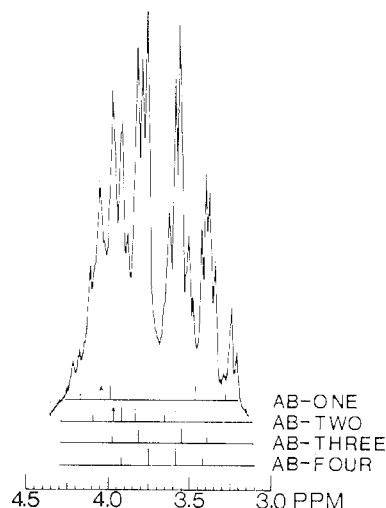Figure 5.—Pmr spectrum of $Co^{III}PDTA$ after acid-catalyzed deuteration (pD 0.9. 95°) for 2 hr.

quent reoxidation yields a species whose spectrum is shown in Figure 6. This redox-induced scrambling procedure causes the same randomizations described above, but now the effect upon AB-four is observable as well as the effect upon AB-three. By comparison with Figure 1, it is apparent in Figure 6 that the downfield portion of AB-four has decreased in intensity and the upfield portion of AB-four has redistributed its intensity, resulting in the appearance of a new peak at 3.53 ppm, indicated by the vertical arrow below the spectrum. This enables us to assign the downfield portion of AB-three to $H_{c'}$ and the upfield portion to $H_{d'}$. Concurrently a partial renewal of the intensity of the upfield portion of AB-two, corresponding to $H_{a'}$, and a partial redistribution of the intensity of the downfield portion of AB-two, corresponding to $H_{b'}$, back to its original unperturbed form (as in Figure 1) has taken place. The partial renewal of the original $H_{a'}$ intensity has, of course, been gained from $H_{c'}$. The partial redistribution of the $H_{b'}$ intensity has resulted in a decrease in intensity of the peak at 3.98 ppm, the chemical shift position of $H_{b'}$. Thus, the assignment of each individual glycinate methylene proton in the $Co^{III}PDTA$ system is now complete and is summarized in Table I.

TABLE I

CHEMICAL SHIFTS FOR GLYCINATE PROTONS IN COBALT(III) AMINOCARBOXYLATES[a]

	R rings[b] (out-of-plane)				G rings[b] (in-plane)			
	R		R'		G		G'	
	H_a	H_b	$H_{a'}$	$H_{b'}$	H_c	H_d	$H_{c'}$	$H_{d'}$
$Co^{III}PDTA$	3.39	4.06	3.76	3.98	3.88	3.50	3.81	3.53
$Co^{III}EDTA$	...	...	3.81	3.94	...	...	3.89	3.57
$Co^{III}CyDTA$	3.37	4.23	...	...	...	...	3.83	3.54

[a] Chemical shifts in ppm relative to DSS. [b] See structure II.

$Co^{III}EDTA$.—The 100-MHz pmr spectrum of Co^{III}-EDTA shows two AB patterns (Figure 7). Previous workers have assigned AB-one to the out-of-plane glycinate protons and AB-two to the in-plane glycinate protons based upon displacement of the in-plane glycin-
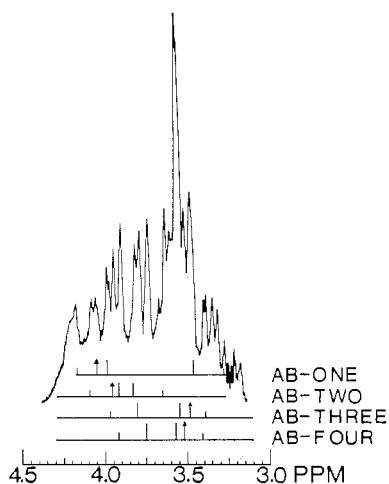
Figure 6.—Pmr spectrum of Co^{III}PDTA after acid-catalyzed deuteration (pD 0.9, 95°) for 2 hr followed by reduction to the Co(II) complex and subsequent reoxidation.

ate rings by halide ions,[3] deuterium substitution of out-of-plane glycinate protons,[5,26] and arguments regarding out-of-plane glycinate chemical shift difference.[4a] The geminal coupling constants for AB-one ($J = -18$ Hz) and for AB-two ($J = -16$ Hz) and the widely differing peak intensities support these assignments so clearly that "tickling" was unecessary. Because of inadequate resolution of AB-one at 60 MHz by all previous workers, the stereospecificity of deuteration of out-of-plane glycinate protons was not discovered, and, therefore, no attempt to assign the individual protons H$_a$, H$_b$, H$_c$, and H$_d$ (labeled as in I) was made.

Previous studies in this laboratory[6] enabled us to predict that the deuteration of out-of-plane glycinate protons would be stereospecific (H$_a$ more rapid than H$_b$) despite the absence of alkyl substitutents on the ethylenediamine backbone deemed necessary by previous workers.[5]
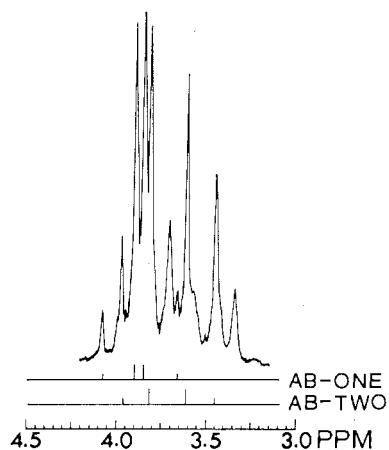
Figure 8 shows the spectrum of Co^{III}EDTA in D$_2$O

Figure 7.—Pmr spectrum of Co^{III}EDTA at 100 MHz.

after 30 min of deuteration at pD 10.0 (room temperature). The high-field portion of AB-one is diminished in intensity, and the low-field portion undergoes a re-

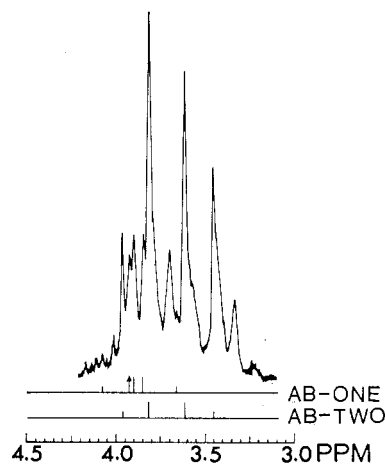(26) D. H. Williams amd D. H. Busch, *J. Amer. Chem. Soc.*, **87**, 4644 (1965).

Figure 8.—Pmr spectrum of Co^{III}EDTA after base-catalyzed deuteration (pD 10.0) for 30 min.

distribution of its intensity, with a new peak appearing at 3.94 ppm, the calculated chemical shift value of the low-field portion of AB-one. Figure 9 shows the spectrum of the same sample after deuteration had con-
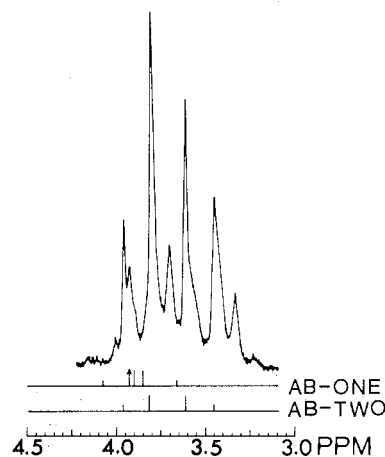

Figure 9.—Pmr spectrum of Co^{III}EDTA after base-catalyzed deuteration (pD 10.0) for 1 hr.

tinued for a total of 1 hr. At this point there is no trace of the upfield portion of AB-one, but the peak at 3.94 ppm remains. At longer deuteration times, the peak at 3.94 ppm eventually diminishes to zero intensity.

These results prove our prediction of stereospecificity in the deuteration of Co^{III}EDTA and enable us to assign the low-field and high-field portions of AB-one to H$_b$ and H$_a$, respectively. The H$_a$ resonances are consistently upfield of the H$_b$ resonances in Co^{III}EDTA, Co^{III}PDTA, and Co^{III}CyDTA.

Since EDTA contains no center of optical activity, the scrambling technique would result in racemization of the cobalt(III) chelate. Therefore, assignments of the individual glycinate protons were made by analogy. The chemical shift differences for coordinated in-plane glycinate protons ($\Delta\delta_{cd}$) generally fall into a fairly narrow range of values (0.28–0.38 ppm), and thus $\Delta\delta_{cd}$ appears to be relatively insensitive to structural changes

such as alkyl substitution at the carbon atoms of the diamine backbone. For both cases where H_o and H_d have been assigned, $Co^{III}PDTA$ and $Co^{III}CyDTA$, the H_o resonances lie downfield of the H_d resonances. Any crossover in the chemical shifts of H_o and H_d for Co^{III}-EDTA seems unlikely, and on this basis the chemical shifts of H_o and H_d shown in Table I are obtained.
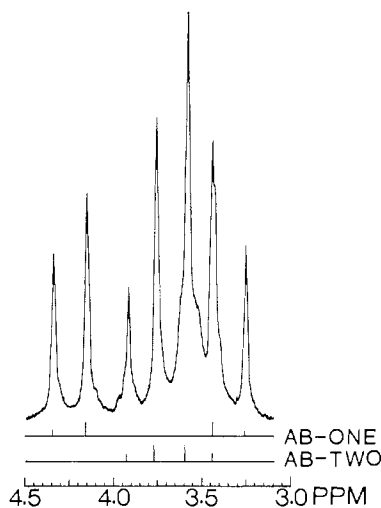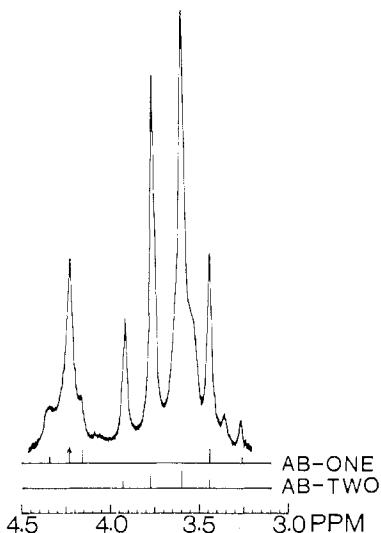

Figure 10.—Pmr spectrum of $Co^{III}CyDTA$ at 100 MHz.

Figure 11.—Pmr spectrum of $Co^{III}CyDTA$ after acid-catalyzed deuteration (pD 0.9, 95°) for 7 hr.

$Co^{III}CyDTA$.—Figure 10 shows part of the 100-MHz pmr spectrum of $Co^{III}CyDTA$. Terrill and Reilley[5] assigned AB-one ($J = -18$ Hz) to H_b and H_a (in order of increasing field strength) and AB-two ($J = -16$ Hz) to

H_c and H_d (in order of increasing field strength) by 60-MHz pmr. Their method required the precipitation of the partly deuterated ligand ("2D-CyDTA") a technique which is laborious and of limited applicability.

Figure 11 shows the spectrum of $Co^{III}CyDTA$ after treatment for 7 hr at 95° in acidic D_2O (pD 0.9). As in previous work, the upfield portion of AB-one decreases in intensity (some 10% remains) while the downfield portion undergoes redistribution of its intensity. This results in the formation of a new peak at 4.23 ppm, the calculated chemical shift value of the low-field portion of AB-one.

After electrochemical reduction and subsequent chemical oxidation of this partially deuterated sample, the pmr spectrum shown in Figure 12 was obtained.
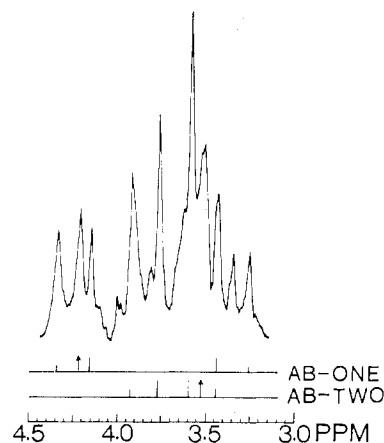

Figure 12.—Pmr spectrum of Co^{III} CyDTA after acid-catalyzed deuteration (pD 0.9, 95°) for 7 hr followed by reduction to the Co(II) complex and subsequent reoxidation.

The new peak at 3.54 ppm, the calculated chemical shift value of the high-field portion of AB-two, results from the random mixing of H_b with H_d due to the ligand scrambling. The assignment of H_c and H_d (Table I) obtained by this method agrees with that of previous workers.[5]