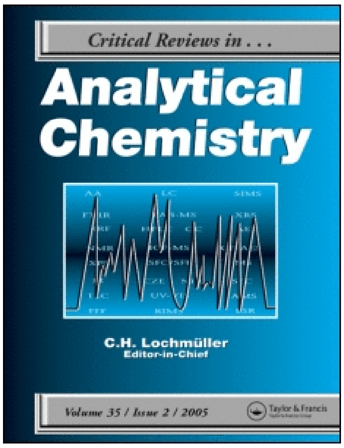

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NMR SPECTROSCOPY OF METAL CHELONATES AND RELATED COMPOUNDS

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I. INTRODUCTION

The purpose of the review is to summarize in a critical way the information that has been obtained concerning the structure, equilibria, and chemical dynamics of metal chelonates utilizing nuclear magnetic resonance. To accomplish this goal, an arbitrary division of topics has been made as well as an arbitrary selection of material. It is considered to be beyond the scope of this review to relate each detail of all reports of the use of NMR where metal chelates or chelating agents are studied. For the most part, the state of knowledge and illustrative applications of NMR may be exemplified by means of a commonly known member of a series. For example, ethylenediaminetetraacetic acid (EDTA) is used as a model for purpose of discussion of the aminocarboxylate compounds. Acetylacetone and 8-hydroxyquinoline are used to illustrate studies on β-diketones and quinolinols respectively.

In the organization of the review, the aminocarboxylates will be considered first. There are several reasons for this. Historically, these were the first compounds studied extensively by those of analytical persuasion. The details of these studies permit a logical development of the techniques. In addition, the aminocarboxylates form a large series of compounds of wide interest. An in depth development of studies of this series perhaps would be of greatest interest. The organization of this section follows a loose chronological order, because in this case it is a reasonable logical order. The first topics are the acid-base equilibria and microscopic protonation schemes. This is followed by structure and lability considerations, ligand exchange reactions and, last, conformation studies.

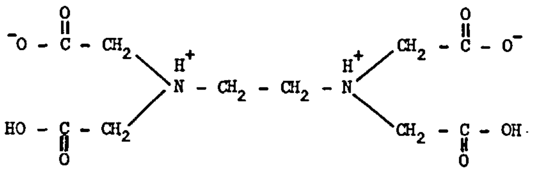
Additional major sections are devoted to quinolinols and β-diketones. These sections are less extensive because the techniques will have been developed in earlier sections and there have been fewer studies.

II. AMINOCARBOXYLATES

The general class of compounds known as aminocarboxylates includes a large variety of chelating agents important in biological systems and analytical applications. A member of the class may contain one or more amino and carboxylate sites capable of simultaneous coordination to a central metal ion to form a chelate. Because these functional groups participate in acid-base equilibria, the chelation equilibrium is pH-dependent. It is understandable that the fundamental property of the acid-base equilibrium was among the first investigations to be made when NMR studies of chelating agents were undertaken.

A. Protonation of Free Ligands

Using the model that ethylenediaminetetraacetic acid (EDTA) represents a multifunctional α-amino acid, Schwarzenbach and Ackermann proposed a double zwitterion structure (I).[1] This proposition was based upon macroscopic ionization constants, and went unconfirmed for many years as a reasonable interpretation without serious challenge. Chapman et al. explicitly pointed out that a method for investigating protonation schemes must be capable of either giving a signal directly from the labile proton or detecting its influence on a site near the functional group.[2] It was also pointed out that NMR had the required capability. There are only two serious disadvantages encountered when using NMR for such studies. First, in the absence of special methods of signal enhancement, the technique requires a concentration of approximately 0.2 M for most ligands of interest. This is well above a desirable level for equilibrium studies. Second, the strong absorption by water in the NMR is an occasional problem. This may be remedied in several ways, the most obvious of which is to utilize D_2O as the solvent. In spite of these two disadvantages, NMR can often provide information not obtainable by any other reasonable means. Certainly the prolific abundance of instruments which was developed in the last ten years played a significant role in the volume of research conducted.



I

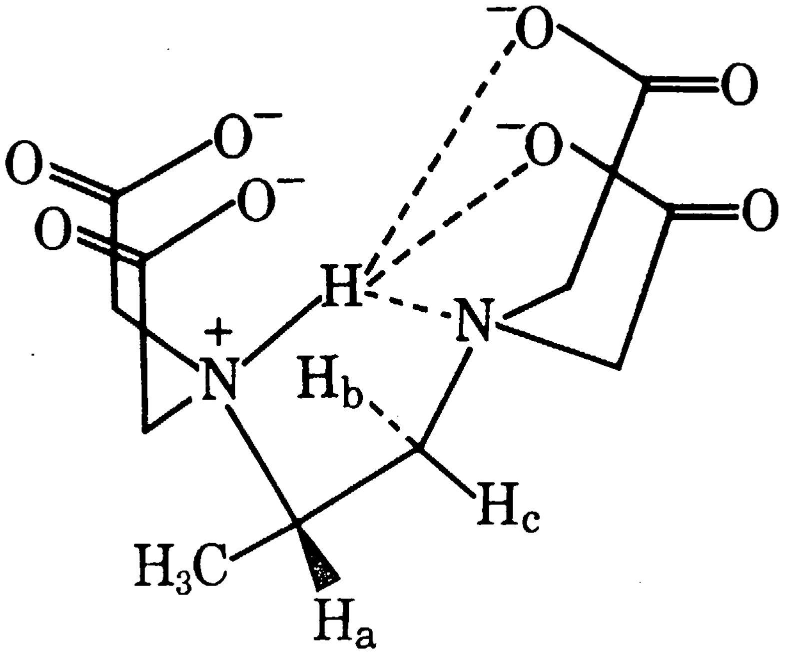
The basis of the application of NMR to the study of microscopic protonation of multifunctional molecules lies in the influence the loss or gain of a labile proton has upon the chemical shift of adjacent nonlabile protons. The chemical shift of these nonlabile protons is determined by many factors including diamagnetic shielding by the electrons. If an adjacent site is protonated, the electron density about the nonlabile protons is reduced and the chemical shift will change to a lower magnetic field. The extent of the change in chemical shift is a function of the nature of the functional site, the distance from this site, and the degree of protonation of the site on a time average basis. The first two of these dependencies are easily accepted, at least qualitatively. The last, however, deserves some comment. If the labile protons did not undergo rapid exchange among all the molecules of the ligand, two spectra would be observed as protonation was carried out by adding an acid to a solution of the unprotonated ligand. One of these would represent the molecules protonated, and would increase in intensity as the titration proceeded. The second would represent the molecules still unprotonated, and its intensity would decrease to zero at the equivalence point. The degree of protonation would be evaluated from the relative intensity of the two spectra. However, in aqueous solutions acidic protons undergo rapid exchange between solvent and ligand sites. The result is that only one NMR spectrum is observed instead of two; it represents an average of the two described above and its chemical shift is a weighted average of the protonated and unprotonated states. The position of the chemical shift between the two extremes may be interpreted as a measure of the fraction of time the site is protonated (f-value). Identification of the proton(s) responsible for a peak whose shift is observed upon changing pH permits both the identification of the site being protonated and the determination of the f-value for that site as a function of pH or the number *n* of equivalents of acid added.

Chapman et al. were the first to apply these principles to elucidate protonation schemes by aminocarboxylates.[2] Their work will not be discussed in great detail because the conclusions were qualitative and were in essence confirmed by later quantitative studies. However, the concepts may be further illustrated by an example from this work. In order to assign the observed NMR peaks, the chemical-shift changes for particular structural arrangements were obtained from model compounds and compared with those for more complicated compounds. For example, dimethylglycine was used as a model. Upon addition of the first proton a chemical-shift change ($\Delta\delta$) of 0.68 ppm was observed for the CH_3 protons and one of 0.80 ppm was observed for the CH_2 protons. When the second proton is added, $\Delta\delta_{CH_3}$ is 0.11 ppm, whereas $\Delta\delta_{CH_2}$ is 0.39 ppm. One would expect that protonation at the nitrogen site would generate a large shift in both the CH_3 and CH_2 protons, while protonation at the carboxylate site would lead to a smaller shift in the more remote CH_3 protons than in the adjacent CH_2 protons. Therefore, a set of values obtained from such models may be used to elucidate, at least qualitatively, protonation of multifunctional molecules. The conclusion of Chapman et al. was that aminocarboxylates protonate first at the nitrogen site. A further contribution was the suggestion that monoprotonated aminopolycarboxylates such as EDTA exist in a structure "stereochemically equivalent to a trifurcated hydrogen bond" as in II. Further evidence for this structure obtained by Sudmeier and Senzel will be discussed later.[3]

Following the general techniques of Chapman et al., a quantitative study of the protonation schemes of a series of polyamines and polyaminocarboxylates was performed by Sudmeier and Reilley.[4a] The chemical-shift change of a given set of nonlabile protons upon protonation of an adjacent site is expressed as

$$\Delta\delta_i^c = \sum_{j=1}^{N} C_{ij} f_i \qquad (1)$$

where $\Delta\delta_i^c$ is the total "protonation shift" (calculated) of the i^{th} observed resonance, C_{ij} is the protonation shift constant for the i^{th} resonance corresponding to total protonation of the j^{th} basic site, and f_i is the fraction of time during which the j^{th} site is protonated. There are two fundamental assumptions implied in Equation 1. First, the shift contribution of a particular basic site is linearly related to the fraction of time the site is protonated. Second, the contributions of protonating different sites are additive. The first assumption is reasonably well documented by the early work of Grunwald, Loewenstein, and Meiboom.[5] The weakness in the method centers about the second assumption. In order to compare calculated values of $\Delta\delta_i^c$ with observed values ($\Delta\delta_i$), it is necessary to obtain the values of C_{ij}. If the protonation of one site perturbs the value of C_{ij} for the protonation of a second site, then these "protonation shift constants" will change as the titration proceeds. The effect of electronic and conformational changes cannot be easily incor-



STRUCTURE II. Sudmeier, J. L. and Senzel, A. J., *J. Amer. Chem. Soc.*, 90, 6860 (1968). With permission.

porated into functions for C_{ij}, and therefore one must resort to the use of model compounds containing the substituents most commonly found in chelating agents. As an example, one set of data for model compounds is shown in Table 1.[4] The normal or isolated methylenic group was arbitrarily set at 1.25 ppm vs. sodium 3-(trimethylsilyl)-propane sulfonate (DSS) in this table. The values in parentheses are modifications to account for chain stiffening due to coulombic repulsion when polyamines are protonated. To calculate a protonation shift constant for a given methylenic group, one takes the difference between the corresponding two groups in the table. For example, the chemical shift change upon protonation of a primary amine group in the β-position with respect to a methylene group whose resonance is observed is the difference between the chemical shift values given for the fifth and fourth entries in Table 1, or 0.25 ppm.

In addition to Equation 1, one may normalize the total time spent on all sites by protons as

$$\sum_{i=1}^{N} f_i = n \qquad (2)$$

where n is the number of equivalents of protons added. Equation 2 permits the elimination of one variable f_i. In many cases in which degenerate sites

TABLE 1

Methylenic Substituent Shielding Constants

(normal $-CH_2-$ at 1.25 ppm vs. DSS)[4,a]

$-CH_2-CH_2-CH_2NH_3^+$	0.00	
$-CH_2-CH_2-NH_2$	0.05	
$-CH_2-CH_2-NH_3^+$	0.10	
$-CH_2-NH_2$	0.15	
$-CH_2-NH_3^+$	0.40	(0.45)[a]
$-NH_2$	1.35	
$-NH_3^+$	1.80	(1.85)[a]
$-CH_3$	0.05	
$-CH_2-NRH$	0.15	(0.20)[a]
$-CH_2-NRH_2^+$	0.40	(0.50)[a]
$-NRH$	1.30	
$-NRH_2^+$	1.85	(1.90)[a]
$-CH_2-NR_2$	0.15	
$-CH_2-NR_2H^+$	0.50	
$-NR_2$	1.15	
$-NR_2H^+$	1.90	
$-NR_3^+$	2.00	
$-CH_2-OH$	0.20	
$-OH$	2.25	
$-CH_2-OR$	0.25	
$-OR$	2.15	
$-CH_2-CO_2^-$	0.30	
$-CH_2-COOH$	0.35	
$-CO_2^-$	0.90	
$-COOH$	1.10	

[a] Modified for application to polyamine compounds

are present (such as EDTA with four equivalent carboxylate sites and two equivalent amino sites) the number of equations is greater than the number of unknowns and techniques for solving over-determined equations must be employed. Sudmeier and Reilley chose to minimize the sum of squares of the differences between the calculated and observed chemical shifts for each of the observed i^{th} resonances as expressed in Equation 3.

$$\sum_{i=1}^{N} (\Delta\delta_i^c - \Delta\delta_i)^2 = \sum_{i=1}^{N} d_i^2 = \text{minimum} \qquad (3)$$

Using the sum of the squares of the i^{th} deviations, standard methods were employed to estimate the standard deviations of the results.

A plot of the observed chemical shift vs. pH for the various methylenic protons in ethylether-diaminetetraacetic acid (EEDTA) is shown in Figure 1.[4a] This example illustrates a case in which the addition of two equivalents of acid (note values of pK_3 and pK_4) results in a smooth change in the chemical shift to lower field. Notice that the *a* and *b* protons have approximately equal change in the chemical-shift values indicating protonation at the nitrogen site, whereas the more remote *c* protons have a much smaller change in chemical shift. Figure 2 shows a similar plot for diethylenetriaminepentaacetic acid (DTPA)[4a]. The plot illustrates an example in which the central amino site (2) is protonated with the first equivalent of acid; presumably, however, coulombic repulsion forces a redistribution upon the addition of the second equivalent of acid so that the terminal amino sites (1) are the ones of major protonation. These interestingly vacillating chemical-shift data for DTPA were reported almost simultaneously by Sudmeier and Reilly[4b] and by Kula and Sawyer.[6]

However, Sudmeier and Reilley used Equations 1 and 2 to calculate chemical shift changes ($\Delta\delta_i^c$) and fit curves such as those shown in Figures 1 and 2 by means of Equations 1 to 3. Table 2 shows the results for a series of polyamines and aminocarboxylates.[4a] The results shown in Table 2 confirm with some extension the early work of

TABLE 2

Percent Protonation at Various Basic Sites[4a]

Compound	f_1	f_2	f_3	f_4	σ
EDTA					
n = 1	53±5	-2±3			±6.5
n = 2	96±2	2±1			±3.0
EEDTA					
n = 2	96±11	2±5			±11.3
EGTA					
n = 2	94±6	3±3			±6.4
HEDTA					
n = 1	42±7	52±6	3±18	0±21	±5.8
n = 2	87±6	97±5	0±15	16±18	±4.8
DTPA					
n = 1	26±1	41±1	7±2	...	±1.0
n = 2	85±5	15±6	0±10	4±8	±3.7
n = 3	80±11	64±13	76±23	...	±13.0
Dien					
n = 1	41±4	18±8			±3.9
n = 2	92±12	16±25			±11.9
n = 3	98±6	104±12			±5.9
Trien					
n = 1	36±6	14±6			±5.2
n = 2	76±10	24±10			±9.2
n = 3	99±7	51±7			±6.4
n = 4	100±1	100±1			±1.0
Tetren					
n = 1	29±1	13±1	16±1		±0.6
n = 2	69±11	11±11	40±15		±8.1
n = 3	99±18	9±19	84±25		±13.7
n = 4	104±6	67±6	58±8		±4.5
n = 5	99±5	101±5	100±7		±3.8

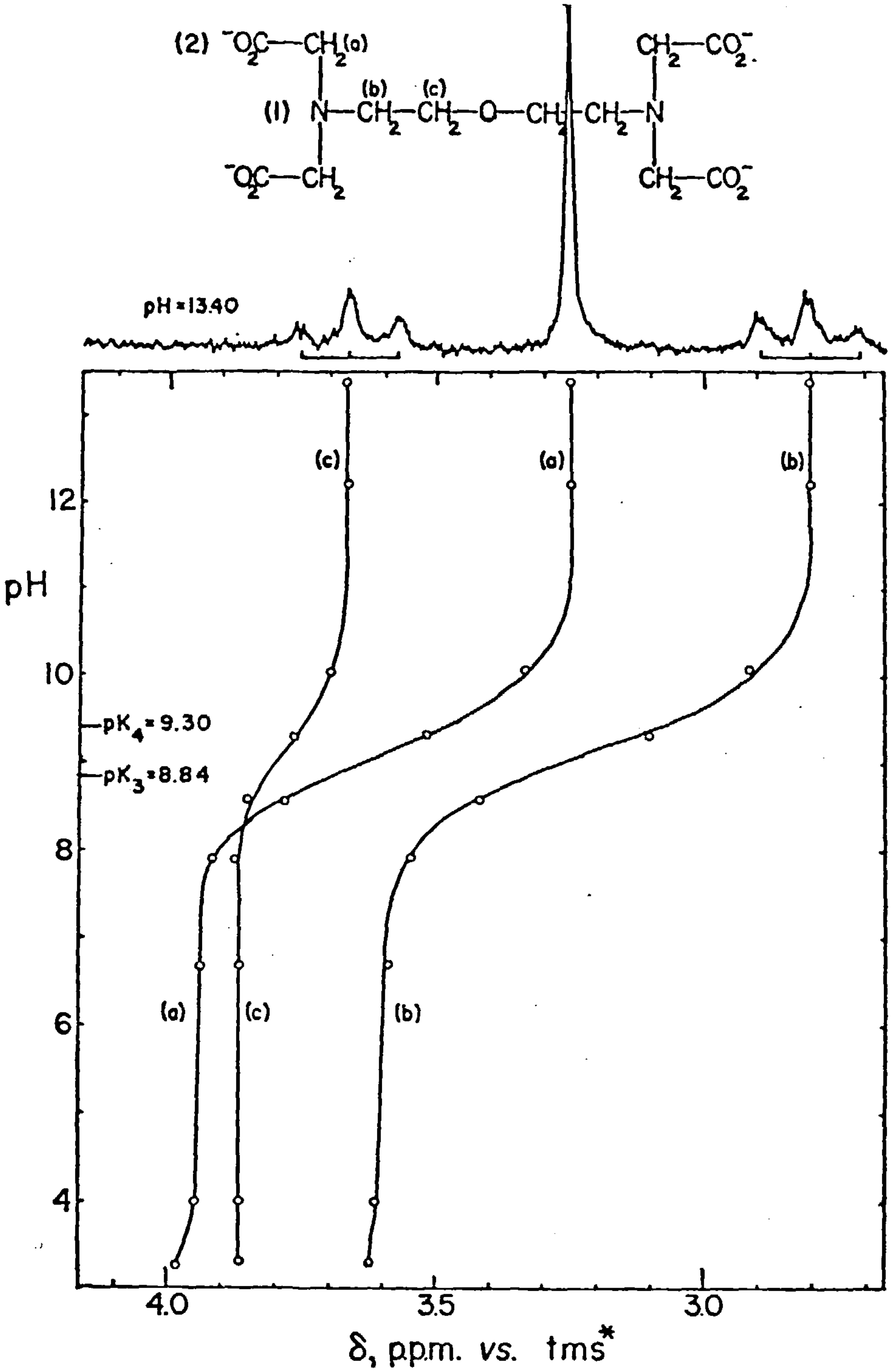


FIGURE 1. Chemical shift of EEDTA at various pH values. Sudmeier, J. L. and Reilley, C. N., *Anal. Chem.*, 36, 1698 (1964). With permission.

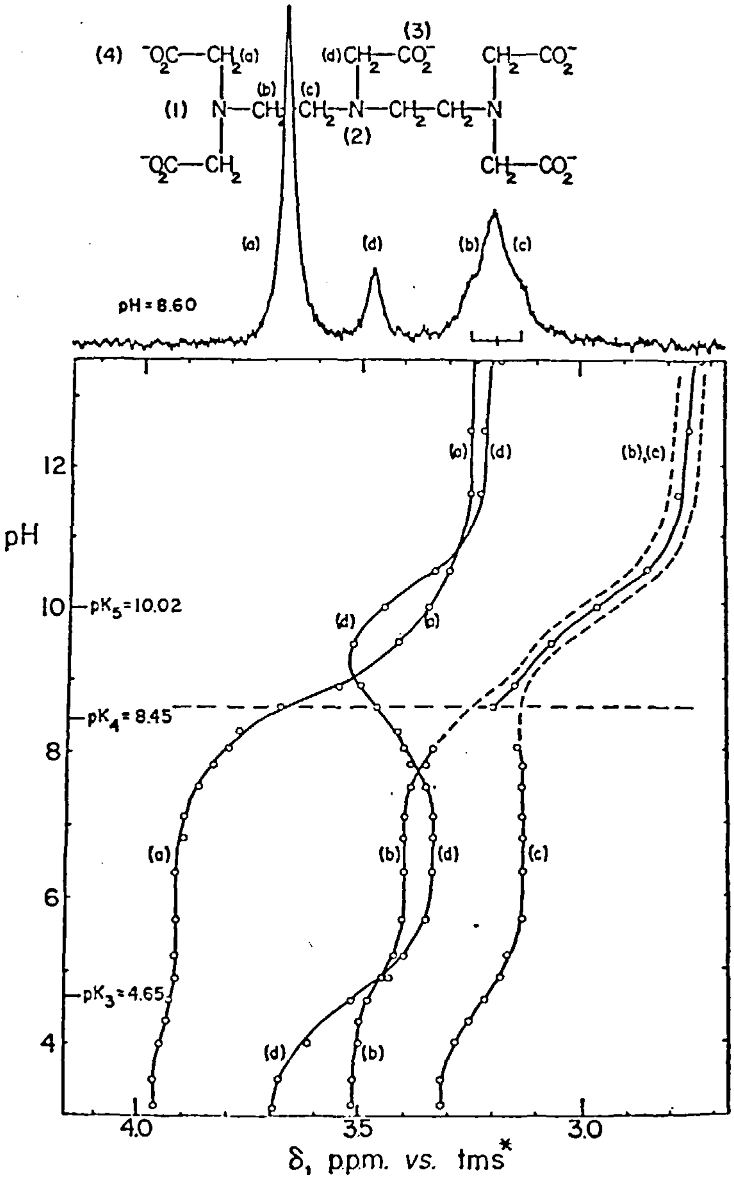


FIGURE 2. Chemical shift of DTPA at various pH values. Sudmeier, J. L. and Reilley, C. N., *Anal. Chem.*, 36, 1698 (1964). With permission.

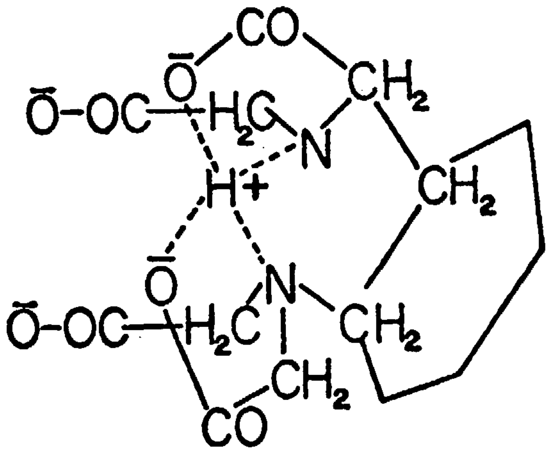
Chapman et al.[2] The rather large uncertainties are unfortunate. Most of the lack of precision can easily be attributed to the assumptions made about the constancy of the protonation shift parameter (C_{ij}). In addition, the values of these parameters are not large (0.05 to 0.5 ppm) and lack of precision is encountered in the measurement. However, these results leave little doubt that the general scheme for the protonation of aminocarboxylates is to protonate the amino groups first and then the carboxylate groups.

Because of the small differences in the chemical shifts of the methylenic protons in polyamines, these results are even less certain than those for aminocarboxylates. This is unfortunate because of the need for answers to the old question about the basicity of amines. An area of study which may answer some of the remaining questions of protonation schemes is the application of ^{13}C NMR to the problem. The obvious disadvantage of ^{13}C NMR is the need for signal enhancement. In the past isotopic enrichment was required.

However, the use of Fourier transform techniques can provide spectra of ^{13}C nuclei at natural abundance with acceptable signal-to-noise ratios in approximately five minutes' instrument time. The use of natural abundance ^{13}C is a major advantage because of the very low ^{13}C-^{13}C spin-spin coupling. ^1H-^{13}C coupling is removed by noise decoupling about the ^1H resonance frequency. The results obtained should be of greater precision because the ^{13}C chemical-shift changes upon protonation are more than ten times those for protons.[7] The effects of protonation upon the ^{13}C chemical shift can be detected with more precision at sites more remote with respect to the functional group.

A study of additional importance is an investigation of protonation in various solvents. The role of solvents in the determination of the relative basicity of amines is known to be important and may be studied conveniently by NMR.

The question of the nature of the proton in mono- and diprotonated aminocarboxylates had been discussed by Schwarzenbach[1] and Chapman.[2] Perhaps the strongest evidence that monoprotated aminocarboxylates have structures equivalent to polyfurcated hydrogen bonds has been obtained by NMR studies. An early study by Sudmeier and Reilley suggested that the tetraanion of *trans*-1,2-cyclohexanediaminetetraacetic acid (CyDTA) preferred a conformation in which the iminodiacetate groups are diequatorial.[4b] Fujiwara and Reilley performed a detailed study of CyDTA and found that the AB quartet of the methylene protons in the acetate group is asymmetrical in the pH range 8 to 11; this is the range of monoprotonated CyDTA.[8] The AB pattern results from the nonequivalence of the methylene protons in their configurationally different positions.[4b] After a careful series of investigations of the effects of pH, radio-frequency power, temperature, and cation upon the spectra, it was concluded that, in this pH range where monoprotonated CyDTA predominates, a configuration represented by III was preferred. This configuration is similar to one required for chelation, which substantiates the suggestion by several researchers that the ligand is preoriented in a conformation suitable for chelation. The asymmetric AB pattern was explained as an interchange between the two types of methylene groups as a result of nitrogen inversion. One pair of acetate methylene protons apparently has accidental coincidence of chemical shift. The second pair exhibits a usual AB pattern. In the pH range in which monoprotonated CyDTA predominates, an AB → CD type exchange occurs through nitrogen inversion at a sufficiently low rate to cause line broadening. There was no evidence to indicate direct bimolecular proton exchange between the monoprotonated and unprotonated (tetraanionic) species.



STRUCTURE III. Fujiwara, Y. and Reilley, C. N., *Anal. Chem.*, 40, 890 (1968). With permission.

To this point only the protonation of the amine and carboxylate sites has been considered. Recently, the protonation of the carbonyl oxygen in aminocarboxylates has been investigated by Sudmeier, Schwarz, and Senzel.[9] An exceptionally strong acid such as FSO_3H - SbF_5 - SO_2 is required. At -80°C the $\gt C = \overset{+}{O}H$ protons are observed by NMR. This technique provides a rapid identification of the number, and in some cases, the types of carbonyl groups in the molecule. For example, in hexadentate octahedral complexes such as Co(III) EDTA there is a pair of in-plane and another pair of out-of-plane carbonyl groups. The NMR spectrum of the $\gt C = \overset{+}{O}H$ protons exhibits two peaks of equal intensity at 12.09 and 12.56 ppm vs. TMS. This potentially useful tool for structure elucidation must be used with care. Co(III) EDTA has a C_2 symmetry axis whereas Co(III) PDTA (propylenediaminetetraacetic acid) has no C_2 axis and one should expect four carbonyl proton peaks. However, the fact that only two peaks are seen may be rationalized by the remoteness of the asymmetric sites.[9]

B. Protonation of Metal Complexes

One of the early papers dealing with protonation studies of EDTA included several metal chelates.[10] Although this paper did not present a quantitative treatment of the protonation data, there were several interesting points made which by and large confirmed earlier results or opinions concerning metal-EDTA chelate behavior as a function of pH. A plot of the chemical shift of the methylene and acetate protons vs. pH showed two breaks when the EDTA salts of lithium, sodium, and potassium were used. There were some variations in chemical shift in the presence of the different metal ions, but these were small compared with the pH effect. The fact that two steps were observed indicated very weak chelation of these ions. However, in the case of the alkaline earths, only one break is observed in the pH curve indicating much stronger chelation of the metal ion. Of significant interest is the result that, in the presence of excess EDTA, two distinct sets of lines are observed over the pH range studied in the case of magnesium. Calcium under these conditions shows two sets of lines between pH 6 to 9.5 and one set above pH 9.5 which is at first broad and sharpens above pH 11.5. Strontium and barium show only single lines at all pH values studied with broadening of the spectra between pH 5 and 10. Other observations were that lead and zinc chelates gave two sets of lines over wide pH range and mercury-EDTA exhibited two sets between pH 4 and 9. The lower pH limits were usually forced by precipitation of the chelate. The distinct sets of lines were attributed to slow ligand exchange of the general type

$$MY + Y^* \longrightarrow MY^* + Y$$

where charge is ignored and the asterisks label the ligand molecules. Furthermore, the results of pH and chemical-shift data permitted the authors to obtain a qualitative order of stability of the chelates. The order of stability was found to be Ca> Sr> Ba> Li> Na> K $\simeq$ Cs which is in agreement with the stability constants. The exact position of Mg in this series could not be determined because of precipitation below pH 5.5. The results showed that Pb, Al, Zn, and Hg all form more stable complexes than the alkali and alkaline earth metals. The results are only qualitative and are of limited usefulness.

Kula performed a more quantitative investigation of the formation of Zn(OH)-EDTA complexes.[11] By observing the effect of pH upon the chemical shift of the Zn-EDTA complex much in the same way as the free ligands were studied, the equilibrium constant

$$K_1 = \frac{[\mathrm{Zn\,(OH)\,EDTA^{3-}}]}{[\mathrm{ZnEDTA^{2-}}]\,[\mathrm{OH^-}]} \qquad (4)$$

was evaluated as $10^{2.0}$. A more detailed and complicated treatment of the line width of the complexed EDTA allowed the evaluation of the equilibrium constant

$$K_2 = \frac{[\mathrm{Zn\,(OH)_3\,EDTA^{5-}}]}{[\mathrm{Zn\,(OH)\,EDTA^{3-}}]\,[\mathrm{OH^-}]^2} \qquad (5)$$

as $10^{-2.2}$. The concentration of the solutions required for NMR measurements and some of the small changes measured in the NMR data as well as inaccuracies in the pH measurements in alkaline solutions limit the accuracy of the results. However, calculation of the value

$$\beta_3 = \frac{[\mathrm{Zn\,(OH)_3^-}]}{[\mathrm{Zn^{+2}}]\,[\mathrm{OH^-}]^3} = 10^{16.3} \qquad (6)$$

showed the NMR results to be compatible with the values of $10^{14.2}$ to $10^{16.1}$ obtained by other means.

Whidby and Leyden have used NMR to evaluate constants for various protonated and hydroxy complexes of Cu (II), Ni(II), and Fe (III).[12] In these cases, advantage may be taken of the paramagnetic broadening of the solvent by rapid exchange of the proton and/or hydroxyl groups from the ligand. Figure 3 shows the effect of pH upon the line width of the solvent. An inflection in the curve occurs at a pH = value equal to pK for an acid-base equilibrium involving the complex. These pK-values were confirmed by potentiometric titrations. The advantage of this technique is that concentrations of approximately 0.05 M in the complex may be used. However, it is restricted to paramagnetic species. There is always some doubt as to the exact exchange process. For example, in hydroxy chelates, either the hydroxyl group or its proton alone may exchange. Studies using O^{17} NMR would be required to determine the exchange process. The following equilibria were studied and the results are given in Table 3.

$$H_2MY \rightleftharpoons HMY + H^+ \qquad K_1 = \frac{[H^+]\,[HMY]}{[H_2MY]} \qquad (7)$$

$$HMY \rightleftharpoons MY + H^+ \qquad K_2 = \frac{[H^+]\,[MY]}{[HMY]} \qquad (8)$$

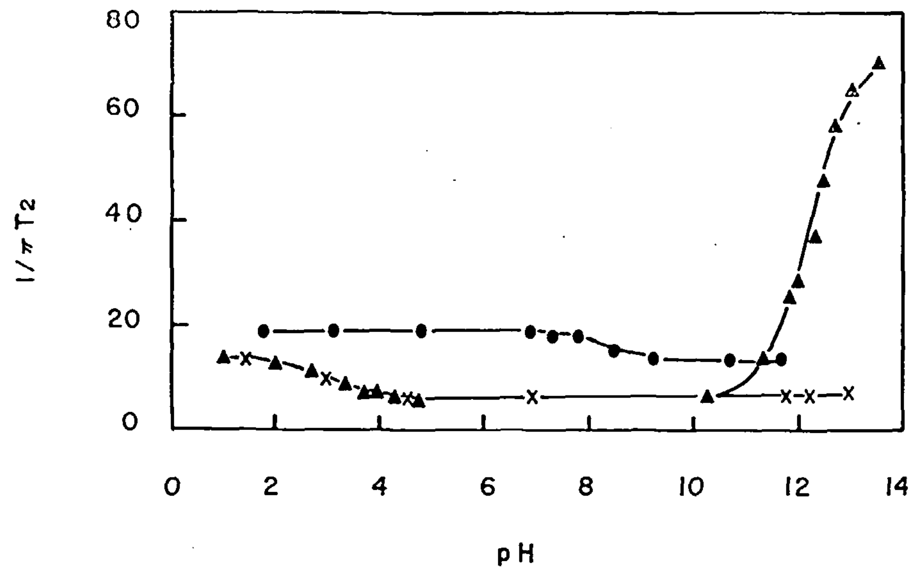


FIGURE 3. $I/\pi T_2$ vs. pH for metal-EDTA solutions. (O) 0.013 M Fe(III)-EDTA; (Δ) 0.053 M Cu(II)-EDTA; (X) 0.070 M Ni(II)-EDTA.

$$MY \rightleftharpoons M(OH)Y + H^+ \qquad K_3 = \frac{[H^+][M(OH)Y]}{[MY]} \tag{9}$$

$$M(OH)Y \rightleftharpoons M(OH)_2Y + H^+ \qquad K_4 = \frac{[H^+][M(OH)_2Y]}{[M(OH)Y]} \tag{10}$$

TABLE 3

pK_a Values For Metal-EDTA Species

Metal ion	pK_1	pK_2	pK_3	pK_4
Cu(II)	2.5	3.3	12.2	–
Ni(II)	2.0	3.3	12.2	–
Fe(III)	–	3.0	8.0	12.0

The effect of chemical shift change of a ligand upon coordination has also been applied to the detection of weak 2:1 chelates of calcium and strontium with EDTA.[13] If the equilibria represented by Equations 11 and 12

$$MY + M \rightleftharpoons M_2Y \tag{11}$$

$$M_2Y + M \rightleftharpoons M_3Y \tag{12}$$

are considered, the corresponding concentration equilibrium constants are

$$K_2 = [M_2Y]/[M][MY] \tag{13}$$

$$K_3 = [M_3Y]/[M][M_2Y] \tag{14}$$

The calculated chemical shift is then given by Equation 15

$$\delta_{calc} = ([M_2Y]/C)\,\delta_{M_2Y} + ([M_3Y]/C)\delta_{M_3Y} \tag{15}$$

where C is the analytical concentration of MY, and $[M_2Y]$ and $[M_3Y]$ are the equilibrium concentrations of the respective species.

Each of these values is calculated with assumed values for K_2 and K_3 and the usual expressions for simultaneous equilibria. The values of δ_{M_2Y} and δ_{M_3Y} represent the chemical shift of the respective species in ppm measured from the resonance of MY. Estimates were made for δ_{M_2Y} and δ_{M_3Y} from limiting values for the observed chemical shift at high metal-to-ligand ratios. The function is relatively insensitive to δ_{M_2Y} and δ_{M_3Y} but quite sensitive to K_2 and K_3. This of course is advantageous inasmuch as the choice of the chemical-shift parameters was not excessively critical. A true value of the chemical-shift parameter cannot be obtained unless either the species can be made to predominate or the formation constants are previously known. The arguments for including the species M_3Y, which represents three metal ions per ligand, will be discussed later.

Figure 4 shows a plot of the relative chemical shift of the acetate protons of EDTA vs. the mole percent of strontium in a strontium-Sr(EDTA) mixture. The circled points are the experimental data, whereas the solid line represents the cal-

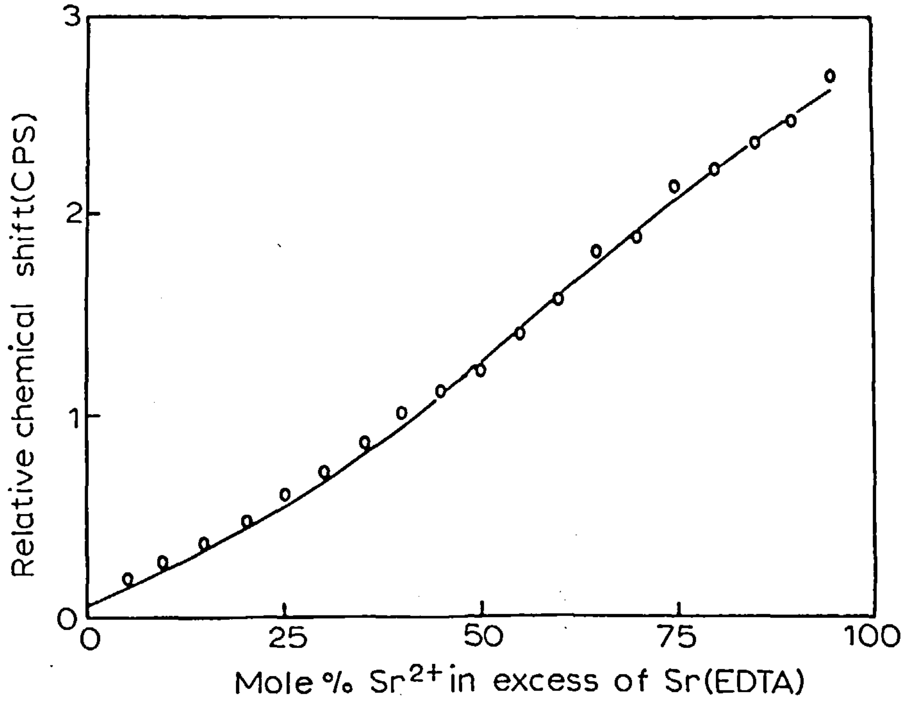


FIGURE 4. Relative chemical shift of acetate protons vs. mol % strontium in a strontium-Sr(EDTA) mixture (pH 12.5). Sudmeier, J. L. and Reilley, C. N., *Anal. Chem.*, 36, 1698, (1964). With permission.

culated curve obtained from Equation 15. The formation constants, K_2 and K_3, which gave the best comparison between the experimental and computed chemical shift values are given in Table 4.

TABLE 4

Stability Constants for Calcium and Strontium Complexes of EDTA

	log K_1	log K_2
Ca	10.57[a]	1.1 ± 0.1
Sr	8.63[a]	1.0 ± 0.1

[a] Sillen, L. G. and Martel, A. E., Stability Constants of Metal-Ion Complexes, The Chemical Society, London, 1964, 634.

The formation constants for the 2:1 metal-to-EDTA species are relatively small as expected. The similar numerical values for K_2 for $Ca_2(EDTA)$ and $Sr_2(EDTA)$ are also reasonable in view of the formation constants of the iminodiacetic acid chelates with these ions. In order to obtain the best correlation between the computed curves shown in Figure 5 and the experimental data it was necessary to include a small value represented by K_3 which is larger than the estimated error of ± 0.1 for all the values given in Table 4. However, there is no evidence at present to permit anything more than speculation about the structure of a species with a calcium: EDTA ratio of 3:1.

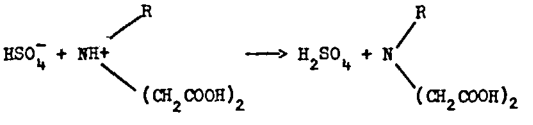
This work illustrates a further application of NMR to the detection of weak chelates in an equilibrium involving several species. However, as in this case, the chemical-shift changes become smaller upon further coordination of the ligand, and the precision suffers.

C. Protolysis Kinetics of Free Ligands

Just as the protonation equilibria play an important role in understanding the chemistry of aminocarboxylates and their behavior as ligands for chelate formation, so do the rates of the protolysis reactions. NMR is of only moderate use in the study of these reactions. The reason for the limitation may be more easily understood if the methods of study are clear. There are two NMR techniques which may be applied with relative ease to the study of protolysis reaction rates. First, one may use a slow-passage high-resolution method which involves the comparison of the shape of the experimental NMR lines with those computed from theory. For example, a simple molecule such as trimethylamine may be studied by the broadening and eventual coalesence of the methyl doublet arising from spin coupling with the NH proton in a highly acidic solution. As the acidity is lowered, the rate of proton exchange increases and the doublet is coalesced.

The second technique involves the use of pulsed NMR in which the relaxation time of the protons in the solvent is determined from the decay pattern of the NMR "echos" which follow a series of pulses of the radio frequency. This technique can usually be made to serve for the evaluation of rates higher than those that can be measured by slow-passage high-resolution methods. However, both require acidic solutions in the case of amine sites so that the rate of proton exchange may be lowered to a measurable value. If the compound to be studied has more than one functional site, those having the lower pK_a-values may require that extremely acidic solutions be used to lower the exchange rate sufficiently.

The protolysis kinetics of the amino sites of IDA (iminodiacetic acid) and MIDA (methyliminodiacetic acid) have been investigated by Leyden and Whidby utilizing the slow-passage method.[14] The acidity required to observe the spin coupling between the labile – NH proton and the $-CH_2$ protons of the acetate groups in IDA and MIDA or the methyl protons in MIDA was achieved with approximately 80% sulfuric acid solutions. Protonation of the carboxyl groups to form $C = OH^+$ species did not occur in these solutions. However, because of the medium, many difficulties were encountered. For example, the NMR lines were viscosity-broadened, and elevated temperatures had to be used to minimize this effect. The concentrations of the solvent species were difficult to obtain and it is questionable whether the representation of the acidity was reasonable. In spite of these difficulties, two significant exchange processes were found. The first is represented by a transfer of a proton to the solvent and values of 2.0 s^{-1} and 2.1 s^{-1} for the pseudo-first-order rate constant were obtained for IDA and MIDA respectively. The second reaction may be illustrated by Reaction 16 and rate



$$HSO_4^- + NH^+(R)(CH_2COOH)_2 \longrightarrow H_2SO_4 + N(R)(CH_2COOH)_2$$

(16)

constants of 4.9 x 10^4 M^{-1} s^{-1} and 1.4 x 10^5 M^{-1} s^{-1} were obtained for IDA and MIDA respectively. All data are at 60°C. These studies also yielded qualitative results on the effect of acidity on the inversion of the nitrogen atom in MIDA. The inversion rate was determined from the AB pattern of the acetate protons. An attempt was made to investigate the protolyses of EDTA, CyDTA, and other similar ligands. However, these compounds were not soluble at the acid concentrations required.

Because the protolysis reaction is so inherently involved in the chelation chemistry of aminocarboxylates, it would be of great interest to know more about these processes. However, it is likely that methods other than NMR must be used to obtain these data. Obviously one would like the data to be obtained in a medium similar to the ones in which chelation studies are made.

D. Protolysis Kinetics of Metal Complexes

Proton-exchange reactions of aminocarboxylates are limited in their consideration to the labile protons of the amino and carboxyl sites. The acetate and ethylenic protons are inert, or at least exchange sufficiently slowly to be considered as inert. In certain chelates, however, this is not the case. Williams and Busch showed that hydrogen atoms on a carbon alpha to a nitrogen coordinated to Co(III) undergo deuteration in alkaline D_2O.[15] Thus, the protolytic reactions of aminocarboxylate chelates may be divided into those that involve -CH protons and those that involve protons of the amino and carboxyl groups.

Terrill and Reilley studied the base-catalyzed isotopic exchange of the α-hydrogen atoms in a series of EDTA chelates with bivalent metal ions.[16] Only the acetate protons were observed to exchange. The rate of exchange was determined by following peak areas during isotopic substitution. The isotopic exchanges of the alkaline earths were observed to follow the order $Mg > Ca > Sr > Ba$. This order is rationalized on the basis of charge-to-radius ratio of the metal ion assuming ionic bonding predominates. On the other hand, the series $Cu^{+2} > Ni^{+2} > Co^{+2} > Zn^{+2}$ is rationalized on the basis of covalent bonding and ligand field considerations. It is interesting that the alkaline earth series is in qualitative agreement with the order of chemical shifts given by Kula et al.[10] Terrill and Reilley also pointed out that good correlation is obtained between the rate of exchange and the gross heat of ligation, which suggests that the nature of the metal chelate in respect to coordinated water molecules is largely the same. That is, the presence of coordinated water molecules would alter the inductive effect of the metal ion on the exchange process. Only lead-EDTA could not be rationalized by these considerations.

An interesting observation was that uncomplexed EDTA undergoes isotopic exchange of the acetate protons which is zero order in base and with a rate faster than barium-EDTA in 1.0 *M* OD^-. The results suggest an intramolecular process in which the proton abstracting sites of the unassociated ligand are the amino and/or carboxylate groups on the same molecule. The more remote nitrogen was found to be more effective in removing the proton than the α-nitrogen. For mono protonated EDTA, exchange was immeasurably slow. The less basic carboxylate sites are apparently ineffective in proton abstraction, although studies of esters or alkylated amino sites were not performed. It should be mentioned that this study may be important in considering reactions other than hydrogen exchange at the acetate sites and illustrates an excellent method of preparation of acetate-deuterated ligands for other studies such as ligand exchange.

Terrill and Reilley extended their study to Co(III) · CyDTA.[17] The NMR spectrum of this complex indicates there are four types of acetate protons. It was found that the acid-catalyzed deuteration was strikingly stereospecific in that certain hydrogen atoms on acetate groups out of the plane of the nitrogen-metal bonds are deuterated more rapidly than their geminal neighbors. The base-catalyzed exchange was complicated by the formation of hydroxyl-chelates and reduction to paramagnetic Co(II). Sudmeier and Occupati found *trans*-Co (III) EDDA $(en)^+$ to be more useful where EDDA represents ethylenediaminediacetic acid and en represents ethylenediamine.[18] Using this compound, which is stable in base, base-catalyzed exchange was found to be more rapid than acid-catalyzed exchange and approximately ten times more stereospecific. A series of compounds gave similar results and confirmed the findings of Terrill and Reilley. The major contribution of Sudmeier and Occupati was to eliminate a possible ambiguity in the spectral assignment of Terrill and Reilley. However, considerable data is available on the rate and activation parameters of α-hydrogen exchange in this paper.

A series of papers by Erickson and co-workers have considered the rates of N-H proton exchange and nitrogen inversion for some amino acid complexes of platinum (II).[19-22] The studies were done both by using the slow-passage method and by following slow spectral changes in D_2O solutions of the complexes. These exchange processes were found to be catalyzed by D_2O and OD^-, and in the pH range 4 to 7 intramolecular catalysis by the acetate group was found. The rates of exchange are in all cases much slower than that typical of the free ligand. A discussion of the correlation between the degree of nitrogen substitution and the macroscopic pK_a of analogous free ligand sites with respect to the rate data is given. However, no report of an attempt to compare the rate laws found by Erickson and co-workers for the complexes with those of free amino acids was given. The nitrogen inversion studies by Erickson and co-workers[21] and others[23] on platinum and cobalt complexes have shown that the rate of proton exchange catalyzed by hydroxide ion exceeds the rate of nitrogen inversion by 10 to 10^4 fold. Erickson explains this by invoking a mechanism for inversion as follows

$$N - H + OH^- \underset{k_2}{\overset{k_1}{\rightleftharpoons}} N:^- + H_2O \qquad (17)$$

$$N:^- \xrightarrow{k_3} {}^-:N \qquad (18)$$

where $N:^-$ represents the deprotonated nitrogen and k_3 is a first-order rate constant for inversion. This mechanism is substantially the same as that suggested by Saunders and Yamada for the inversion of amines.[24] Erickson and co-workers assumed $K_a \simeq 0.01K_w$ for the N-H dissociation in the complexes, for few have been measured. Assuming that the value of the hydroxide-catalyzed proton exchange rate constant for a series of complexes may have the form of a simple Bronsted relation

$$k_{OH^-} = G(K_a)^\beta \qquad (19)$$

where G and β are constants, the authors estimated values of k_3 to be 10^5 to 10^6. These values compare remarkably well with those of nitrogen atoms contained in five- and six-membered rings.[25] However, the results obtained by Erickson should be modified to include the mechanism for nitrogen inversion in aqueous acid proposed by Morgan and Leyden for acyclic amines.[26] This mechanism considers the possibility that, even though the amine is deprotonated, the strong hydrogen bond between the nitrogen atom and solvent water molecules inhibits inversion until that hydrogen bond is broken. This mechanism easily provides a qualitative explanation of faster proton exchange than inversion and yields a quantitative description of the rate of nitrogen inversion as a function of pH. The mechanism has been found recently by Pitner and Martin to hold for the inversion of nitrogen atoms in platinum complexes.[27]

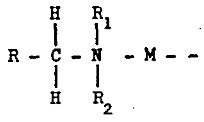
The study of protolytic kinetics, both C-H and N-H, and associated reactions such as nitrogen inversion in metal complexes is relatively new. NMR should play an important role in this research. A better understanding of the effects of metal bonding upon acidity of the C-H and N-H protons, the mechanism of configurational changes, K_a values for the weakly acidic N-H sites and many other properties of metal chelates should be generated by such studies. However, the studies of the complexes should be accompanied by studies of the free ligands.

E. Structures and Bond Labilities of Metal Complexes

The protonation studies have established reasonably well the structure of free aminopolycarboxylates in solution. The next step was to ascertain the gross structure of the various complexes. The extensive x-ray crystallographic studies by Hoard[28] and others had contributed a great deal to the elucidation of the structures of aminopolycarboxylate chelates in the solid state. However, there is always a reluctance to extrapolate these data to the solution phase. The solution chemist is interested in the dynamics of structural change as well as in average structures. The answers to many questions about the effect of solvent, extraneous reagents, and, of course, the central metal ion, among others, upon something as simple as an EDTA titration may lie in the lifetime of a metal ligand bond.

Day and Reilley[29] applied NMR to the investigation of individual metal ligand bond lifetimes. Before this it had long been accepted that aminocarboxylates formed multidentate chelates. From x-ray data, it was known that in the solid state a given ligand such as EDTA formed different numbers of metal-ligand bonds depending upon protonation of the ligand.[30] Mixed aquo- and hydroxy-complexes were also known. However, no direct results had been obtained to determine the lifetime of these metal-ligand bonds.

Consider the effect that bond lifetime would be predicted to have on the NMR spectrum of an aminopolycarboxylate chelate. If all metal-ligand bonds have short lifetimes, the spectrum of the nonlabile protons in the molecule will be relatively simple because of internal averaging. This is similar to the fast proton exchange in a partially protonated ligand. As in protonation, the chemical shift of the chelate will be different than the free ligand. An average spectrum would be observed as a result of rapid ligand exchange. On the other hand, if one or more metal-ligand bonds is sufficiently long-lived to be considered permanent by NMR detection, then chemical shifts which are identical in the free ligand may not average in the chelate and a more complex spectrum may result. As an example, two protons on a carbon atom adjacent to an asymmetric nitrogen are expected to be nonequivalent if the nitrogen atom does not undergo inversion. A structure such as



$$\begin{array}{ccccc} & H & & R_1 & \\ & | & & | & \\ R - & C & - & N & - M - - \\ & | & & | & \\ & H & & R_2 & \end{array}$$

where R_1, R_2 and RCH_2 are all different and the nitrogen-metal bond has a long life-time meets these requirements. The two protons on the carbon adjacent to the nitrogen are expected to yield the AB-type splitting pattern. The mere presence of an AB pattern confirms a qualitatively long nitrogen-metal bond lifetime. A more detailed examination of these AB patterns may yield quantitative rate data as well as information about the confirmation of the structure and will be discussed later.

A further tool is the presence of abundant isotopes of the central metal ion which have nuclear spins greater than one half. Such isotopes may result in metal-proton spin coupling which is useful.

Day and Reilley proposed four situations to consider.[29] The first of these is the case in which both the metal-oxygen and metal-nitrogen bond lifetimes are short. In such a case of labile bonds, there should be two sharp peaks corresponding to the ethylenic and acetate protons. If there is a significant abundance of an isotope of the central metal ion with spin one half or greater, splitting of the ethylenic and acetate protons may occur unless there is complete ligand exchange. In the event of rapid bond breaking and reforming, the coupling would persist as long as the ligand bonds are reformed with the original metal ion. An example is ^{207}Pb which has a spin one half and a natural abundance of 21%. The NMR spectrum of Pb-EDTA is shown in Figure 5a. In some cases such as the alkali metals only an average spectrum of free and complexed ligand is observed indicating rapid ligand exchange. The alkaline earth metals on the other hand exhibit ligand exchange rates which are pH dependent.[10]

The second situation is one in which the lifetime of the metal-oxygen bonds is short while that of the metal-nitrogen bond is long. This case allows for rapid averaging of the acetate protons. However, the long lifetime of the metal-nitrogen bond inhibits inversion of the nitrogen atom and results in a nonequivalence of the two protons of the acetate groups. An AB pattern is thereby expected. Again, the presence of a metal isotope

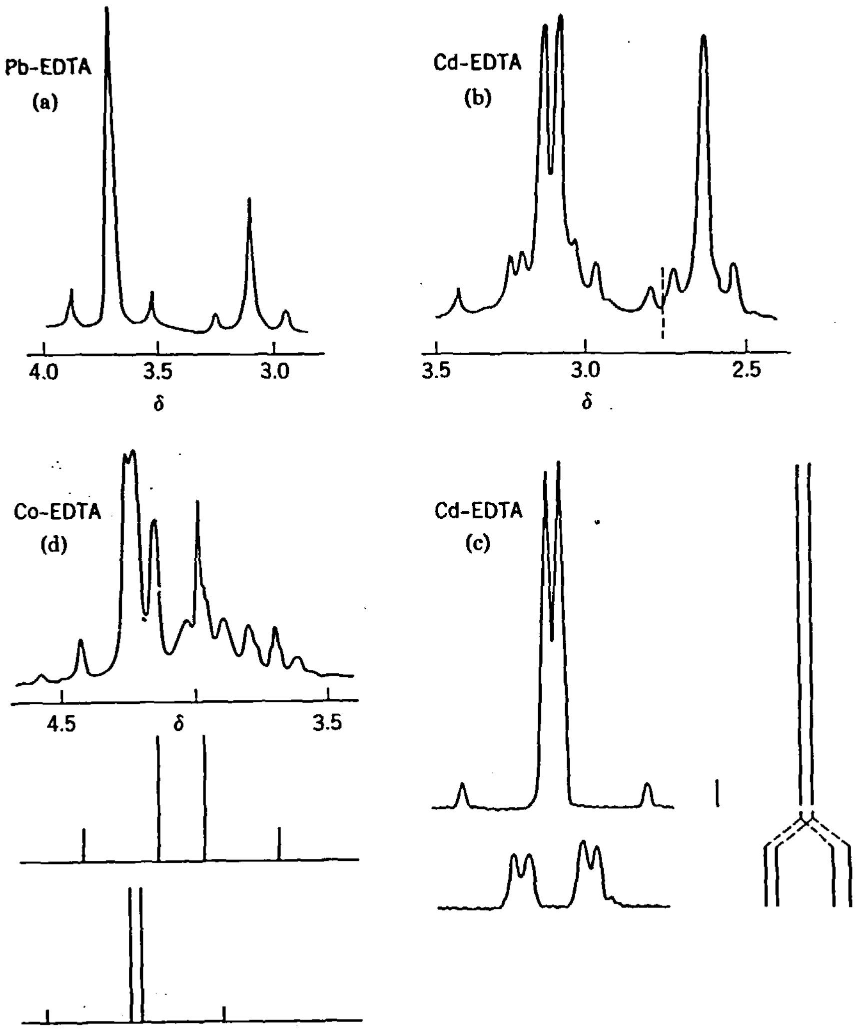


FIGURE 5. PMR spectra at 60 mc sec^{-1} of 0.5 to 1.0 M aqueous solutions of metal-EDTA complexes; reference: sodium 3-(trimethylsilyl)-1-propanesulfonate. (a) Pb-EDTA; acetate and ethylenic proton resonances at ca. 3.7 and 3.1 δ, respectively with side bands from ^{207}Pb shown. (b) Cd-EDTA; PMR for acetate protons to left of broken line and for ethylenic protons to right. (c) Cd-EDTA; acetate portion of PMR spectrum; on top the AB pattern due to Cd with 1 = 0; on bottom ABX pattern due to Cd with 1 = ½. (d) Co(III)-EDTA; two AB patterns for the acetate groups shown below spectrum; remaining peaks due to ethylenic protons. From *Spectroscopy and Structure of Metal Chelate Compounds,* Nakamoto, K. and Mc Carthy, P. J., Eds., John Wiley & Sons, New York, 1968. With permission.

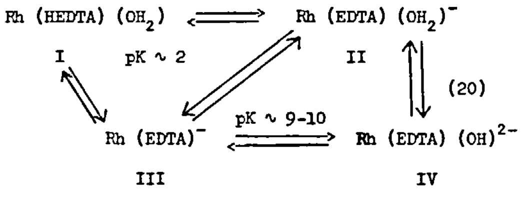
with a spin greater than one half would have persistent coupling with the protons on the ligand. An example of this case is cadmium-EDTA whose spectrum is given in Figure 5b which shows the acetate AB pattern and an ethylenic singlet. The spectrum is complicated by the presence of 25% isotopes of spin one half. Figure 5c shows the AB pattern due to cadmium with I = 0.

The third possibility is one in which the lifetime of the metal-oxygen bond is long, whereas that of the metal-nitrogen bond is short. Consideration of the structure of metal-EDTA complexes suggests that this is an unlikely case. Although it is likely that certain metal-nitrogen bond lifetimes are inherently shorter than metal-oxygen bonds to the same metal ion, it is unlikely that the metal-nitrogen bond in an EDTA chelate could be broken without prior rupture of the metal-oxygen bonds. There have been no unambiguous confirmations of this type of situation. However, Kula has suggested that this is the case with some Mo(VI) chelates which will be discussed later.[31]

The final combination is the case in which both the metal-oxygen and the metal-nitrogen bond lifetimes are long. In this case, the most complex spectra are expected because the acetate groups are not equivalent. Furthermore, the two protons on each acetate group are not equivalent because of the adjacent asymmetry about the nitrogen atom. The complex is substitution-inert and, in the case of an octahedral complex where all ligand atoms are bound, dl-forms are present. For a given optical form, two of the acetate groups are "in plane" with the nitrogen-to-metal bonds and are identical, and the other two acetate groups are "out-of-plane" and are identical. An example of this case is cobalt (III)-EDTA whose spectrum is shown in Figure 5c. Two AB patterns are observed for the acetate protons. The ethylenic protons may not be equivalent because of the rigidity of the complex.

Chan, Kula, and Sawyer investigated the NMR spectra of molybdenum (VI) chelates of EDTA, NTA (nitrilotriacetic acid), and MIDA (methyliminodiacetic acid).[32] The results show that the metal to ligand ratios of the chelates are 1:1 for NTA and MIDA and 2:1 for EDTA. The acetate protons in the MIDA and EDTA complexes give rise to AB patterns. The authors interpret this as an indication that the carboxylate groups are "coordinated rigidly to the metal ion in these chelates." It is possible that rapid breaking and reforming of metal-oxygen bonds occurs without nitrogen inversion. Protonation of MIDA in 50% H_2SO_4 gives rise to an AB pattern for the acetate protons because of slow nitrogen inversion.[14] Kula was able to demonstrate that J_{AX} and J_{BX} were different in the ABX pattern obtained by N-H proton coupling with the acetate protons in Mo(VI) – IDA.[31] He calculates H_X-N-C-$H_{A \text{ or } B}$ angles to be 12° between AX and 110° between BX and interprets this as conclusive evidence that the bond lifetime of the Mo-O bonds were long (>0.2 sec). His interpretation was that this long Mo-O bond lifetime was required to preserve the distinct J_{AX} and J_{BX} values. He further concluded that nothing could be said with respect to the Mo-N bond lifetime because, assuming long Mo-O bond lifetimes, the spectrum would be the same regardless of the lability of the Mo-N bond. It is possible that this interpretation is correct. However, Kula's reasoning is based upon the assumption that no, or very slow, rotation about the H_2C-N bond is required to observe the difference in J_{AX} and J_{BX}. If the Mo-N bond lifetime is long, on the other hand, rapid rupture and rotation about the H_2C-N bond could occur and still preserve the difference in J_{AX} and J_{BX} as long as there was a preferred rotamer on the time average. The preferred rotamer would likely be the one suitable for Mo-O bonding. The interpretation of the molybdenum (VI) chelate data should be reconsidered. A molybdenum (V) – EDTA chelate has been studied and combined x-ray and NMR studies of it suggest a bridged structure.[33]

Rhodium (III)-EDTA is one of the more interesting chelates with EDTA to be investigated. Smith and Sawyer report that the NMR spectrum of the protonated complex Rh(HEDTA)(OH_2) indicates the presence of four different types of acetate protons in a chelate in which the ligand is pentadentate.[34] Two acetate AB patterns are observed and two acetate singlets which result from accidental degeneracy of the two protons in each group. The ethylenic portion of the ligand gives rise to an ABCD pattern. The pH dependence of the spectrum is attributed to the equilibria:



By analogy with the spectra of Co (III)-EDTA, the sexadentate chelate is thought to be predominate at pH5. In all cases, the metal-oxygen and metal-nitrogen bond lifetimes are long. Rh(EDTA) is sensitive to ultraviolet radiation and optically active solutions of this chelate are rendered inactive after two hours' exposure. There are changes observed in the NMR spectrum during this process which have been investigated recently.

Sudmeier and co-workers used Rh(III) PDTA to investigate the photolysis effect.^{35} The complex (-)$_{5461}$Rh(III)D-(-)-PDTA lost its optical activity upon exposure to ultraviolet light, but regained it after storage in the dark for several days. This cycle may be repeated many times. The NMR spectrum shows a new methyl doublet at the expense of the original methyl resonances following irradiation. The ratio of the areas of these peaks coincides with the loss of optical rotation. The CD and absorption spectra of the photolysis product showed no evidence of a change in the denticity of the ligand or coordination number of the rhodium ion. The authors proposed that the photolysis product contains a 1:3 ratio of diastereomers. The proposal is substantiated on the observation that the corresponding enantiomers of Rh(EDTA) would be equal and no dark recovery of optical activity would be expected as observed after three days. These interpretations are contrary to the early proposal of Dwyer and Garvan of a photoaquation reaction.^{36} However, the explanation of Sudmeier and co-workers seems to be very sound.

The type of PMR spectrum observed for the chelates of EDTA has been shown to be very useful in evaluating individual bond lifetimes. Qualitatively, the more complicated the spectrum, the more inert the complex. For example, in an EDTA chelate, the presence of an AB pattern for the acetate protons implies a long metal-nitrogen bond lifetime. However, when more complicated ligands are used, the free ligand may give rise to complicated spectra. Day and Reilley investigated the effects of ligand structure on the bond lifetimes of chelates by using CyDTA [(*trans*-1,2-cyclohexylenedinitrilo)tetraacetic acid] and BDTA [(*meso*-2,3-butylenedinitrilo)-tetraacetic acid] in addition to EDTA.^{37} In molecules such as CyDTA and BDTA the presence of asymmetric carbon atoms within the ligands gives rise to AB patterns for the acetate groups adjacent to these sites.^{4b} Thus AB patterns may be present even in the free ligand. Caution must be used, however, for the two acetate protons may be accidentally degenerate and give rise to a single peak. The spectrum of the chelate may be further complicated by slow interconversion between configurations. Figure 6 shows the structure of octahedral complexes of CyDTA and two configurations of *meso*-BDTA. The intercon-

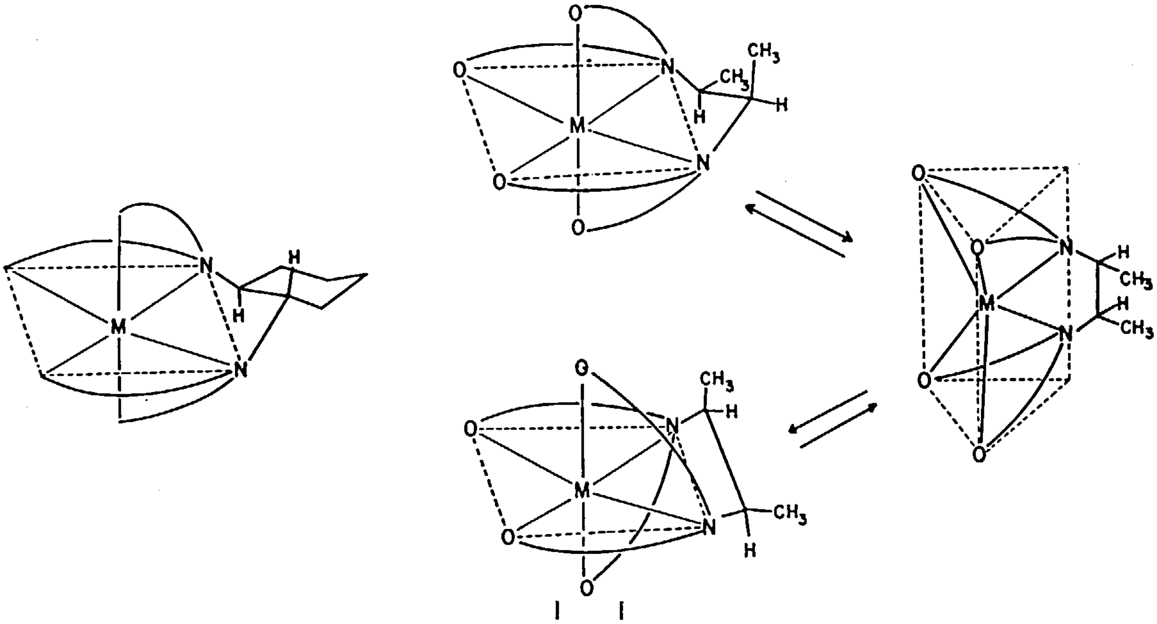
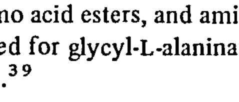


FIGURE 6. Structures of octahedral metal complex of CyDTA and two octahedral configurations of meso-BDTA. Intermediate structure in interconversion of two configurations also shown. Day, R. J. and Reilley, C. N., *Anal. Chem.*, 37, 1326 (1965). With permission.

version of all the BDTA chelates studied was found to be rapid as evidenced by a single sharp methyl resonance. The spectra of some of the complexes studied are shown in Figures 7 and 8. The total interpretation of these data is beyond the scope of this review. In brief, metal ions such as Mg, Ca, Zn, and Pb which show short metal-nitrogen bond lifetimes in EDTA chelates exhibit longer metal-nitrogen bond lifetimes in their chelates with *meso*-BDTA. The metal-oxygen bond lifetimes remain short. Strontium and barium form chelates in which both metal-nitrogen and metal-oxygen lifetimes are short. Co(III) forms an inert chelate. For *meso*-BDTA, if both types of bonds are labile in the chelate, only one type of acetate and one type of methyl group will be observed. If the metal-oxygen bond is labile but the metal-nitrogen bond has a long lifetime, two acetate groups will be observed but only one type of methyl group will be observed. If both types of bonds are nonlabile, there will be four types of acetates and two types of methyl groups.

In the case of CyDTA, the conformation of the cyclohexane ring places additional restrictions upon the chelate which permits only one configuration. The symmetry of the octahedral chelate leads to the result that as long as the metal-nitrogen bond has a long lifetime, there will be two types of acetate protons (those in-plane with the nitrogen-metal bonds and those out-of-plane). In this case, the bond lifetime for the metal-oxygen bonds can only be evaluated by subtle changes in the value of J_{AB} resulting from variation in the strain upon the acetate groups. Kula and Sawyer suggested the addition of paramagnetic metal ions to the solution.[38] A labile acetate group will spend some time coordinated to the paramagnetic ion which will broaden the -CH_2- resonance of that group.

In addition to the study of individual metal-ligand bond lability, NMR may be used to determine the site on a ligand at which metal bonding first occurs during chelation. For example, a series of investigations was performed by Li, Mathur, and others in which paramagnetic metal ions were added to solutions of amino acids, peptides, amino acid esters, and amides.[39-42] The results obtained for glycyl-L-alaninate may be used as an example.[39]



$$H_2NCH_2\overset{O}{\overset{\|}{C}} - \overset{H}{N} - \underset{CH_3}{\underset{|}{C}}HCOOH$$

Figure 9 shows the PMR spectra of 0.5*M* glycyl-L-alaninate and its metal complexes. The assignments for the free anion are an alaninate -CH quartet (2.30 ppm), a glycyl CH_2-singlet (3.15 ppm), and an alaninate -CH_3 doublet (5.13 ppm) vs. benzene as an external reference. When the solution is made 0.5 *M* in $CdCl_2$, the chemical shifts change as a result of complex formation with the order of change $CH_2 > CH > CH_3$. This order suggests that the site of coordination is in the glycyl moiety. Moreover, the addition of 10^{-4} *M* Cu (II) to 0.5 *M* glycyl-L-alaninate broadens the -CH_2- resonance so drastically that it alone becomes unobservable, further substantiating coordination with the glycyl residue.

A technique which is similar in principle to the use of paramagnetic ions to determine coordination sites may be used to determine the extent of metal ion coordination to macromolecules. In these cases, a halide ion is used as the probe.[43,44] The ^{35}Cl isotope is convenient because of the relative ease of obtaining its magnetic resonance spectrum. The line width of this resonance from aqueous NaCl is greater in the presence of proteins which have heavy metals bound to their functional (sulfhydryl) sites than in the presence of free proteins. This is a result of quadrupole relaxation of the ^{35}Cl spin states. For this relaxation to effect the spectrum, the bulk chloride-ion population must be involved, thereby requiring rapid chloride-ion exchange between the bulk and the available coordination sites on the metal bound to the protein.

A knowledge of the relative efficiency and rate of different chelating agents toward the complete and selective removal or "de-poisoning" of proteins would be of great usefulness in the design and selection of chemotheropeutic agents for heavy metal poisoning. Sudmeier and Pesek[45] have made use of the decrease in the line width of the ^{35}Cl resonance to investigate the merits of several ligands toward the de-poisoning of bovine serum albumin. They reported that EDTA removed cadmium but not mercury. 2,3-Dimercapto-1-propanol (BAL), D-cysteine, DL-penicillamine, D-penicillamine, and 3-mercaptopropionic acid remove both cadmium and mercury. The latter four chelating agents undergo oxidation. These studies appear to be greatly informative. However, quantitation of the data is difficult and a serious question may arise as to whether the metal is in fact removed from the

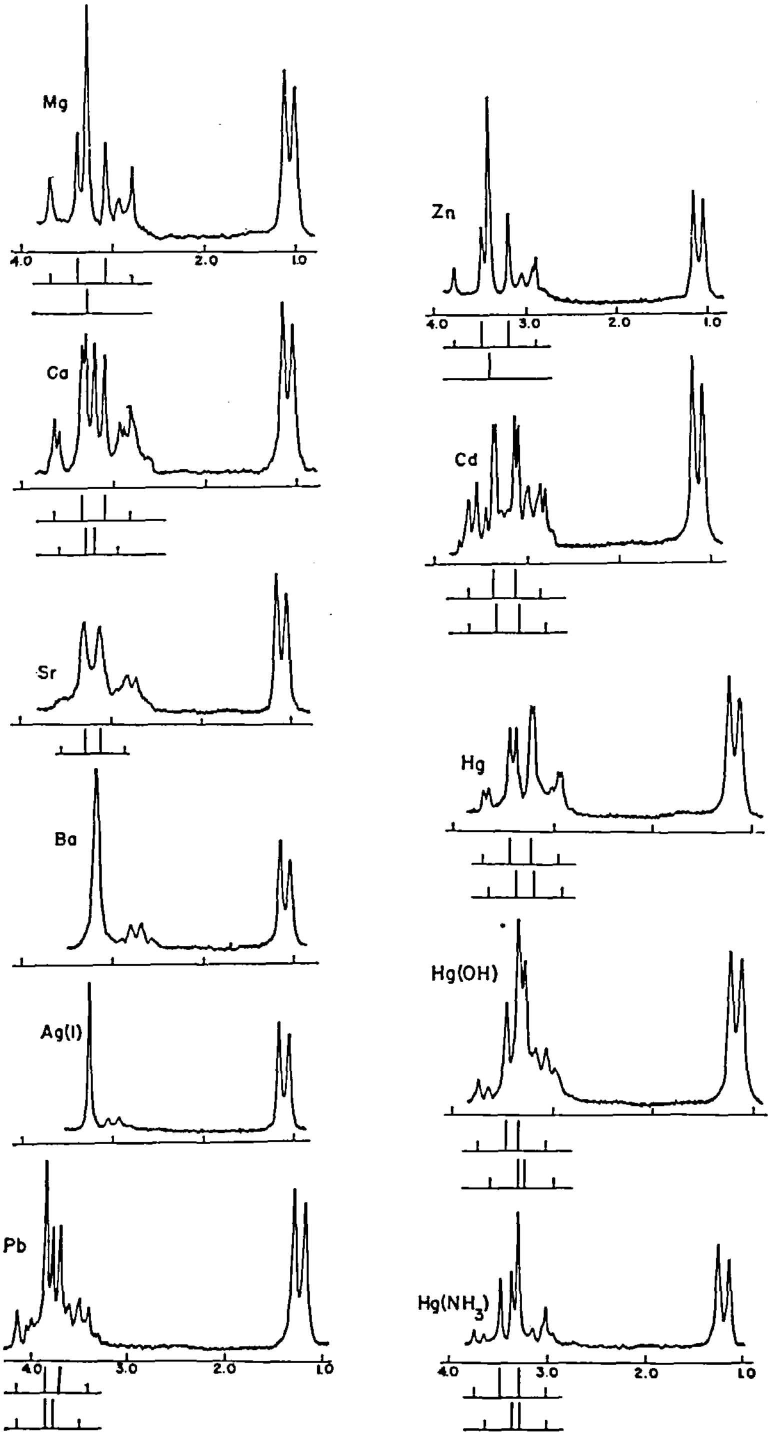


FIGURE 7. Proton NMR spectra of some metal-BDTA complexes. Assignments for acetate AB patterns indicated below each spectrum. Chemical shifts vs. DSS. Day, R. J. and Reilley, C. N., *Anal. Chem.*, 37, 1326 (1965). With permission.

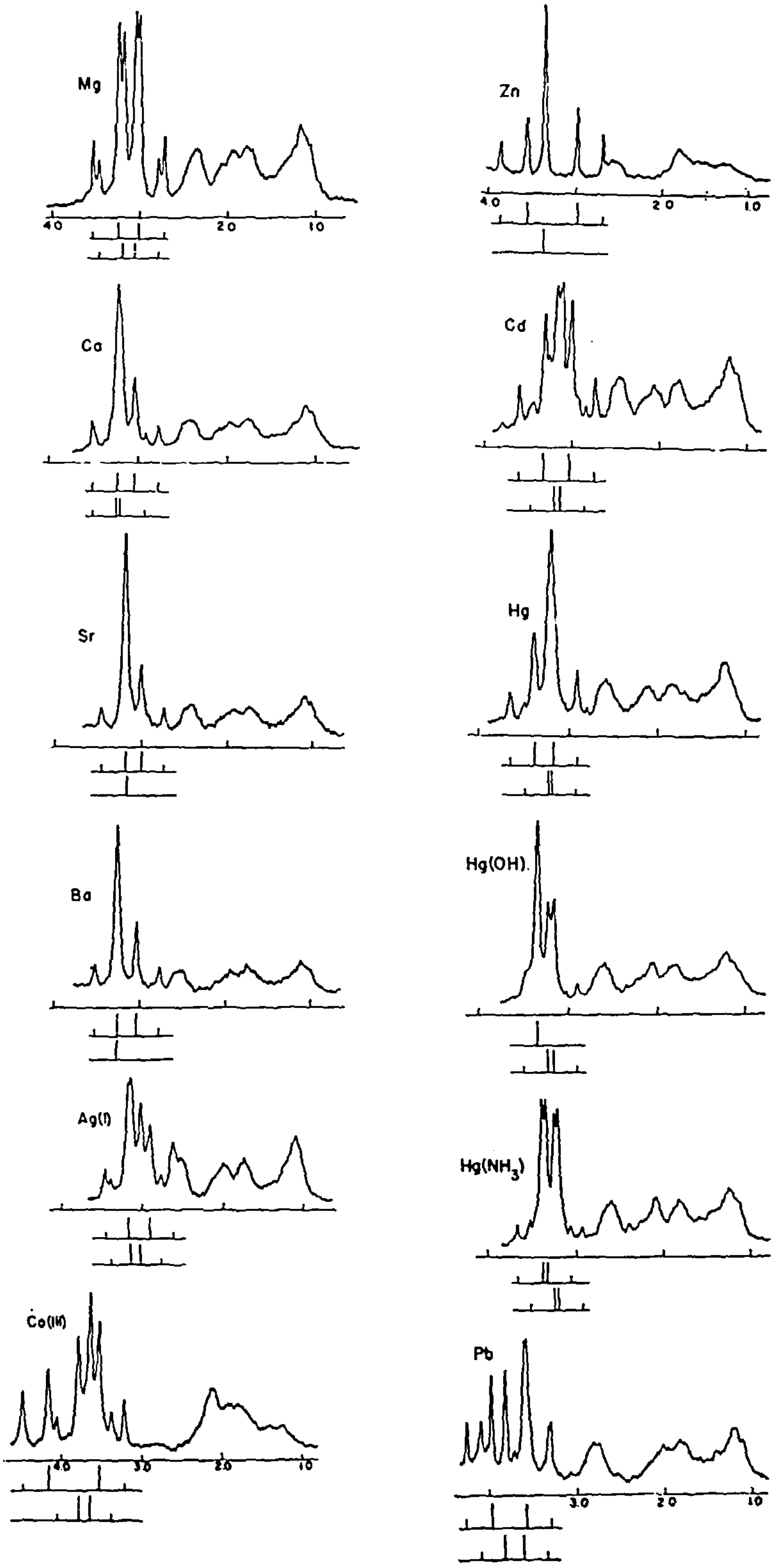


FIGURE 8. Proton NMR spectra of some metal-CyDTA complexes. Day, R. J. and Reilley, C. N., *Anal. Chem.*, 37, 1326 (1965). With permission.

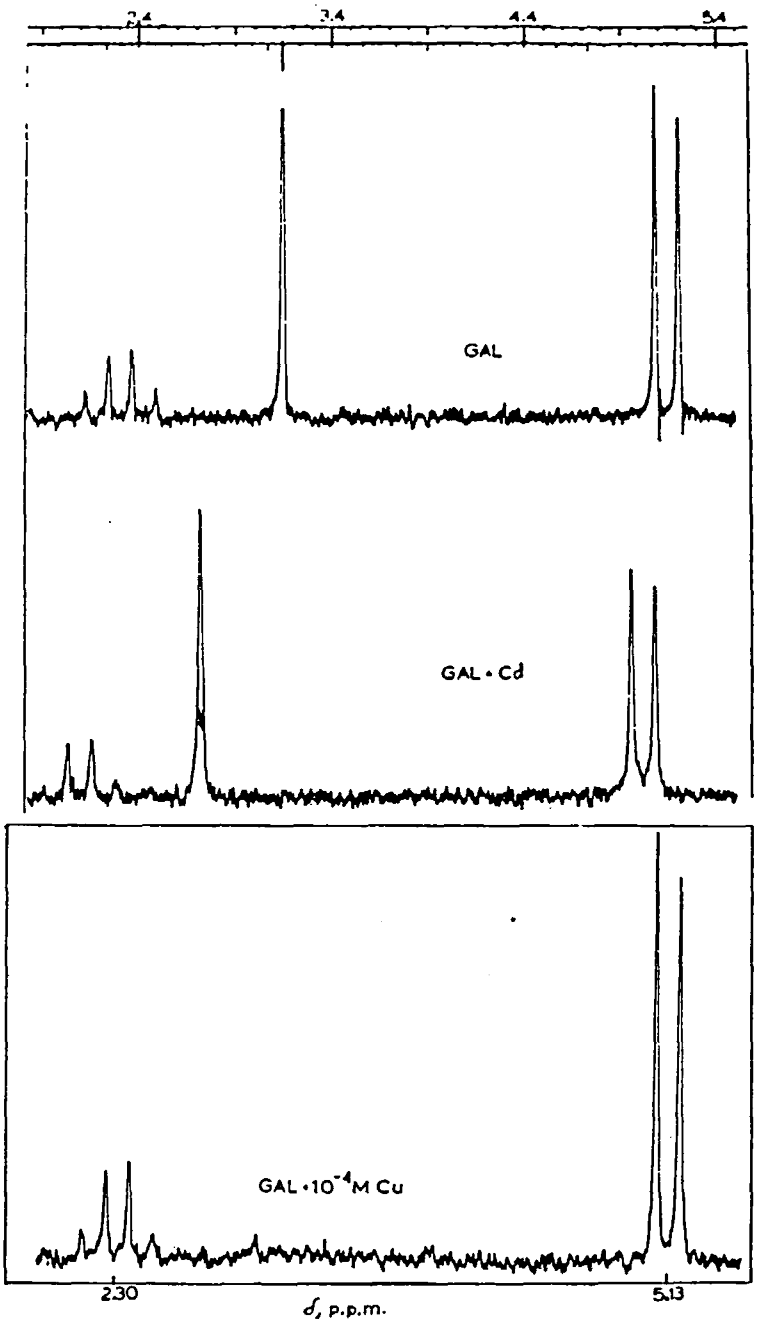


FIGURE 9. NMR Spectra of 0.5 M glycylalaninate, its cadmium complex, and with 10^{-4} M Cu^{+2}. Li, N. C., Scruggs, R. L., and Becker, E. D., *J. Amer. Chem. Soc.*, 84, 4650 (1962). With permission.

protein. If the chelating ligand forms a mixed complex with the metal-protein complex, the chloride ion may not find available sites for coordination. The efficiency of the quadrupolar relaxation would decrease and the resonance line width would decrease. This could be misinterpreted as a removal of the metal from the protein. This work is of great interest and potential, but will require a detailed investigation.

F. Exchange Reactions Involving Metal Complexes

The chemical dynamics of ligand-exchange reactions of aminocarboxylate chelates was the subject of extensive investigations utilizing techniques other than NMR.[46] By these techniques, only unsymmetrical exchange reactions could be studied. That is, a ligand on a metal complex was exchanged for a chemically different though structurally similar ligand. For example, one ligand may be optically active (as is PDTA) whereas the other (EDTA) may be optically inactive. The exchange process could then be studied by means of optical activity of the ligand molecules.[47] However, care must be taken in relating these results to symmetrical exchange reactions. If the ligand-exchange reaction is sufficiently slow, isotopic tagging may be employed to investigate symmetrical reactions by separation and isotopic analysis of the reaction mixture. However, most reactions involving ligand exchange of alkali, alkaline earth and transition metal complexes of aminocarboxylates are much too fast for study by these means.

Ligand-exchange reactions of multidentate ligands may be divided conveniently into two categories: (a) nucleophilic substitution reactions (S_N) in which excess ligand is added to a metal complex and the ensuing reaction is represented as

$$MY_A + Y_B \rightleftharpoons [Y_BMY_A] \rightleftharpoons MY_B + YA \quad (21)$$

where Y_BMY_A represents an intermediate in which both ligands are coordinated to the metal ion, and (b) electrophilic substitution reactions (S_E) represented as

$$M_AY + M_B \rightleftharpoons [M_AYM_B] \rightleftharpoons M_BY + M_A \quad (22)$$

involving an intermediate in which both metal ions are coordinated to the ligand. A generalization of the S_E mechanism would include the dissociation of a protonated complex.

Unlike other techniques, NMR readily permits the study of symmetrical exchange reactions because the free and complexed ligands, and even parts of these ligands, are labeled by their nuclear spins. This permits the study of both intermolecular and intramolecular (such as individual bond lifetime) exchange reactions. An additional advantage is that because the measurement is based upon the shape of the NMR spectrum resulting from exchange, the studies are done using solutions which are at chemical equilibrium. Of course, slow exchange studies may be done by isotopically labeling the ligand with deuterium and observing changes in spectral areas with exchange. Both of these methods are easily applied using conventional NMR spectrometers. The limitations are essentially based upon whether the exchange rate can be adjusted to fall into the range of rates which may be determined by NMR. This range, sometimes called the NMR kinetic "window," is determined by the separation of resonance frequencies of the protons in their different environments. This separation may manifest itself as a chemical shift or as a spin-spin splitting pattern. For example, the collapse of spin-spin multiplets ($J \sim 0.5$ to 20 cps) would permit studies of rate constants on the order of 10^1 to $10^3 s^{-1}$, the exchange between diamagnetic environments in which chemical shift differences of 0.5 to 100 cps are observed would permit rate constants of 10^1 to $10^4 s^{-1}$ to be determined, and exchange between a diamagnetic environment and a paramagnetic one which gives rise to chemical shift changes of 10^2 to 10^4 cps would permit rate constants of the order of 10^4 to $10^7 s^{-1}$ to be investigated. Frequently the control of pH, reactant concentration, temperature, or other parameters can be used to bring the rate of the reaction which is to be studied into the appropriate range. The major disadvantage of NMR for kinetic and equilibrium studies is the high concentration of solute (e.g., 0.1 *M*) needed to obtain spectra with good signal-to-noise ratios. This problem may be eliminated by the use of signal averaging techniques such as Fourier transform NMR. Occasionally, the NMR lines are broadened by the viscosity of the solutions. This is a result of a decrease in the transverse relaxation time and may require empirical correction, or in some cases may be removed by operating at an elevated temperature which lowers the viscosity.

Among the first studies of aminocarboxylate ligand exchange reaction rates done by NMR were those of Pearson and co-workers[48,49] who investigated amino acid complexes of paramagnetic ions. The exchange rates show the order Mn>Fe>Co> Ni<<Cu with the tetrahedral copper complexes showing a second order (S_N2) mechanism and iron, cobalt and nickel (and probably manganese) showing mixed first and second order rate laws. These octahedral complexes likely undergo a solvent-assisted dissociation (S_N1) mechanism.

Although Kula had given some qualitative values of exchange rates involving hydroxy complex of Zn(II)EDTA,[11] the first detailed study of ligand exchange of an EDTA chelate using NMR was done by Sudmeier and Reilley.[50] Cadmium-EDTA was chosen because it is diamagnetic and earlier work suggested that its reaction rates fall in the NMR kinetic "window."[37] The metal-nitrogen bond lifetime was known to be long because AB patterns are observed for the acetate protons. Rupture of one of these metal-nitrogen bonds permits nitrogen inversion. Upon reforming the bond following inversion the A and B protons exchange their magnetic environments. If the rate of this process is rapid, a broadening of the AB pattern will result and the rate of the individual metal-nitrogen bond breaking may be determined (assuming the nitrogen inversion step is rapid). Intermolecular exchange rates are obtained from the line shape of excess (free) EDTA in the solution which becomes broadened by exchange between two diamagnetic environments: free and complexed.

The following exchange reactions may be considered:

$$MY^{-2} \xrightleftharpoons[k_{-1}]{k_1} M^{+2} + Y^{-4} \qquad (23)$$

$$MY^{-2} + \overset{*}{Y}^{-4} \xrightleftharpoons[k_{-2} = k_2]{k_2} M\overset{*}{Y}^{-2} + Y^{-4} \qquad (24)$$

$$MY^{-2} + HY^{-3} \xrightleftharpoons[k_{-3} = k_3]{k_3} M\overset{*}{Y}^{-2} + HY^{-3} \qquad (25)$$

$$MY^{-2} + H_2\overset{*}{Y}^{-2} \xrightleftharpoons[k_{-4} = k_4]{k_4} M\overset{*}{Y}^{-2} + H_2Y^{-2} \qquad (26)$$

$$MHY^{-} \xrightleftharpoons[k_{-5}]{k_5} M^{+2} + HY^{-3} \qquad (27)$$

$$MHY^{-} + H\overset{*}{Y}^{-3} \xrightleftharpoons[k_{-6} = k_6]{k_6} MH\overset{*}{Y}^{-} + HY^{-3} \qquad (28)$$

$$MHY^{-} + H_2\overset{*}{Y}^{-2} \xrightleftharpoons[k_{-7} = k_7]{k_7} MH\overset{*}{Y}^{-} + H_2Y^{-2} \qquad (29)$$

Table 5 shows a summary of the rate constants obtained for these reactions. A detailed consideration of the charge, size, and number of coordination sites on the attacking ligand in the S_N type reactions suggested a mechanism for exchange not unlike that given by Pearson. A representation of this mechanism is shown in Figure 10. Figure 11 shows a proposed mechanism for the S_E type reaction and invokes the 2:1 metal-complex intermediate. These compounds are known to be stable for molybdenum[32] and evidence for a similar structure with calcium and strontium has been presented earlier.[13]

Almost simultaneous with the publication of the Cd(II)-EDTA exchange studies, Kula and

TABLE 5

Experimental Rate Constants for Ligand Exchange

	CdEDTA		CaEDTA	SrEDTA	CaHEEDTA
	(28°C) (Ref. 48)		(32°C, μ~1.6 M) (Ref. 50)		
k_1 sec^{-1}			<1	3×10^1	3×10^1
k_{-1} M^{-1}sec^{-1}			$<2.5 \times 10^9$	8.1×10^8	1.3×10^8
k_2 M^{-1}sec^{-1}	1.3×10^2	(μ = 2.5)	1.2×10^2	1.1×10^3	1.1×10^3
k_3 M^{-1}sec^{-1}	<3	(μ = 2.5)	5	1.6×10^2	2.3×10^2
k_4 M^{-1}sec^{-1}	1.4	(μ = 1.6)	2×10^5	1×10^5	--
k_5 sec^{-1}	1.2	(μ = 1.6)	5×10^4	2×10^4	--
k_{-5} M^{-1}sec^{-1}	1×10^9	(μ=1.6)	1×10^7	5×10^5	--

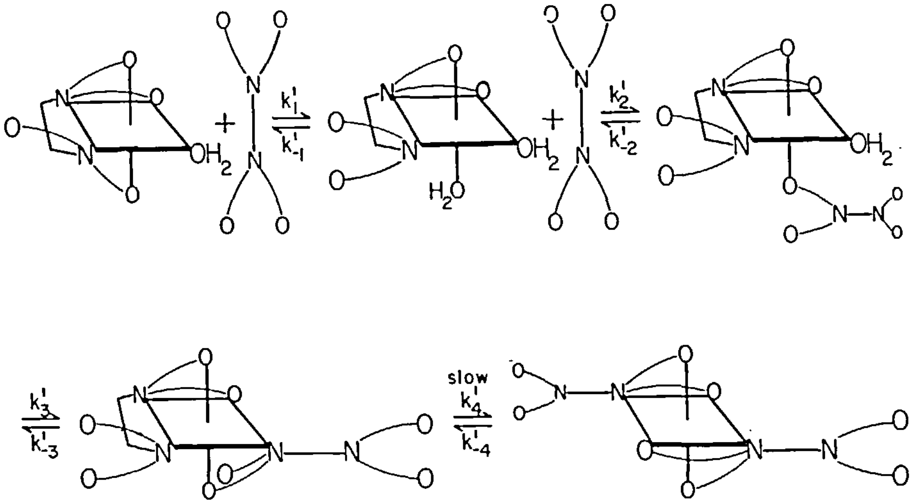


FIGURE 10. Proposed mechanism of S_N reactions of Cd-EDTA and EDTA. Sudmeier, J. L. and Reilley, C. N., *Inorg. Chem.*, 5, 1047 (1966). With permission.

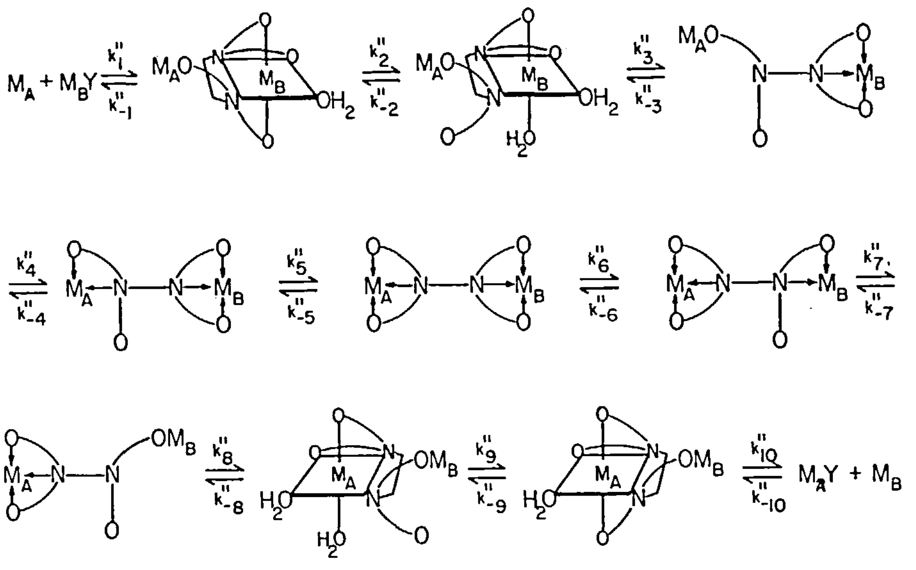


FIGURE 11. Proposed mechanism of S_E reactions of Cd-EDTA. Sudmeier, J. L. and Reilley, C. N., *Inorg. Chem.*, 5, 1047 (1966). With permission.

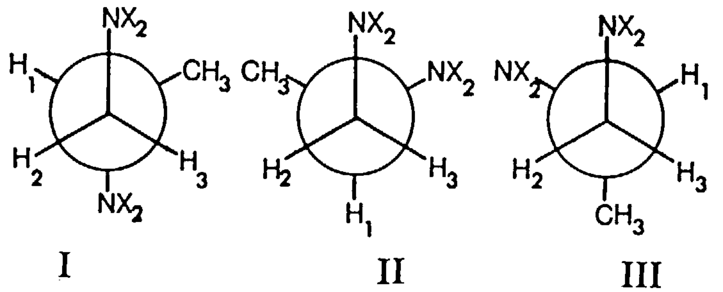
co-workers published the results of a similar study of Ca(II)-EDTA exchange[51] and later the results of Sr-EDTA and Ca-HEEDTA [N'-(2-hydroxyethyl)-ethylenediamine-N, N, N'-triacetic acid].[52] The latter ligand, while structurally related to EDTA, is capable of only pentadendate coordination. The results of these studies are also given in Table 5. A mechanism less detailed than, but similar to, that proposed by Sudmeier and Reilley was put forth by Kula and co-workers. The reader may compare the rate constants for these complexes as his interests direct. The most obvious trend shows the alkaline earth complexes to have much larger nucleophilic exchange rates, probably due to the inherent M-N bond lability and the subsequent availability of coordination sites on metal for attacking ligands.

Carr, Reilley, and co-workers[53] have studied exchange reactions of lead and copper complexes of EDTA and PDTA utilizing optical rotatory dispersion in conjunction with NMR, and Ni(II)-EDTA using NMR.[54] In general, the mechanism is analogous to that for the Cd(II), Ca(II) and Sr(II) chelates of EDTA. Carr is presently investigating the activation parameters of these reactions, but as yet has found no obvious relationship between these data and the metal-ligand combinations.[55]

The disadvantages of NMR as applied to ligand-exchange studies, which were mentioned earlier, are trivial compared to the power of the technique. To date only a limited number of cases have been studied. The similarity of the reaction mechanism tends to discourage new researchers from entering the field. No applications of pulsed NMR methods are known to the author. Although this technique has certain restrictions, it would certainly be useful, for example, to determine the role of water in ligand exchange, particularly in the case of paramagnetic ligands. It should be mentioned that indiscriminate usage of buffers and salts should be avoided in ligand-exchange studies as the presence of even weakly coordinating ions such as sodium can alter the results.

G. Conformational Analysis of Metal Complexes

The conformations of coordinated ligands have been a subject of interest for several years. The use of NMR had made it possible in some cases to determine the conformations of ligands in metal complexes by application of the Karplus relationship between vicinal proton-coupling constants and the dihedral angle[56] and the large contact shifts observed in certain paramagnetic metal complexes. As an example of the use of the Karplus relationship, Sudmeier and Senzel performed a study of free and complexed PDTA (dl-propylenediaminetetraacetic acid).[3,57] The introduction of a methyl group into the ethylenic chain of EDTA to form PDTA also introduces asymmetry, which in turn leads to observable spin coupling between the ethylenic-CH_2- and -CH protons. Additional coupling to the-CH_3 protons produces a complex $ABCX_3$ spectrum, which of course contains more information related to conformation than the single peak observed for the ethylenic proton resonance in the case of EDTA. The point of this is to consider possible configurations of the ligand, and to evaluate their relative populations. Usually, three classical rotamers such as I, II, and III are proposed as shown in Figure 12. The determination of the relative populations of these rotamers is based upon the relationship between the vicinal coupling constants J_{12} and J_{13} and their corresponding dihedral angles. There is one serious limitation to this procedure. Although the experimental values of J_{12} and J_{13} may be readily and accurately determined, the parameters needed to calculate rotamer populations include the coupling constants for the *anti* (J_a) and *gauche* (J_g) configurations of the coupled protons. Obviously these cannot be obtained from the compound under study. Frequently, values obtained from rigid ring structures of similar functional groups are used. However, the coupling constants are subject to considerable variation with substituents. Certain empirical expressions have been proposed and used with some success to allow corrections to J_a and J_g for the electronegativities of substituents.[58] The success is limited to the observation that qualitatively no shocking results have been obtained for relative rotamer populations. However, the significance of the numerical values frequently reported is an open question. In spite of these restrictions, Sudmeier and Senzel showed convincingly that monoprotonated PDTA had a conformation structurally equivalent to a "proton chelate" as represented by Structure II, which does not imply a polyfurcated



$X = -CH_2CO_2$

FIGURE 12. Representations of the classical staggered rotamers of PDTA. Sudmeier, J. L. and Senzel, A. J., *Anal. Chem.*, 40, 1693 (1968). With permission.

hydrogen bond, but an "averaged" equivalent structure. In addition, these authors demonstrated the presence of weak 1:1 chelates of K^+, Rb^+, and $(CH_3)_4N^+$ in solution in which the metal ion (cation) is coordinated to both functional groups. Formation constants of 1.3, 0.14, and 0.12 respectively were estimated for these chelates. Cesium was used as the cation of the "free" PDTA, for it showed the least tendency of all alkali metals to orient the PDTA in a conformation equivalent to chelation. This technique is obviously valuable, at least qualitatively, in determining rotamer preference in aminocarboxylate ligands and detection of weak chelates.

The Karplus relationship may also be used to determine ligand conformation in substitution inert compounds such as cobalt(III)-trisdiamines. For years the resolution of the NMR spectra of these compounds was of such low quality as to render accurate spectral analysis impossible. This lack of resolution has been attributed to intermediate inversion rates of the puckered five-membered chelate rings, to the presence of residual paramagnetism, and to spin coupling of protons to long-lived nuclear spin states of cobalt-59 ($I = ½$, 100% abundant). By nuclear spin decoupling of the ^{59}Co, Sudmeier and Blackmer showed that the latter explanation is correct.[59] Figure 13 shows the $-CH_2-CH_2-$ portion of the spectrum of D-$[Co(en)_3]Cl_3$ at 100 MHz. Figure 13A shows the broad peak centered at 2.84 ppm versus DSS to have a half-width of ~ 23 Hz. Figure 13B shows the spectrum after nitrogen deuteration in which the half width is reduced to ~ 18 Hz because of removal of H-N-C-H spin coupling. Figure 13C shows the spectrum of the N-deuterated complex after the addition of 0.5 *M* K_3PO_4. The increased resolution is attributed to an increase of the rotational correlation time, τ_c, caused by phosphate complexing (which in turn reduces T_1 for cobalt-59 through increased efficiency in quadrupole relaxation). Figure 13D shows the effect of irradiation about the cobalt-59 resonance of the N-deuterated complex. The AA′BB′ pattern indicates the presence of only two types of protons and suggests rapid interconversion of the δ and λ puckered forms of each diamine ring. A further conclusion is that the Co-N-C-H coupling constant is greater for the equatorial than for the axial protons.

The application of the Karplus relationship to conformational studies, long known in organic compounds, shows considerable promise in metal chelate ligand-conformation studies. Related research is needed to determine the effects of typical functional-group substituents upon the coupling constants as well as the effect of protonation on these coupling constants in the absence of conformational changes. Because so much research utilizing other methods, such as optical rotatory

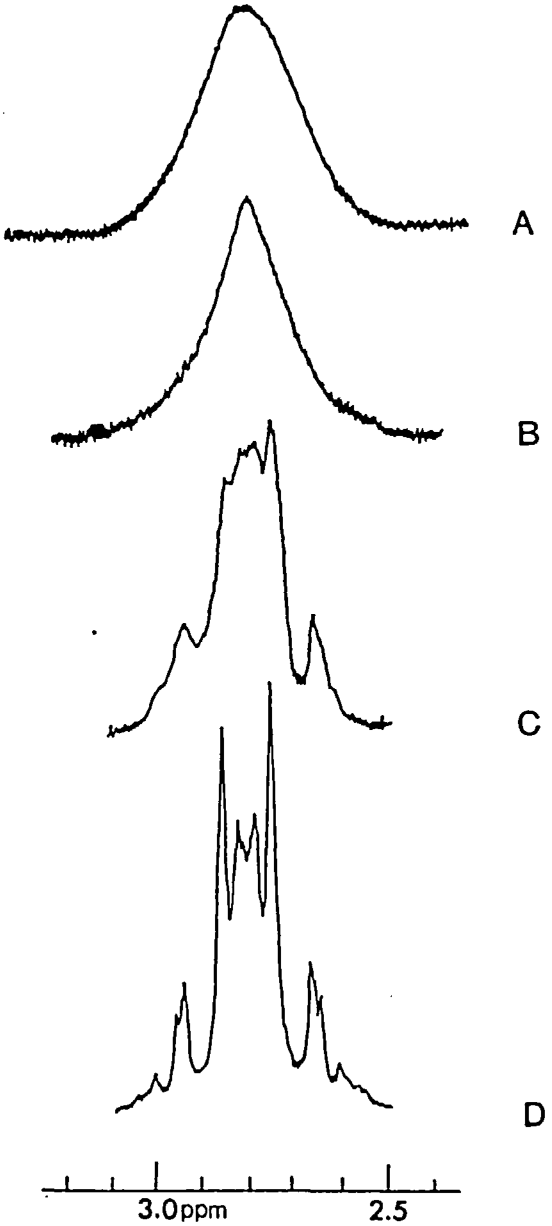


FIGURE 13. Pmr spectra of D-[$Co(en)_3]Cl_3$ at 100 MHz: A, in H_2O;B, in D_2O;C, in D_2O solution of 0.5 M K_3PO_4; D, in D_2O solution potassium phosphate with Co-59 decoupling. Sudmeier, J. L. and Blackemer, G. L., *J. Amer. Chem. Soc.*, 92, 5238 (1970). With permission.

dispersion and circular dichroism, has been done on Co(III) complexes, it is advantageous that the spin decoupling methods reported by Sudmeier and Blackmer will permit NMR studies of these compounds to be performed. In addition to the use of vicinal proton-proton coupling to elucidate ligand conformation in complexes, recent data obtained for vicinal (M-N-C-H) metal-proton coupling constants for cobalt[59] and platinum[19,22] complexes should be of increasing value.

Until recently the application of NMR to the study of metal chelates of analytical importance has been essentially limited to diamagnetic chelates, although there have been studies of a few paramagnetic chelates.[60] The primary justifications for this are that there was a great deal to investigate concerning the more easily studied diamagnetic chelates and that, in all probability, few researchers recognized the potential of the results of study of paramagnetic chelates. Moreover, studies of paramagnetic chelates require instrumentation more flexible than the commercial instruments available to most researchers at the time.

In diamagnetic complexes, proton chemical-shift differences between the free and coordinated ligands are of the order of one ppm. In corresponding paramagnetic complexes, chemical shifts greater than 100 ppm are observed for protons close to the metal ion.[61] (Errors in the interpretation of spectra reported in reference 61 were corrected by Erickson et al.[72]). These large shifts result from spin interactions between the protons and the unpaired electron. The mechanism for these shifts have been the subject of extensive experimental and theoretical investigation.[62] Two mechanisms are to be considered, the Fermi contact interaction and the pseudo-contact interaction. The former arises from unpaired electron spin density at the observed proton through a spin polarization in σ bonds or by delocalization in π bonds. In σ=bonded systems, the spin density at a given proton is sensitive to the dihedral angle between the metal ion and the proton (M-N-C-H-), thus providing information concerning ligand conformation.[63,65]

The pseudo-contact interaction arises from a nonzero dipolar interaction of electron-spin and orbital contributions with the proton nuclear spin. Unlike Fermi contact interaction, this process is transmitted through space.

The power of the Fermi contact and pseudo-contact interactions has been convincingly demonstrated by Holm and co-workers on four-coordinate complexes with Ni(II),[65] and by Eaton, Phillips, and co-workers on N-substituted Ni(II) aminotroponeimineates.[66] When the Fermi contact term predominates, as it does in many Ni(II) complexes, the interpretation of the data is reasonably straightforward and the temperature dependence of the chemical shifts is useful in obtaining relative energies of conformations. Complexes in which pseudo-contact interactions are significant present a more complicated situation because of a complex spin-delocalization mechanism or a significant temperature dependence of the population of the Kramer's doublets.[67] Careful selection of complexes of the alkyldiamine variety may permit useful studies.

An outstanding series of papers from Reilley and co-workers represents a significant development of the use of paramagnetic chelates to study ligand conformations.[68-72] It is beyond the scope and purpose of this review to summarize the information contained in these papers. An example from an early report will be used to illustrate the methods and the type of results it yields.

When the Fermi contact term predominates, the chemical shift of the *i*th group of protons, δ_i, (in ppm) is given by

$$\delta_i = -A_i \left(\frac{\gamma_e}{\gamma_H}\right) \frac{g\,\beta\,S\,(S+1)}{6SkT} \qquad (30)$$

where A_i is the corresponding nuclear spin-electron spin hyperfine splitting constant (in gauss) and S is the total spin of the paramagnetic complex. Other symbols have their usual meaning. It is readily seen from this equation that the observed chemical shift should be inversely proportional to temperature. The only deviations from this with Ni(II) complexes have been shown to involve a configuration equilibrium between tetrahedral and square planar forms, the latter usually being diamagnetic.[73] X-ray studies with ethylenediamine complexes indicate that these coordination complexes could exist in two conformations designated as k and k′ by Corey and Bailar.[74] In the case of unsymmetrical N-substituted derivatives, the two conformations may not be equally populated, but rapid interconversion between these configurations may be expected. The temperature dependence of the

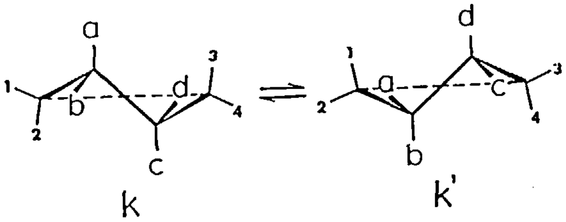


FIGURE 14. k and k′ forms of coordinated ethylenediamine rings.

contact shift is expected to follow Equation 30 if there are no changes in configuration other than rapid interconversion between equally populated states of k and k′; this is the case with Ni(II) complex of ethylenediamine. However, the temperature dependence of the contact shift of unsymmetrical N-alkyl derivatives is greater than that predicted, and may be explained as a shift in the $k \rightleftarrows k'$ equilibrium with temperature.[68] Evaluation of these data permits the calculation of relative free energies of the conformations, as well as the upper limits of the rate of nitrogen inversion which is slow ($< 10^4 s^{-1}$) because of long-lived Ni-N bonds.

Reilley and co-workers have performed a detailed study of the configuration, conformation, and interconversion dynamics of the EDTA, PDTA and CyDTA chelates of Ni(II).[72] The authors concerned themselves with the questions of both optical and coordination isomers with concern over the ligand-centered and metal-centered isomers. With ligands such as EDTA, only two equally probable isomers are possible (Δ or Λ) based upon the absolute configuration of the ligand. However, in the case of ligands with asymmetric centers, such as PDTA, additional optical isomers are possible. The choice of nickel as the metal ion for use in the investigation is made because of its favorable paramagnetic properties. The dependences of the paramagnetic shift of the proton resonances on ring conformation and stereospecific deuteration of the acetate protons are of great aid in the interpretation of the spectra. For example, peaks which disappear upon deuteration may be assigned to the acetate protons. From the differential rates of deuteration, in-plane and out-of-plane protons as well as axial and equatorial protons may be assigned. An interesting result of this treatment is that the axial in-plane acetate proton was not observed in any of the three complexes studied. No completely satisfactory explanation for this was given. A comparison of the observed chemical shift of the acetate and ethylenic protons with those expected for 5- and 6-coordinate species suggested that the EDTA and PDTA complexes were essentially 6-coordinate, but the CyDTA chelate was intermediate between 5- and 6-coordinate. Nitrogen inversion in the CyDTA complex was not observed after one week. However, nitrogen inversion was observed in the PDTA complex and both nitrogen inversion and $\Delta - \Lambda$ interconversion were observed for the EDTA chelate.

These studies by Reilley and co-workers have provided many answers to the questions related to metal-complex geometry. However, there are still many areas of research to be considered. So far only the nickel chelates have received major attention because of the favorable properties of this cation. The work to date must be considered the cream from the top. Further studies on different cations will be more difficult but will be necessary if the knowledge of chelate geometry is to become generalized rather than limited to nickel chelates.

These examples only illustrate the most important methods of the use of conformational analysis by NMR. The application of the Karplus relationship for vincinal proton splitting has served the chemist interested in the conformation of organic molecules and metal complexes well. An excellent chapter on the latter application has recently been published.[75] However, the low sensitivity of the proton coupling, and its inability to detect subtle configurational changes many chemical bonds removed, limit its usefulness. Certain metal chelates, namely, those of platinum and mercury, have been studied by metal-proton couplings analogous to the Karplus relationship. These larger couplings are more sensitive and may be observed at protons as many as four chemical bonds removed from the metal. Beyond doubt, paramagnetic contact and pseudo-contact shifts have found and will find extensive applicability to the study of ligand configuration in chelates. The techniques require only a versatile commercial research spectrometer. However, even this technique will have its limitations. First, only paramagnetic complexes may be studied. Second, certain paramagnetic chelates may not be useable for studies because of long electron-relaxation times and the requirement that the complexes must not be labile. Finally, studies with ligands in which pseudo-contact interactions are large will be complicated and in most cases will require significant temperature variations and other means of separating the Fermi contact interaction and the pseudo-contact interaction. In the case of non-labile diamagnetic compounds, expertise in areas other than NMR spectroscopy may be required. For example, the isolation of isomers of (iminodiaceto)diethylenetriaminecobalt (III) by Legg and Cooke required over three week's elution time on a chromatographic column.[76]

III. QUINOLINOLS

The quinolinols, particularly 8-quinolinol (oxine), are an important class of compounds for direct analytical application. A considerable amount of study has been made of the formation constants, solubilities, and solvent-extraction distribution equilibria of metal complexes of these compounds.[77] Only limited structural studies have been performed using x-ray methods. Among the principal points to be studied were the nature of bonding and structure of the chelates, and the question whether "solvated" complexes such as U(VI) oxinate, which contain an uncoordinated ligand in the solid state,[78] exist in solution.

Baker and Sawyer obtained the NMR spectra of 8-quinolinol and its 5-chloro- and 2-methyl-derivatives in dimethylsulfoxide shown in Figure 15.[79] The spectra were analyzed and assignments guided by analogy with quinoline and its derivatives. Spectra of the oxinate complexes of Pt (II), Zn(II), Mg(II), Hg(II), Pb(II), Sn(II), Al(III), Rh(III), and Co(III) were investigated. Most of these presented little experimental difficulty. However, the magnesium oxinate spectrum was recorded using a computer of average transients because of the low solubility of that complex. In general, the coupling constants in the oxine are not greatly affected by chelation. However, the chemical-shift values of ligand protons are shifted substantially upon coordination. The extent of these shifts was found to depend on the nature of the metal-ligand bond and also on the geometry of the chelate in question.

A careful investigation of the spectra of the oxinate species H_2Ox^+, HOx, and Ox^- showed that shifts of 0.05 ppm and 0.15 ppm for protons 2 and 7 respectively were a result of intramolecular hydrogen bonding and comparison of these values with those expected (determined from α-naphthol) indicate a 15 to 20% population of the keto tautomer.

Baker and Sawyer suggested that metal bonding to the oxygen and nitrogen of oxine represent independent effects, and this led them to propose five limiting cases of metal-ligand bonding to oxine. Case 1: the metal-oxygen bond is covalent and the nitrogen is coordinated strongly; the resulting effect is equivalent to total protonation of both oxygen and nitrogen. The resulting spectrum will be similar to that of oxine in strongly acid solutions (H_2Ox^+). Case 2: The metal-oxygen bond is ionic and the nitrogen is coordinated strongly. The spectrum in this case will be intermediate between those of oxine in acid and in base. Case 3: The metal-oxygen bond is ionic and there is no metal-nitrogen bond. The complex is essentially an ionic salt and the spectrum will be that of oxine in basic solution (Ox^-). Case 4: The metal-oxygen bond is covalent and there is no coordination to nitrogen. The spectrum will be essentially that of oxine (HOx), but corrected for the effects of hydrogen bonding. Case 5: An intermediate case, obtained by averaging Cases 1 and 3 (or 2 and 4), in which both ligand bonds contain partially delocalized electrons. Each of these cases was adapted to tetrahedral (T) and square planar (SP) configurations. The effects of geometry upon chemical shift arise mainly from anisotropic shielding-deshielding contributions of neighboring ligands. Using these cases, the bonding and structure of the complexes of oxine studied were classified.

$PtOx_2$ showed strong oxygen and nitrogen bonding and square planar configuration. The NMR spectrum was similar to Case SP-1, with some characteristics of SP-5.

$ZnOx_2$ was intermediate to Cases T-3 and T-5. Both metal-ligand bonds appeared labile.

$MgOx_2$ is also labile and the NMR spectrum parallels Case T-3 with proton 2 appearing about 0.3 ppm upfield from its calculated position. This proton is closest to the metal atom and any sepcific interactions will manifest themselves most at position 2.

$SnOx_2$ and $PbOx_2$ are similar and both are intermediate between Case T-4 and Case T-5 with labile metal-nitrogen bonds and more inert metal oxygen bonds.

$AlOx_3$, $CoOx_3$, and $RhOx_3$ exist in the *trans*-configuration in solution as represented by Figure 16. The three ligands, A, B, and C have different

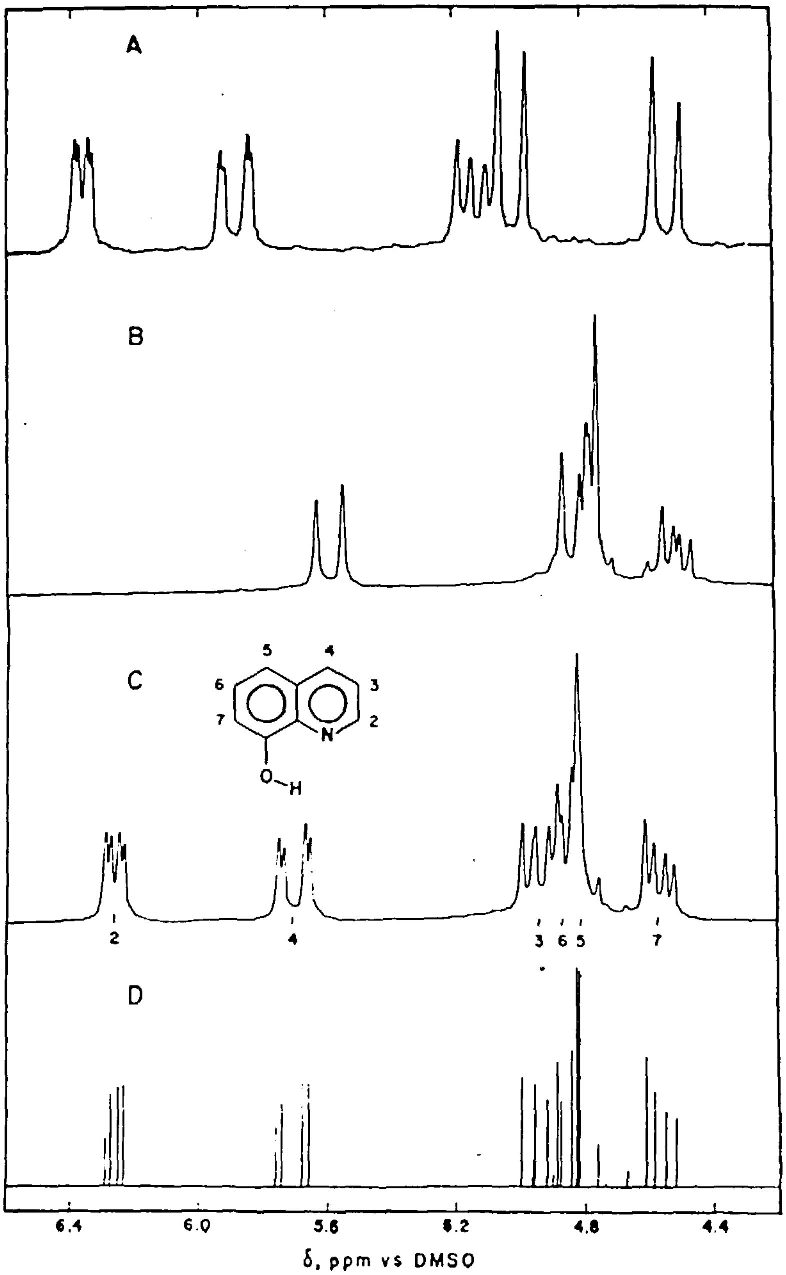


FIGURE 15. Proton NMR spectra for 8-quinolinol (Curve C) and its 5-chloro- (Curve A) and 2-methyl- (Curve B) derivatives in dimethylsulfoxide. Curve D represents the computed line spectrum for oxine. Baker, B. C. and Sawyer, D. T., *Anal. Chem.*, 40, 1945 (1968). With permission.

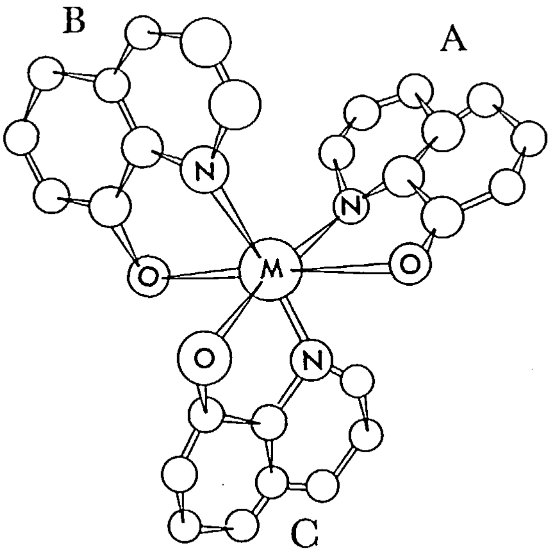


FIGURE 16. Trans (1,2,6) octahedral configuration of $M(III)(Ox)_3$. Baker, B. C. and Sawyer, D. T., *Anal. Chem.*, 40, 1945 (1968). With permission.

chemical shifts. The spectra of all three complexes are very similar and are closely related to cases 2 and 5, which suggests strong nitrogen coordination and a highly labile metal-oxygen bond. Aluminum oxinate undergoes rapid ligand exchange in the *trans*-isomer at elevated temperatures. No rate or mechanism was reported. The cobalt complex is highly inert even at elevated temperatures.

A study of the oxinates of thorium(IV) and uranium(VI) was performed.[80] The major purpose of this investigation was to determine whether the so-called "solvated" complexes, $MOx_n \cdot HOx$, which had been found in the solid state exist in solution. A detailed NMR and thermal decomposition study established that these complexes of thorium and uranium exist only in the solid state and dissociate to $ThOx_4$ and UO_2Ox_2 in solution.

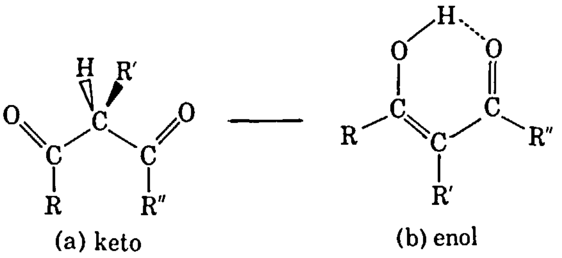
A few additional reports of the NMR spectra of quinolinols have been given,[81] including the ^{14}N spectra.[82] However, the studies have been of limited scope. The fact that many of these complexes are insoluble, the limited configuration possibilities, and the relative complexity of the spectra make them unattractive to study. This is not to underestimate their place in chemistry, for they have biological as well as analytical significance. Further study is certainly justified; the more that is known about the nature and lability of metal-ligand bonding, the better the design and application of metal complexes to analytical problems. Ligand-exchange rates of some oxinates are known to be accessible to NMR and should be investigated. The work of Baker and Sawyer has only touched the surface of this series of compounds.

IV. β-DIKETONES

β-Diketones form a class of compounds of importance in analytical chemistry. They are used as chelating agents for solvent extraction and their fluoro-derivatives form volatile metal complexes which permit selective trace metal determinations by gas chromatography. Recently, certain rare-earth-metal complexes have been shown to be valuable reagents which greatly facilitate the analysis of complex NMR spectra of certain types of compounds.[83] There have been a number of NMR studies of compounds of this class.

A. Keto-Enol Tautomerism

Prior to the availability of NMR, studies of the keto-enal tautomerism of β-diketones were performed by bromine titration and infrared and ultraviolet spectroscopy. Frequently, interpretation of the data was time consuming and, in the case of the bromine titration, the equilibrium was shifted during the determination. On the other hand, NMR provides a direct measure of the relative population of the tautomeric forms by integration of the spectra of the two tautomers. The keto-enol equilibrium is represented below where (a) is the keto tautomer and (b) is the enol tautomer.



(a) keto

(b) enol

STRUCTURE IV. Burdett, J. L. and Rogers, M. T., *J. Amer. Chem. Soc.*, 86, 2105 (1964). With permission.

The stability of the enol form depends greatly upon strong intramolecular hydrogen bonds, hence only monomeric forms are expected. Solvent interactions are negligible as indicated by the unchanged chemical shifts of the hydrogen nuclei in acetylacetone as found in the pure liquid and when it is dissolved in acetic acid and triethylamine.[84,85] The 60 MHz NMR spectrum of acetylacetone (2,4-pentanedione) is shown in Figure 17. The interchange between tautomers is sufficiently slow at room temperature to permit the observation of separate signals for the keto and enol forms present in approximately a 4:1 ratio. The spectral assignments are enol-CH_3 (2.0 ppm), keto-CH_3 (2.16 ppm), keto-CH_2 (3.61 ppm) enol-CH (5.56 ppm) and the enol-OH (15.56 ppm).[86] These assignments can be confirmed by variable-temperature studies and solvent effects. For example, dilution in cyclohexane increases the percentage of enol tautomer. The enolic -OH hydrogen chemical shift in acetylacetone occurs at about 15.5 ppm indicating the extensive hydrogen bonding,[85,86] but is shifted as much as 7 ppm to higher field by the addition of triethylamine.[85] Addition of compounds which form strong hydrogen bonds with one of the tautomers and changes in the dielectric constant of the solvent will influence the equilibrium. Reeves found a value of 2.7 kcal $mole^{-1}$ for the enthalpy of keto-enol conversion for acetylacetone from a temperature study of the equilibrium.[85]

In symmetrical β-diketones, only one resonance is observed for the terminal alkyl groups indicating equivalence of the enolic -OH hydrogen. In the case of *p*-methyl- and *p*-methyoxybenzoylacetone, the equilibrium strongly favors the retention of the carbonyl structure most remote from the benzoyl derivative. However, a small amount of a second enol has been detected.[87] This evidence is difficult to rationalize in view of the observation with acetylacetone. It is likely that one of the enol forms of these 1,3-diketones is stabilized by the benzoyl group.

B. Protolysis and Keto-Enol Interconversion Kinetics

The hydrogen exchange in β-diketones represents an interesting case. First there is exchange of the enolic -OH proton and a second type of exchange which involves the keto-enol interconversion. Intramolecular exchange of the enolic hydroxyl proton has been demonstrated to be rapid at room temperature because of the equivalence of the methyl protons.[85] Intermolecular exchange of this hydrogen is apparently very slow. However, when acetylacetone and acetic acid are mixed in various ratios, two broad resonance lines exist for the carboxyl and enol-hydroxyl protons. The two signals coalesce at 64°C in a solution containing 58 mol % acetylacetone with an estimated lifetime of a proton before exchange of 1.5×10^{-3} s.[84] A detailed study of the kinetics of protolysis of these compounds is needed.

The process of keto-enol interchange involves a hydrogen-atom transfer. Because separate resonance spectra are observed for the two tautomers, the rate constant of interchange for acetylacetone can only be estimated as less than $2 \times 10^3\ s^{-1}$. Heating to 150°C does not increase the rate sufficiently to coalesce the spectrum.[87] The observed rate of interchange can be increased by the addition of compounds capable of forming hydrogen bonds with the carbonyl oxygen. Amines greatly increase the rates of keto-enol interchanges.[88] A possible mechanism is the formation of a hydrogen bonded molecular complex. That tertiary amines are less effective in catalyzing the interchange implies that the N-H protons participate in the mechanism. Perhaps this participation involves a transfer of the amine hydrogen atom to the α-CH carbon in the enol tautomer, with subsequent conversion to the keto tautomer. The absence of an N-H proton in the tertiary amine is consistent with this proposal.

Much of the work on the dynamic processes of acetylacetone and related compounds is insufficiently quantitative. A detailed study of the mechanism of the keto-enol interconversion would be useful. The thermodynamic values associated with the enolization and with the dissociation of the enol to form the anion could be determined readily by NMR in conjunction with other methods. These values could then be compared with the thermodynamic quantities associated with coordination.

C. Metal Complexes

There have been extensive NMR studies of the metal chelates of β-diketones, and only a representative discussion of them will be given here, for a significant portion of the relevant literature has been reviewed elsewhere.[89,90] To a great degree, the transition metal complexes of acetylacetone (acac) were used to illustrate the power (and the pitfalls) of NMR for the purpose of structural and conformational studies. Piper and co-workers noted that tris-complexes of unsymmetrical bidentate ligands should yield a *cis* isomer with a threefold rotation axis and ligands in identical environments, and a *trans* isomer devoid of symmetry and having each ligand in a different

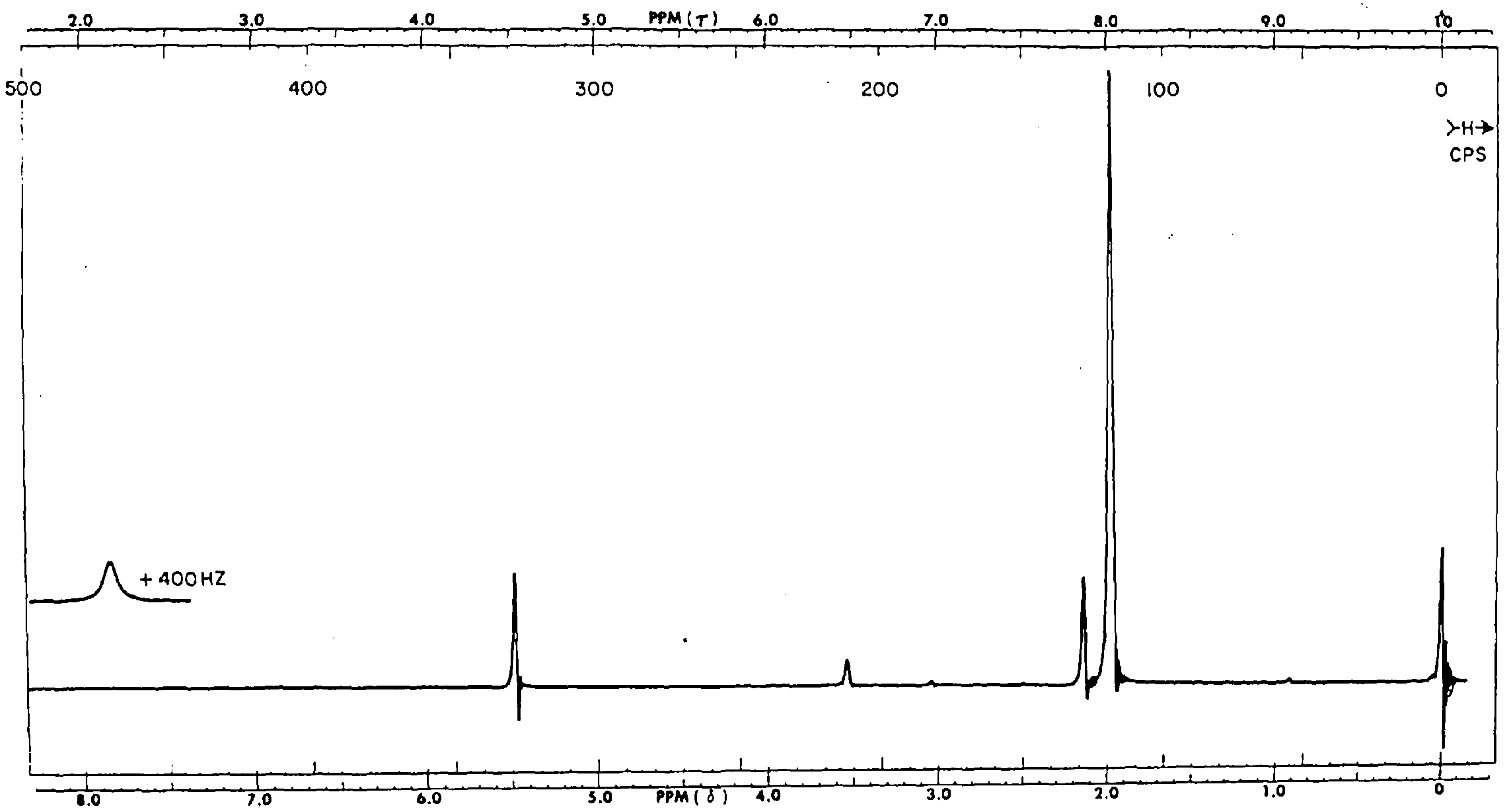


FIGURE 17. 60 MHz NMR spectrum of 2,4-pentanedione (acetylacetone). From *Spectral Exercises In Structural Determination of Organic Compounds*, by Robert H. Shapiro. Copyright (c) 1969 by Holt, Rinehart and Winston, Inc. Reproduced by permission of Holt, Rinehart and Winston, Inc.

environment.[91] After the synthesis of tris-complexes of Cr, Co, Rh, Al, Mn, and Fe with benzoylacetone, two isomers were resolved by chromatography for Cr, Co, and Rh. The two isomers of the Co and Rh complexes were identified by NMR. The *trans* isomer showed three methyl peaks, whereas the *cis* isomer showed only one. The paramagnetic chelates of Cr, Mn, and Fe could not be studied (at the time) and the Al chelate showed only the *trans* isomer. An example of the occasional pitfalls of using only NMR for this type of study is represented by *cis* $[Co(acac)_2en]^+$ studied by IR, visible, UV, and NMR spectroscopy.[92] This chelate should show different resonance peaks for the methyl groups on the acetylacetone. However, only a single sharp resonance is observed because of accidental coincidence of the chemical shifts.

Following the work of Piper, many researchers began to apply NMR to the study of the structure of β-diketone complexes. By and large, imaginative compounds were synthesized in wide variety and their structures were elucidated by NMR. In most cases, standard spectral parameters were used. One feature which sets these complexes apart is the "pseudo-aromatic" character of the chelate rings, which has been reviewed in detail by Collman.[89] This property is in part evidenced by the fact that the γ-protons on tris-acac complexes undergo certain substitution reactions. In addition, anomalous chemical shifts observed for the -CH= protons in complexes of silicon suggest a ring current deshielding similar to benzene.[93] However, more recent reports suggest that there is an alternative explanation.[94] The question of pseudo-aromatic character in acetylacetone chelates appears to be open in the minds of some and closed in those of others.

D. Shift Reagents

One of the most significant developments in the NMR of β-diketone compounds lies not in the direct investigation of the compounds, themselves, but in the effect that the presence of certain rare earth complexes has upon the spectrum of other molecules. Sievers and Eisentraut prepared a series of rare earth complexes of 2,2,6,6-tetramethyl-3,5-heptanedione (dipivaloylmethane), which may be represented as $M(thd)_3$ according to the abbreviation adopted by these authors.[83] (Many authors prefer the abbreviation DPM). These complexes were prepared because of their volatility and were used for gas chromatographic determination of the rare-earth elements. Hinckley prepared the pyridine adduct of the europium complex, $Eu(thd)_3 \cdot 2py$, and obtained the spectrum of an 0.05 *M* solution of it which was also 0.1 *M* in cholestrol in carbon tetrachloride.[95] The results are shown in Figure 18. Spectrum 1 is of cholesterol monohydrate in carbon tetrachloride taken with a spectrometer operating at 100 MHz. Spectrum 2 is the spectrum after addition of the $Eu(thd)_3 \cdot 2py$. Spectrum 3 is an expansion of the 1-2 ppm portion. Notice that the metal-complex resonance is at higher field than TMS and does not interfere with the spectrum of interest. The striking effect of these reagents in causing large chemical-shift changes has suggested the term "shift reagents."

The shift reagents provide an exceptionally inexpensive method of resolving complex spectra. For example, to increase the chemical shift between the *a* and *d* resonances to the extent shown in Figure 18 would require a 270-MHz field. This would only be accomplished with an exceptional superconducting magnet. Also, the increased field would enhance all chemical shifts equally. The shift reagents do not do this, which is frequently an advantage. Other shift reagents, such as Co(II) acetylacetonate, have been applied in this way to the study of proteins.[96] However, most of the compounds that have been utilized excepting the rare-earth chelates, have severe limitations. Selective shifts are not always induced and the degree of Fermi contact interaction with the molecule of interest may vary from so little as to cause no effect to so much as to broaden the NMR line so that it is not observable.

In the first year and a half after the discovery of the *thd* derivatives as shift reagents, over a dozen short communications appeared describing its use. However, the literature concerning the theory and mechanism of the shift process is sparse. In brief, the nature of the shift may be either a Fermi contact or a pseudo-contact interaction or both. It is generally accepted that the pseudo-contact term predominates when rare-earth ions are involved.[97] This shift experienced at the j^{th} proton under this circumstance may be represented by

$$\frac{\Delta H_j}{\Delta H} = -k\,[(3\cos^2\phi_j - 1)/R_j^3] \qquad (31)$$

where k is a composition constant, ϕ_j is the angle

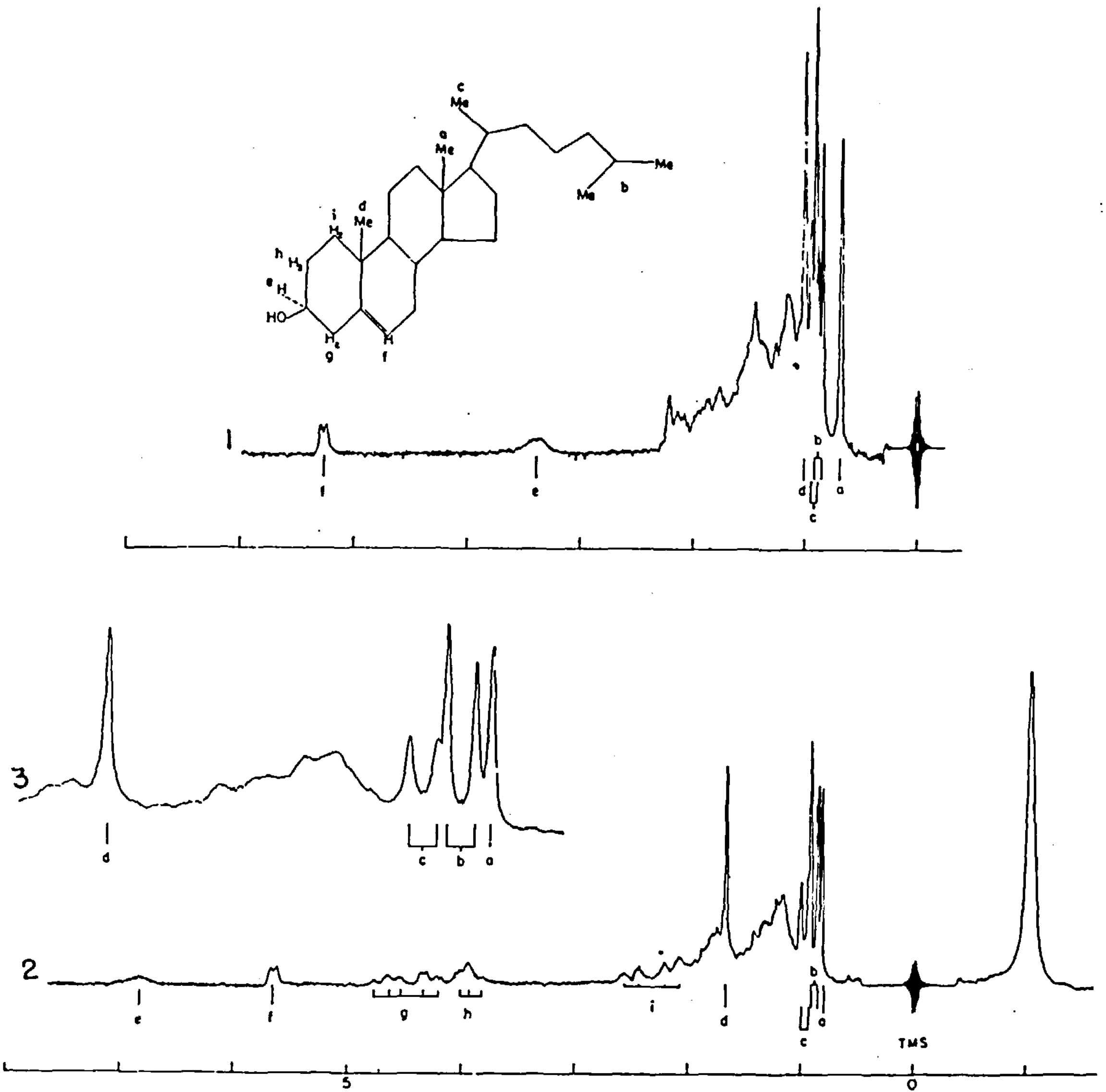


FIGURE 18. Spectrum 1 is of cholestral monohydrate in CCl_4. Spectrum 2 is of a CCl_4 solution 0.05 M in $Eu(DPM)_3 \cdot 2py$ and 0.1 M in cholesterol monohydrate. Spectrum 3 is an expansion of that region of spectrum 2 which includes the methyl resonances. Assignments are indicated by letter on the accompanying molecular diagram. The resonance 1 ppm upfield from tetramethylsilane (TMS) is due to the metal complex. Hinckley, C. C., *J. Amer. Chem. Soc.*, 91, 5160 (1969). With permission.

between the crystal-field axis of the complex and the radius vector from the metal to the j^{th} proton (contact angle), and R_j is the distance between the metal ion and the j^{th} proton. In order for the line broadening to be small, it is necessary for the metal ion used to have a short electron-spin relaxation time. This condition is met for the rare-earth elements, with the exception of gadolinium. The magnitudes of the observed shifts are found experimentally to depend upon the concentration of the shift reagent, the metal ion involved, and the nature of the functional groups of the organic molecule under study. The shift effect requires some type and degree of coordination of the metal ion to the molecule of interest. $Eu(thd)_3$ has a solubility of approximately 40 mg/ml in CCl_4 or $CDCl_3$. However, in the presence of a substrate in which shifts are observed, the solubility increases substantially, as expected if some type of bonding is involved.

$Eu(thd)_3$ is unstable in acid. It may be recovered from stable solutions by silica gel TLC.[98]

Hinckley discovered the potential of $Eu(thd)_3$ as a shift reagent when he attempted to prepare complexes of the steroid cholesterol and the bipyridine adduct of $Eu(thd)_3$. In running the NMR of these, large downfield shifts in the cholesterol spectra were observed. Because the steroid has only one site available for association, it was reasoned that this hydroxyl site must be the position of metal complex-cholesterol interaction. The observed changes in chemical shift after the addition of $Eu(thd)_3$ were found to decrease with increasing distance between the paramagnetic center and the affected proton. Thus the protons closest to the point of association experienced larger shifts than those further away. Furthermore, a plot of the observed shift against the reciprocal of the cube of the distance between the metal and the proton ($1/R^3$) was found to be linear. This would indicate that Equation 31 holds, namely that the magnitude of the observed shift is dependent on the reciprocal of the cube of the distance and the contact angle. In the case of a rigid steroid like cholesterol, the interspatial distance may be directly obtained from molecular models with the angular term automatically included. Hinckley next reported the application of $Eu(thd)_3 \cdot 2$ py to the NMR of *d*-camphor.[99] Later tris-(dipivalomethanato)europium(III) itself was used as a shift reagent. Selective shifts were again observed but now were increased by a factor of four at comparable concentrations.[98] The absence of pyridine eliminated ligand competition. It was stated at this time that amines gave the largest shifts, followed by alcohols, ketones, esters, and ethers in decreasing order of strength. However, no spectra of amines have been shown.

The next communication reported the use of the praseodymium analog, $Pr(thd)_3$, as a high field shift reagent.[100] In this case, the spectrum of *n*-pentanol was reported before and after addition of $Pr(thd)_3$. The praseodymium complex in this instance shifted the peaks of *n*-pentanol upfield from TMS. Thus, by having the capability of producing shifts in either direction at will, by use of the Pr or Eu complex, the utility of the method is greatly increased.

Several papers have appeared describing the use of $Eu(thd)_3$ as an aid in structure determination. Complicated cyclic and heterocyclic alcohols have been studied, including *trans*- and *cis*-4-tert-butylcyclohexanols, isoborneol, and borneol.[101,102] Analyses of the NMR spectrum of the steroid androstan-2β-ol and the terpenoid friedelan-3β-ol have been reported. In each of these cases only a hydroxyl group was available to act as an association site. Because of the extreme complexity of the spectra it was necessary to run the solution containing $Eu(thd)_3$ at 220 MHz and to employ spin decoupling. $Eu(DPM)_3$ has also been used as an aid in studying heterocyclic sulfoxides,[103] steroids,[104] dieldrin, endrin (epoxides),[105] and other types of compounds.

Walters has investigated the use of shift reagents in analyzing the NMR spectra of mixed nitrogen- and phosphorus-containing ligands.[106] Figure 19 shows the NMR spectrum of $H_2NCH_2H_4P\phi_2$ in the presence of various amounts of $Eu(thd)_3$. The spectrum of this simple molecule is complicated by the P-H spin coupling and the overlapping lines of the ring proton resonance. Addition of the shift reagent results in great simplification of the spectrum. Figure 20 shows a plot of the change in chemical shift against the concentration of the shift reagent for the various proton types. A series

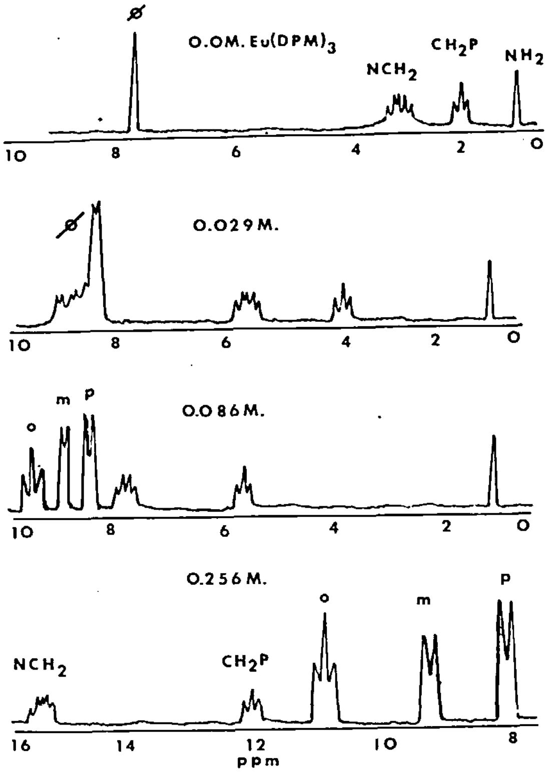


FIGURE 19. 60 MHz spectra of 0.784 $H_2NC_2H_4P\phi_2$ with various amounts of $Eu(DPM)_3$ added.

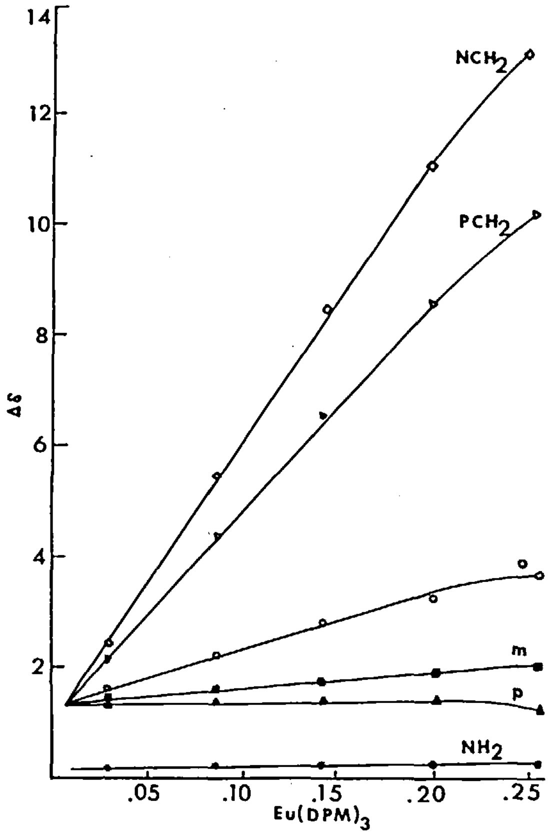


FIGURE 20. Chemical shift change of 0.784 $H_2NC_2H_4P\phi_2$ protons vs. molarity of $Eu(DPM)_3$.

of these ligands, as well as several arsenic-containing ligands, were studied. Although a complete explanation of all the results has not been realized, some trends were established. For example in the N-P and N-As ligands, the interaction with the shift reagent is at the nitrogen atom for tertiary amines, whereas it lies at the phosphorus or arsenic atom in the primary amines. When the substrate and shift reagent are present at equal concentrations, the chemical-shift change is the same no matter what these concentrations are. When N-alkyl groups are present, there is an increase in the line broadening. If the alkyl groups are larger than methyl groups, no interaction was observed.

By and large, the reports published to date on the use of shift reagents are limited studies. Questions about the general usefulness of the reagents and the effects of molecular size and geometry, of the presence of conjugation of heteroatoms, and of multisite coordination, as well as about steric restrictions to coordination have only been touched upon, if discussed at all. So far, studies have been limited to nonpolar solvents because the shift reagents are insoluble in polar solvents. Perhaps one could substitute an ionogenic group on the dipivaloylmethane to render the complex soluble in solvents such as water. The sites of coordination of polyfunctional ligands, such as peptides, could then be studied more conveniently. A study of the effect of steric restrictions might yield methods for discriminating among primary, secondary, and tertiary alcohols and amines by differential shifts of resonances from these substrates. Application of shift reagents to the determination of terminal groups in polymers represents an example of their analytical potential.[107]

SUMMARY

The use of nuclear magnetic resonance for studying the structures, equilibria, and chemical dynamics of metal chelates is considerably more recent than its application to the study of organic compounds, and the early studies were performed by inorganic chemists rather than those whose interests lie in analytical chemistry. These studies were directed toward the geometrical isomers of metal chelates in the newly and rapidly expanding fields of inorganic chemistry. The initial studies of the aminopolycarboxylates opened up new applications of NMR, which provided a better understanding of the chemistry of metal chelates. The old question of the protonation scheme of these compounds was answered in a quantitative way with exceptional experimental simplicity. The fact that NMR provides a probe not only of molecular dimensions, but also into the behavior of individual metal-ligand bonds, provided a method of classification of the various types of metal-ligand bonding. It is important to observe that these studies were performed using aqueous solutions which is the home of metal-chelate chemistry for analytical applications. Earlier studies were in large part performed with aprotic solvents or solvents of low dielectric constant. The presence of the large solvent water peak was avoided by many researchers. However, much important information is available in the spectral features not blocked by the water peak.

The rates and mechanisms of symmetrical ligand-exchange reactions had been of interest for many years. The use of NMR to study these reactions gave the area new life. Compared to other methods for the investigation of fast reactions, NMR is perhaps the most convenient when applicable. Again, these studies were performed using aqueous solutions. The application of Fourier transform methods, which permits studies of more dilute solutions, should encourage further investigations of this type.

There is little doubt that one of the most interesting applications of NMR to the study of metal chelates lies in the conformational analysis of paramagnetic complexes. The use of such data as chemical shift and angular dependence of vicinal coupling in diamagnetic complexes provided considerable early information about the geometries of metal chelates. The ability to detect subtle conformational relationships in the paramagnetic chelates provides significant additional data. However, this technique must be used in conjunction with other methods to realize its maximum power.

The application of NMR to the study of chelates and chelating agents which contain functional groups other than aminocarboxylates, employs techniques similar to those given above. Compounds such as oxinates and β-diketonates have received more limited investigation of their fundamental properties. The geometries of β-diketone complexes have been thoroughly investigated. However, the results of these studies are not easily related to the conditions in solutions, which are of most importance to analytical applications. The studies of the oxinates have been only touched upon.

Although the applications of NMR to the field of metal-chelate chemistry have accelerated rapidly in the past ten years, there are many research opportunities. Many questions may be investigated utilizing simple NMR instrumentation such as the spectrometers frequently designated as instruments designed for teaching purposes. For example, there are questions related to the paramagnetic shift reagents which may be investigated with simple spectrometers. Ligand-exchange studies, spin coupling between the metal atom and protons on the ligands and its relationship to conformation, metal-ligand bond lability of ligands containing ether linkages and sulfhydryl groups, and protonation schemes of ligands are just a few further examples. For those who have more sophisticated instrumentation, the opportunities are even greater. Fourier transform techniques will enable greater precision to be obtained in protonation studies by use of ^{13}C resonance, kinetics may be investigated at lower solute concentrations, and structure and bonding may be studied in more detail by ^{13}C, ^{14}N and other nuclei resonance. It will be of great interest to observe the contributions which NMR makes to the field of metal-chelate chemistry in the next ten years.

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