Conclusion

The very important multispin case cannot be readily solved without resort to numerical methods. However, previous work[2] has shown that the decay in such cases is multiexponential, too complicated for any but qualitative conclusions. In that spirit, the present work makes several suggestions for the interpretation of NOE experiments when the effect of indirect saturation is likely to be important. For steady-state experiments, values should be corrected for saturation using eq 12. Rather than comparing half-lives in transient experiments, it would be better to determine "effective half-lives", the time when the intensity falls to $\cos^2\theta/2$. Even then, one can expect the half-lives of nearby resonances to be shorter than expected from geometry because of a mixing of "T_2 type" processes, as in eq 9. Also, one should watch for, and if necessary correct for, terms oscillating at ω_e.

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Appendix

While the approximate roots of the cubic equation (7) given earlier are remarkably accurate, more accurate formulas may be needed, especially when $R_e > \rho$. The next order of approximation is given below. Defining $r = (R_2 - \rho)/2$ and $E = 8r^3(\cos^2\theta)^2 \sin^2\theta/\omega_e^2$, the more accurate roots are $-R_e'$, $-R_a' \pm i\omega_e'$ with

$$R_e' = R_e - E,\ R_a' = R_a + E/2$$

and

$$\omega_e' = \omega_e[1 - x^2 \sin^2\theta(1 + 3\cos^2\theta)]^{1/2}$$

$x = r/\omega_e$ is the parameter of smallness. In all cases tested, the residual error is an order of magnitude less than the correction.

The constants A, B, and C in eq 13 are given to accuracy $\sim 16x^4$ by

$$\begin{aligned} A &= 1 + 12x^2\cos^2\theta\sin^2\theta \\ B &= (1 - Z)B_0 - WC_0 \\ C &= (1 - Z)C_0 + WB_0 \\ B_0 &= 1 - 12x^2\cos^4\theta \\ C_0 &= x(3\cos^2\theta + 1) - 30(x\cos^2\theta)^3 \\ Z &= R_aR_1/(R_a^2 + \omega_e^2) \\ W &= R_1\omega_e/(R_a^2 + \omega_e^2) \\ R_1 &= \sigma + \rho \end{aligned}$$

Since ω_1 must be large to saturate the irradiated spin and since we have already assumed that Δ of the observed spin is large enough to prevent significant overlap of these resonances, it is unlikely that these corrections will be needed in any case of practical utility. However, the cautious investigator will use them to ensure that the corrections are indeed small.

Equation 12 of this paper is not exact because the high-power limit neglects the "feedback" of magnetization from the observed spin to the irradiated spin. The exact result is obtained if the power factor (the third term of the denominator of eq 12) is divided by

$$1 - (\sigma^2R_2/(\sigma\omega_1^2 + R_2\rho^2))$$

a factor which will be 1 when $\omega_1 \gg \sigma$.

References and Notes

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Aqueous Lanthanide Shift Reagents. 8. Chiral Interactions and Stereochemical Assignments of Chemically and Isotopically Chiral Ligands[1]

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Abstract: The phenomenon of NMR spectral resolution of enantiomeric nuclei of α-hydroxycarboxylates by paramagnetic lanthanide ions in aqueous solution is described in detail and its modes of application are demonstrated. Phenomenological equations are presented describing the spectral resolution of enantiotopic groups, the "self"-resolution of nonracemic mixtures of enantiomers by the mere addition of lanthanide ions, and the resolution of racemic mixtures in the presence of another chiral ligand. Spectral resolution occurs in mixed-ligand complexes of 3:1 ligand/metal stoichiometry but is absent in the 2:1 complexes, indicating that steric crowding around the central lanthanide ion forcing the ligands into stereochemically persistent positions is one of the important structural features of the tris chelates in aqueous solution. Approaches to the configurational assignment of chemically and of isotopically chiral ligands are described. Using these approaches the configurations of (+)-citramalate and of (−)-citramalate are assigned as L and D, respectively, and that of glycolate-*d* made by the action of lactate dehydrogenase on glyoxylate-*d* as L. These assignments are in agreement with determinations by other methods.

Introduction

Enantiomeric nuclei, i.e., nuclei that are interchangeable via reflection in a plane of symmetry, are isochronous in NMR spectra. When enantiomeric molecules, or molecules containing enantiotopic groups, are placed in chiral environments, the enantiomeric nuclei become diastereotopic and in principle anisochronous. The phenomena whereby the enantiotopic carboxyls of citric acid or the methylene hydrogens of ethanol

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become chemically nonequivalent in enzymatic reactions are prominent and well-known examples.[2] The active sites of enzymes provide the chiral environment necessary for the conversion of the enantiotopic groups into diastereotopic and chemically distinct ones. Thus, e.g., it has been shown that the ^{31}P NMR spectrum of adenosine-pentaphosphate-adenosine, originally composed of two bands, is resolved into five resonances upon binding to the enzyme adenylate kinase, for which it is a competitive inhibitor.[3] More recently chiral interactions involving small molecules in aqueous solutions have also been demonstrated. The ^1H NMR spectrum of a racemic mixture of norepinephrine has been resolved into enantiomers upon complexation with the cobaltous chelate of ATP[4] and both enantiomeric mixtures and enantiotopic protons of α-hydroxycarboxylates have been resolved using paramagnetic lanthanide ions.[1] In these cases the chiral environment is provided by chiral ligands, whereas the asymmetric magnetic environment is a result of the anisotropic magnetic susceptibility of the central paramagnetic ion of the complex. These are chiral interactions at what is likely to be the lowest level of molecular organization and complexity. Chiral lanthanide shift reagents that are soluble in *organic* solvents are based on the tris chelates of β-diketonate derivatives of camphor. These reagents can resolve ^1H NMR spectra of racemic mixtures of Lewis bases[5] and in some cases of enantiotopic protons as well.[6]

Apart from the obvious application to the analysis of enantiomeric mixtures, chiral interactions mediated by lanthanide ions in aqueous solution offer a number of possibilities for the stereochemical assignment of chiral ligands.[1] These possibilities arise from the formation of mixed-ligand complexes of higher than 1:1 ligand/metal stoichiometry and from the presence of rapid chemical exchange between complexed and uncomplexed ligands in these systems. This should be contrasted with the slow exchange of the β-diketonate ligand of the tris chelates. Past efforts directed toward the elucidation of the stereochemistry of α-hydroxycarboxylate products of enzymatic reactions are well documented.[2] Usually they involve elaborate stereochemically specific chemical synthesis. The facilities of a nuclear reactor have been used for the configurational assignment of glycolate-*d* made by the action of lactate dehydrogenase on glyoxylate-*d*.[7]

The purposes of the work reported in this article are to examine the nature of the complexes responsible for the lanthanide-mediated chiral interactions in aqueous solution and to demonstrate the modes of their application to the configurational assignment of chemically and of isotopically chiral ligands as well as to the analysis of enantiomeric mixtures.

Experimental Section

Lanthanide chlorides were obtained from Research Chemicals, Phoenix, Ariz. Other chemicals were supplied by Sigma Chemical Co., St. Louis, Mo. Sodium or lithium salts or the free acids were used. The latter were neutralized with NaOD to give solutions with a pH value well above the pK of the acid. Stock solutions, 0.2 M in concentration, were made up in D_2O containing 0.025% v/v *tert*-butyl alcohol, the methyl resonance of which served as an internal reference for the chemical shifts. A sample of lithium glycolate-*d* was kindly provided by the authors of ref 7. Its ^1H NMR spectrum exhibited a triplet with an H–D coupling constant of 2.55 ± 0.05 Hz and a ca. 7% impurity of natural glycolate shifted 0.02 ppm downfield from the central component of the triplet.

Most of the experiments were done at 60 MHz on the Varian T-60 spectrometer. The experiments with glycolate were done at 100 MHz on a Varian XL-100 spectrometer equipped with the Nicolet Fourier transform accessories.

Results

General Considerations. The preliminary work has shown that for the NMR manifestation of lanthanide-mediated chiral interactions in aqueous solution the formation of complexes of 2:1 or higher ligand/metal stoichiometry and bidentate coordination are prerequisites. Absence of axial symmetry of the magnetic susceptibility tensor of the central ion has also been suggested.[1] It is obvious that the attachment of a ligand containing a prochiral center and enantiotopic groups to a chiral complex, i.e., a complex that already contains a chiral ligand, will transform these groups into diastereotopic ones. In the case of carboxylate ligands, rotation about the OOC–C_α bond must be restricted in order to eliminate the possibility of averaging out the chemical-shift difference between the two groups. Thus, attempts to observe spectral resolution by lanthanide-mediated chiral interactions have been successful so far only with ligands containing the bidentate α-hydroxycarboxylate moiety. This type of ligand is the only one considered in this article. However, the same principles should apply to other systems that may exhibit similar phenomena.

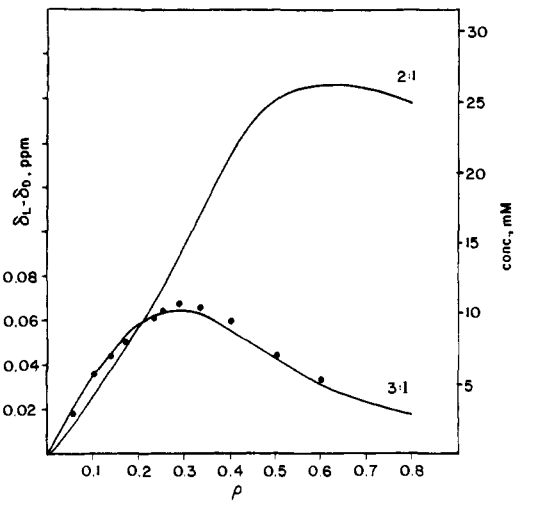


Figure 1. The chemical-shift difference (points, left scale) between the methyl resonances of the lactate enantiomers in a solution containing 80 mM L-lactate and 20 mM D-lactate as a function of the $EuCl_3$/lactate ratio. The solid curves (right scale) describing the concentrations of the 2:1 and 3:1 ligand/metal complexes in this system were calculated with the formation constants of ref 9.

Questions regarding the nature of the complex species that lead to NMR spectral resolution of enantiomeric nuclei and the description of the phenomenon were addressed by investigating the following three systems: (a) nonracemic mixtures of enantiomers, e.g., L- and D-lactate, the resonances of which are resolvable by the mere addition of paramagnetic lanthanide ions; (b) α-hydroxyisobutyrate, the enantiotopic methyl protons of which are resolvable in the presence of chiral ligands of similar chemical nature, e.g., L-lactate; (c) racemic mixtures, the resonances of which are resolvable in the presence of another chiral ligand.

Stoichiometry. The ligands under consideration are known to form complexes of as high a ligand/metal stoichiometry as 4:1.[8] Thus a question arises regarding the stoichiometry at which NMR spectral resolution occurs. In the first series of experiments the spectral resolution of a nonracemic mixture of lactate containing 100 mM total ligand of a L/D ratio of 4.0 was monitored as a function of $EuCl_3$ concentration. In a related series, the resolution of the methyl resonance of α-hydroxyisobutyrate in the presence of a molar excess of L-lactate was measured. In both cases maximum resolution was observed when the Eu/ligand ratio, ρ, was 0.3. The results of the former experiment are plotted as a function of ρ in Figure 1. Also shown in Figure 1 are the concentrations of the 2:1 and 3:1 complexes in this system as calculated with the formation constants determined by Choppin and Chopoorian.[9] A very good agreement with the 3:1 complex is indicated. On the other

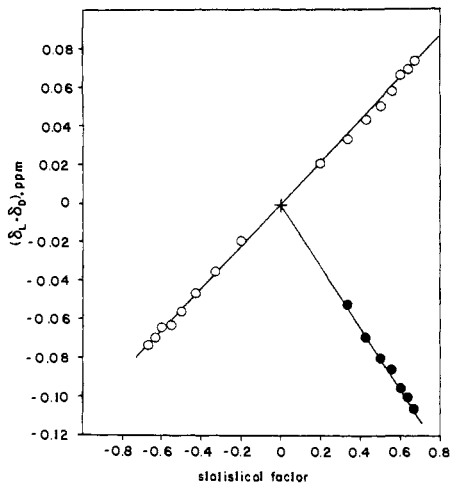


Figure 2. The chemical-shift difference between the methyl resonances of the lactate enantiomers in nonracemic mixtures containing 100 mM total ligand and 25 mM $EuCl_3$ (open circles) or 30 mM $PrCl_3$ (filled circles) as a function of the statistical factor ($f_{L3} - f_{D3}$) (see text and eq 4).

hand, the 2:1 complex exhibits a maximum at $\rho = 0.6$, much higher than the observed maximum in spectral resolution. It should be pointed out that the positions of these maxima are not too sensitive to small variations in the formation constants or to pH variations in the range 4.2–6.3. These results clearly indicate that spectral resolution due to lanthanide-mediated chiral interactions occurs at the level of 3:1 ligand/metal complexes.

Phenomenological Equations. In describing the phenomena of spectral resolution due to lanthanide-mediated chiral interactions only the contributions of 3:1 complexes to the chemical shifts will be considered, since it has already been established that these are the only complexes responsible for the phenomena. In deriving the equations rapid ligand exchange relative to the chemical-shift difference, Δ, between complexed and free ligand is assumed and all shifts are referred to the uncomplexed state of the ligand.

A. Resolution of Enantiomeric Mixtures. The phenomenon of "self"-resolution of the spectra of enantiomeric mixtures by the mere addition of lanthanide ions is observed only with nonracemic mixtures and is due to a chemical-shift difference between the homoliganded ML_3 complex (or its enantiomer MD_3) and the mixed MD_2L complex, where the chirality of the ligands is indicated. The contribution of the 3:1 complexes to the chemical-shift differences is given by

$$\delta_L = 3ML_3\Delta_3/L_t + 2MDL_2\Delta_2/L_t + MD_2L\Delta_1/L_t \quad (1)$$

$$\delta_D = 3MD_3\Delta_3/D_t + 2MLD_2\Delta_2/D_t + ML_2D\Delta_1/D_t \quad (2)$$

where the subscript t indicates total concentration and the square brackets denoting concentrations are omitted for convenience. In the case of no enantiomeric preference in the formation of the mixed MDL_2 and MD_2L complexes, one can calculate their relative abundances on statistical grounds taking into account the L/D ratio, whereas the total concentration of the 3:1 complexes will depend on the complex formation constants and the total metal and ligand concentrations. For the treatment of such a case it is convenient to introduce the fraction of an enantiomer in a given complex out of the total present in 3:1 complexes:

$$f_{L3} = 3ML_3/\sum_3L, f_{L2} = 2MDL_2/\sum_3L, f_L = MD_2L/\sum_3L \quad (3)$$

where $\sum_3L = 3ML_3 + 2MDL_2 + MD_2L$. Similar expressions will describe the fractions f_{D3}, f_{D2}, and f_D with $\sum_3D = 3MD_3 + 2MLD_2 + ML_2D$. The assumption of no enantiomeric preference in complex formation implies that $\sum_3L/L_t = \sum_3D/D_t = F$. Also $f_{L3} + f_{L2} + f_L = f_{D3} + f_{D2} + f_D = 1$. With these equalities in mind and substituting eq 3 and the analogous expressions for the fractions of the D enantiomer into eq 1 and 2 and taking the difference of the latter one obtains the following expression for the spectral resolution:

$$\delta_L - \delta_D = F(f_{L3} - f_{D3})(\Delta_3 - \Delta_1) \quad (4)$$

where account was taken of the fact that $f_D - f_L = f_{L3} - f_{D3}$. For L/D ratios of 2, 3, 4, and 5 the function ($f_{L3} - f_{D3}$) has values of 0.333, 0.500, 0.600, and 0.667, respectively. The same values but with negative sign are obtained for L/D ratios of 0.5, 0.333, 0.25, and 0.20, respectively.

The resolution of the methyl resonances of lactate in the presence of $EuCl_3$ at $\rho = 0.25$ was measured as a function of the L/D ratio in the range of 0.2–5.0. The results are plotted agatnst the statistical function ($f_{L3} - f_{D3}$) in Figure 2. A good linear relationship with a zero intercept is obtained, showing that the assumptions made in deriving eq 4 are fulfilled in this system. The slope of the line is 0.108 ppm. The fraction F as estimated from published formation constants is 0.1. Thus, from eq 4, one calculates $\Delta_3 - \Delta_1 = 1.08$ ppm. A similarly good linear relationship is observed with the results obtained when $PrCl_3$ was applied at $\rho = 0.3$ to nonracemic lactate (see Figure 2). Thus, eq 4 is a good description of the phenomenon of NMR spectral resolution of nonracemic enantiomeric mixtures by paramagnetic lanthanide ions in aqueous solution.

B. Resolution of Enantiotopic Nuclei. When a prochiral ligand, X, forms a mixed lanthanide complex with a chiral ligand, C, the enantiotopic groups on the former become diastereotopic and spectral resolution ensues.[1] The resolution is due to the chemical-shift differences between the R and S groups in the mixed 3:1 complexes MC_2X and MCX_2:

$$\delta_S - \delta_R = 2MCX_2(\Delta_2^S - \Delta_2^R)/X_t + MC_2X(\Delta_1^S - \Delta_1^R)/X_t \quad (5)$$

The relative contribution of the two terms in eq 5 may be estimated from the statistical distribution of the respective complexes, provided that there is no chemical preference in the complexation of C and X. Introducing the fractions

$$f_1 = MC_2X/\sum_3X \text{ and } f_2 = 2MCX_2/\sum_3X$$

where $\sum_3X = 3MX_3 + 2MCX_2 + MC_2X$, one obtains

$$\delta_S - \delta_R = [f_1(\Delta_1^S - \Delta_1^R) + f_2 \times (\Delta_2^S - \Delta_2^R)]\sum_3X/X_t \quad (6)$$

The value of f_1 increases with increasing the C/X ratio. Thus, for C/X ratios of 1, 2, 3, 4, and 5 the values of f_1 are 0.25, 0.444, 0.563, 0.64, and 0.694, respectively, whereas f_2 decreases with values of 0.5, 0.44, 0.375, 0.32, and 0.278, respectively. The spectral resolution of the enantiotopic methyl groups of α-hydroxyisobutyrate by $EuCl_3$ at $\rho = 0.25$ or $PrCl_3$ at $\rho = 0.3$ in the presence of the chiral ligands L-lactate or D-mandelate was measured at different C/X ratios in the range 1.0–5.0. In all the cases the resolution increased with increasing the C/X ratio. A linear dependence with a zero intercept is obtained only when the resolution is plotted against the combination of statistical factors $f_1 + 0.5f_2$. These plots are shown in Figure 3. The predominance of the contribution of the MC_2X complex is likely to arise at the chemical-shift level, rather than being due to difference in complex formation, since literature data indicate that α-hydroxyisobutyrate forms stronger complexes than lactate. Thus, it seems that the relationship $(\Delta_2^S - \Delta_2^R) = 0.5(\Delta_1^S - \Delta_1^R)$ may be a general feature in this system. For such a case eq 6 takes the form

$$\delta_S - \delta_R = (f_1 + 0.5f_2)(\Delta_1^S - \Delta_1^R)\sum_3X/X_t \quad (7)$$

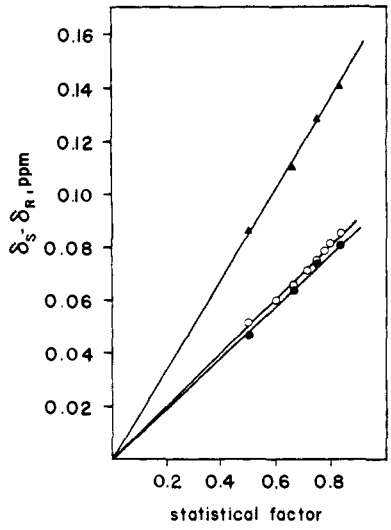


Figure 3. The spectral resolution of the methyl protons of α-hydroxyisobutyrate induced by 25 mM $EuCl_3$ (open symbols) or 30 mM $PrCl_3$ (filled symbols) in the presence of L-lactate (circles) or D-mandelate (triangles) as a function of the statistical factor ($f_1 + 0.5f_2$) (see text and eq 7). The total ligand concentration was kept constant at 100 mM while the chiral/prochiral ratio was varied from 1 to 5.

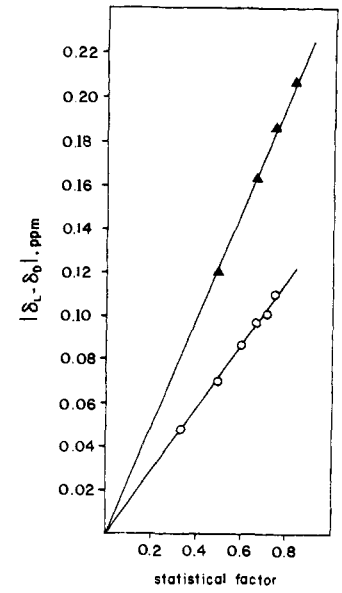


Figure 4. The spectral resolution of the methyl resonances of racemic lactate induced by D-mandelate and 30 mM $PrCl_3$ (filled triangles) and of the α protons of racemic mandelate induced by L-lactate and 20 mM $EuCl_3$ (open circles) as a function of the statistical factor ($f_1 + f_2$) (see text and eq 12). The total ligand concentration was kept constant at 100 mM while the mandelate/lactate ratio was varied from 1 to 5 and from 2 to $\frac{1}{3}$, respectively.

C. Resolution of Racemate. The ^1H NMR spectrum of a racemate can be resolved in the presence of paramagnetic lanthanide ions by the addition of a molar excess of the pure enantiomer of another chiral α-hydroxycarboxylate. Here only the shift contributions due to mixed ligand complexes containing the pure enantiomer of C need to be considered, since for a racemate those due to ML_3, MD_3, MDL_2, and MD_2L will cancel out in the difference $\delta_L - \delta_D$. For a racemate of total concentration X_t one has $L_t = D_t = X_t/2$ and the chemical-shift contributions of interest are

$$\delta_L = (2MCL_2\Delta_{2L}{}^C + MC_2L\Delta_L{}^C + MCDL\Delta_L{}^{CD})/(X_t/2) \quad (8)$$

$$\delta_D = (2MCD_2\Delta_{2D}{}^C + MC_2D\Delta_D{}^C + MCLD\Delta_D{}^{CL})/(X_t/2) \quad (9)$$

Taking into account the equality of the respective concentration factors in eq 8 and 9, the chemical-shift difference is

$$\delta_L - \delta_D = [2MCL_2(\Delta_{2L}{}^C - \Delta_{2D}{}^C) + MC_2L(\Delta_L{}^C - \Delta_D{}^C) + MCDL(\Delta_L{}^{CD} - \Delta_D{}^{CL})]/(X_t/2) \quad (10)$$

It is convenient to introduce the fractions

$$f_1 = MC_2L/\sum_3L, \quad f_2 = 2MCL_2/\sum_3L, \text{ and } f' = MCDL/\sum_3L$$

where $\sum_3L = 3ML_3 + 2MCL_2 + 2MDL_2 + MC_2L + MCDL$. These fractions may be estimated from the statistical distribution of the respective complexes, provided that there is no chemical preference in complex formation. Such estimates show that $f' = f_2$. Under these conditions the spectral resolution takes the form

$$\delta_L - \delta_D = [f_1(\Delta_L{}^C - \Delta_D{}^C) + f_2(\Delta_{2L}{}^C + \Delta_L{}^{CD} - \Delta_{2D}{}^D - \Delta_D{}^{CL})]\sum_3L/(X_t/2) \quad (11)$$

For C/X ratios of 1, 2, 3, 4, and 5 the value of f_1 increases in the sequence 0.25, 0.444, 0.563, 0.64, and 0.694, whereas that of f_2 decreases, its respective values being 0.25, 0.222, 0.188, 0.16, and 0.139.

The spectral resolution of the methyl resonances of racemic lactate by $PrCl_3$ at $\rho = 0.3$ in the presence of D-mandelate was measured at different C/X ratios in the range 1.0–5.0. Similarly the resolution of the α proton of racemic madelate by $EuCl_3$ at $\rho = 0.2$ in the presence of L-lactate was measured at C/X ratios in the range 0.5–3.0. Linear plots with zero intercept are obtained only when the spectral resolution is plotted against the combination of statistical factors $f_1 + f_2$, as shown on Figure 4. This finding indicates that in these systems the equality $(\Delta_L{}^C - \Delta_D{}^C) = (\Delta_{2L}{}^C + \Delta_L{}^{CD} - \Delta_{2D}{}^D - \Delta_D{}^{CL})$ holds. With it eq 11 takes the form

$$\delta_L - \delta_D = (f_1 + f_2)(\Delta_L{}^C - \Delta_D{}^C)\sum_3L/(X_t/2) \quad (12)$$

Noteworthy are certain similarities in the three types of spectral resolution as described by eq 4, 7, and 12. All of them contain a statistical factor and a chemical-shift difference. In the case of "self"-resolution of a nonracemic mixture this is the chemical-shift difference of the L enantiomer between the ML_3 and MD_2L complexes. For the resolution of enantiotopic groups it is the chemical-shift difference between the *S* and *R* positions in the MC_2X complex. Similarly the resolution of racemic mixtures in the presence of another chiral ligand contains the chemical-shift difference between the enantiomers in the MC_2L and MC_2D complexes.

Analysis of Enantiomeric Mixtures. The resolution of ^1H NMR spectra of nonracemic mixtures by paramagnetic lanthanides, which is governed by eq 4, permits the application of the phenomenon to the analysis of such mixtures. Note, however, that identical spectral traces will be obtained for two mixtures having the same ratio of enantiomers irrespective of the identity of the predominant enantiomer. The symmetry with respect to the origin of the line in Figure 2 is due to the sign reversal of the statistical factor ($f_{L3} - f_{D3}$) in eq 4: it is positive when the L enantiomer is in excess but negative when the other enantiomer is more abundant. A simple way of overcoming this degeneracy would be the addition to the mixture under investigation of a known amount of one of the pure enantiomers. An alternative approach is to use a pure enantiomer of another substance for which the sense of resolution, i.e., the sign of $(\Delta_L{}^C - \Delta_D{}^C)$ in eq 12, has been predetermined. By this approach racemic or nearly racemic mixtures can also be analyzed. This latter mode of application is analogous to some extent to the use of the chiral tris-β-diketonates that are soluble in organic solvents. Here, however, the chiral

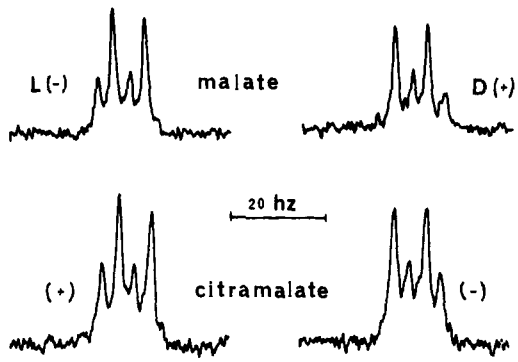


Figure 5. The methyl proton resonances (at 60 MHz) of enantiomeric mixtures (L/D = 2) of lactate (50 mM) in solutions containing 100 mM of a malate or a citramalate enantiomer and 20 or 30 mM, respectively, of $PrCl_3$.

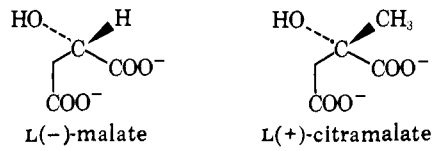
shift reagent is formed in situ rather than being synthesized separately.

Configuration of Chemically Chiral Ligands. The configuration of chiral ligands can be determined by "fingerprinting" the sense of resolution they induce in the spectra of nonracemic mixtures of other α-hydroxycarboxylates and comparing that to the effect of a homologous ligand of known configuration. This approach is based on eq 12. The sign of the chemical-shift difference $(\Delta_L{}^C - \Delta_D{}^C)$ will depend on the configuration of the ligand C, which is responsible for the spectral resolution. Denoting the configuration of the latter by the superscripts *l* and *d* and considering the complexes responsible for spectral resolution it becomes clear that $MC_2{}^dL$ is the enantiomer of $MC_2{}^lD$ and $MC_2{}^dD$ is the enantiomer of $MC_2{}^lL$. Therefore,

$$(\Delta_L{}^{C^l} - \Delta_D{}^{C^l}) = -(\Delta_L{}^{C^d} - \Delta_D{}^{C^d})$$

and the sign of $(\delta_L - \delta_D)$, i.e., the sense of resolution, when C is in the L configuration will be opposite to that with C in the D configuration. Thus the sense of resolution is an indicator of the configuration of the ligand C. These effects are depicted in Figure 5. The application of a molar excess of D-malate in the presence of $PrCl_3$ to a mixture of lactate enantiomers resolves the methyl resonances of the latter with the L enantiomer being shifted further downfield. The sense of resolution is reversed when a similar experiment is carried out with L-malate. Thus, the sense of resolution is a "fingerprint" of the configuration of the malate ligand and may be used for the configurational assignment of ligands homologous to malate.

Citramalic acid is homologous to malic acid: it has a methyl group instead of a hydrogen atom on the α carbon. The citramalate enantiomers are products of enzymatic reactions and are usually labeled according to the sense of optical rotation. The results of applying a molar excess of each of the citramalate enantiomers to enantiomeric mixtures of lactate in the presence of $PrCl_3$ are shown in Figure 5. It is seen that the effect of (+)-citramalate is the same as that of L-malate, while the effect of (−)-citramalate corresponds to that of D-malate. This establishes the configuration of the enantiomers as L(+)-citramalate and D(−)-citramalate.



L(−)-malate L(+)-citramalate

Configuration of Isotopically Chiral Ligands. Stereochemically specific deuterium labeling of glycolate has been achieved by using glyoxylate-*d* as a substrate for lactate dehydrogenase.[7] The configuration of the isotopically chiral glycolate-*d* thus obtained can be determined using lanthanide-mediated chiral interactions, provided that the sign of the chemical-shift factor $(\Delta_1{}^S - \Delta_1{}^R)$ in eq 7 is the same as that of the corresponding factor $(\Delta_L{}^C - \Delta_D{}^C)$ in eq 12 when the ligand C used to obtain the spectral resolution in both cases is of a given, e.g., L, configuration and the observed nuclei are in analogous chemical positions in the molecule, i.e., α protons in this particular case. That this should be so is suggested by the similarity between the complexes responsible for chiral resolution in the two cases: MC_2X in the former and MC_2L and MC_2D in the latter. The phenomenon of spectral resolution of enantiotopic nuclei necessitates a given and well-defined steric arrangement of the liganding atoms around the central ion. This arrangement is likely to be independent of the side chains on the ligands and thus be the same for any α-hydroxycarboxylate. Indeed it was found, e.g., that in the presence of $PrCl_3$ the resolution of the α protons of a number of α-hydroxycarboxylates by L-lactate is the same: the D enantiomer is always shifted further downfield. The application of a molar excess of L-lactate to glycolate in the presence of $PrCl_3$ results in the resolution of the glycolate protons into an AB quartet with $J_{HH} = 16.5 \pm 0.1$ Hz (see Figure 6). The downfield components can be assigned to the *S* hydrogen, since the hydrogen of a D enantiomer is in the *S* position, and the upfield components to the *R* hydrogen. When a similar experiment is carried out with an equimolar mixture of glycolate-*d* and natural glycolate, the resonance of glycolate-*d* appears along with the upfield components of glycolate (see Figure 6), i.e., the hydrogen of this glycolate-*d* is in the *R* position and the deuterium in the *S* position. As expected the trend is reversed when D-lactate is used to achieve spectral resolution. The asymmetry in the effects of L- and D-lactate seen in the spectra in Figure 6 is due to an isotope effect on the chemical shift, the resonance of glycolate-*d* being shifted 0.02 ppm upfield from that of the natural glycolate. Thus, this enzymatically made glycolate-*d* may be labeled as L-glycolate-*d*.

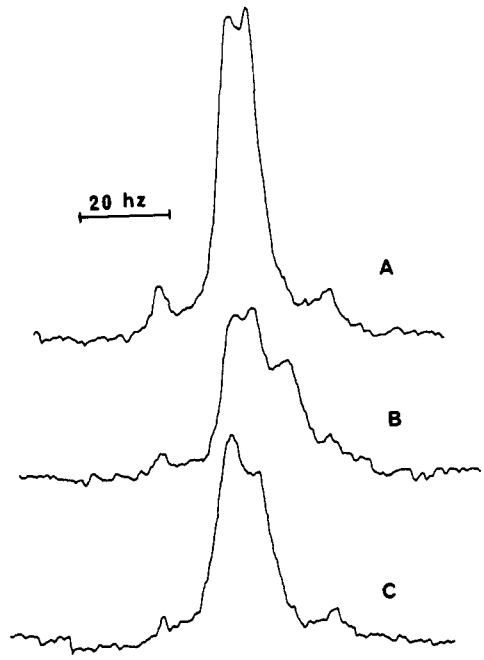


Figure 6. The 100-MHz spectra of the glycolate protons in solutions containing 18 mM total glycolate, 90 mM lactate, and 21.6 mM $PrCl_3$: (A) natural glycolate and L-lactate; (B) equimolar mixture of natural glycolate and glycolate-*d* and L-lactate; (C) equimolar mixture of natural glycolate and glycolate-*d* and D-lactate.

Discussion

The phenomenon of NMR spectral resolution of enantiomeric nuclei by lanthanide-mediated chiral interactions is

due to the ability of the lanthanide ions to form complexes of higher than 1:1 ligand/metal stoichiometry. Spectral resolution is observed when mixed-ligand complexes are formed and is a result of the anisotropic magnetic susceptibility of the central ion. The results of this work have shown that chiral resolution occurs in complexes of a 3:1 ligand/metal stoichiometry but is absent in the 2:1 complexes. Obviously the latter do not satisfy some of the conditions that lead to spectral resolution. One of the important requirements seems to be the structural rigidity or integrity of the complex during a period longer than the reciprocal of the lanthanide-induced chemical-shift difference between the enantiomeric nuclei, i.e., on a time scale of the order of 10^{-3} s. Such a rigidity will prevent the possible averaging out of these differences. The mean lifetimes of the complexes are, however, much shorter as can be inferred from the fact that the total lanthanide-induced shift in the ligand is at least an order of magnitude larger than the resolution. Thus, the requirement for spectral resolution is the formation of complexes of a given and persistent stereochemical arrangement of the ligands around the central ion. This requirement is more likely to be met when there is substantial crowding of liganding atoms around the central ion, thus preferring the 3:1 over the 2:1 complexes. Although the lanthanide ions have relatively high coordination numbers (up to 12), which would tend to confer structural flexibility on the coordination sphere, crystallographic studies have shown that there are great similarities in the coordination polyhedra of complexes with different ligands.[10] In the anhydrous tris glycolate complex of europium one oxygen atom of each carboxylate occupies a corner of a distorted trigonal prism, while the hydroxyl oxygens are at equatorial positions on the faces of the prism.[11] While such an arrangement need not be preserved in aqueous solution, it does indicate the type of steric crowding present in 3:1 complexes that is likely to force the ligands into stereochemically persistent positions. The NMR phenomena described in this work strongly suggest that this is one of the structural characteristics of the tris-α-hydroxycarboxylate complexes of the lanthanides in aqueous solution.

The conditions under which the phenomenon of NMR spectral resolution of enantiomeric nuclei can be applied to the analysis of enantiomeric mixtures and to the configurational assignment of chemically and of isotopically chiral ligands have been established. The experimental requirements are indeed modest and engagingly simple. Thus, e.g., only 5 mg of glycolate-*d* was used in preparing the samples the spectra of which are shown in Figure 6 and, except for the latter spectra, the simplest commercial spectrometer was sufficient. It should be emphasized that the facility of application of these approaches stems, inter alia, from the fact that in these systems the ligand exchange rate is fast relative to the lanthanide-induced chemical shifts. As a corollary to the applications, the phenomenon of spectral resolution by lanthanide-mediated chiral interactions offers the unique possibility of direct observation of only one type of complex species, the tris chelates, in an equilibrium mixture containing at least three types of complexes differing in their ligand/metal stoichiometries.

The configurational assignments of the citramalate enantiomers are in agreement with a determination based on analogy with mevalolactone.[12] and with the results of CD studies.[13] The configuration of glycolate-*d* is in accord with the intuitive expectation (the normal substrate of lactate dehydrogenase is L-lactate) and in agreement with the results of neutron diffraction.[7] Thus, the tentative conclusions reached from the preliminary results[1] and the initial assumptions of the present work are now on firm grounds. It should be emphasized that at present our approach to stereochemical assignments is based solely on experimental analogies and is valid only for homologous ligands.

The phenomenological equations describing the three modes of spectral manifestation of lanthanide-mediated chiral interactions, i.e., the "self"-resolution of nonracemic mixtures, the resolution of enantiotopic groups, and the resolution of racemic mixtures in the presence of another chiral ligand, should be applicable to other systems in which similar phenomena might occur and may serve as a guide in the search for such systems.

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References and Notes

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