NMR Chemical Shift Reagents in Structural Determination of Lipid Derivatives: II. Methyl Petroselinate and Methyl Oleate [1]

JOHN P. WINEBURG and **DANIEL SWERN**, Fels Research Institute and Department of Chemistry, Temple University, Philadelphia, Pennsylvania 19122

ABSTRACT

When *tris*(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedionato)europium (III)–$Eu(fod)_3$–forms a complex with a sufficiently basic functional group in a donor molecule, the change in the magnetic environment of protons near the coordination site causes their nuclear magnetic resonance (NMR) signals to shift to different positions. Consequently $Eu(fod)_3$ and other compounds that similarly affect NMR signals have been designated chemical shift reagents (csr). Because of their ability to shift proton signals, csr substantially increase the amount of structural information that can be obtained from NMR spectroscopy, frequently converting complicated splitting patterns into first-order spectra. Some generally useful experimental and interpretive csr techniques are described here using methyl petroselinate and methyl oleate as examples. Csr studies of methyl petroselinate reveal that the position of the double bond is at C-6, and that there is no chain substitution or branching before C-9. Csr studies of methyl oleate reveal that the position of the double bond is at or beyond C-9, and that there is no chain substitution or branching before C-6. Some suggestions are presented for expanding the amount of structural information that can be obtained by csr studies of unsaturated lipid derivatives.

INTRODUCTION

When the nuclear magnetic resonance (NMR) spectrum of a simple molecule is determined, its chemical structure can often be elucidated by first order interpretation of the spectral data. The number of different types of protons in the molecule can be determined by integration of peak areas while information about proton environment can be obtained from the chemical shift, multiplicity and coupling constants of distinguishable peaks.

Unfortunately, lipid derivatives generally afford NMR spectra that preclude a simple first order interpretation. The 100-MHz NMR spectra of methyl petroselinate and methyl oleate in carbon tetrachloride are presented in Figures 1 and 2 respectively. With only a few exceptions, it is obvious that only general structural information can be obtained from these spectra. Thus, the fact that the signals for the α-methylene and terminal methyl protons (protons 2 and 6 respectively) of both methyl petroselinate and methyl oleate are triplets means that there is no substitu-

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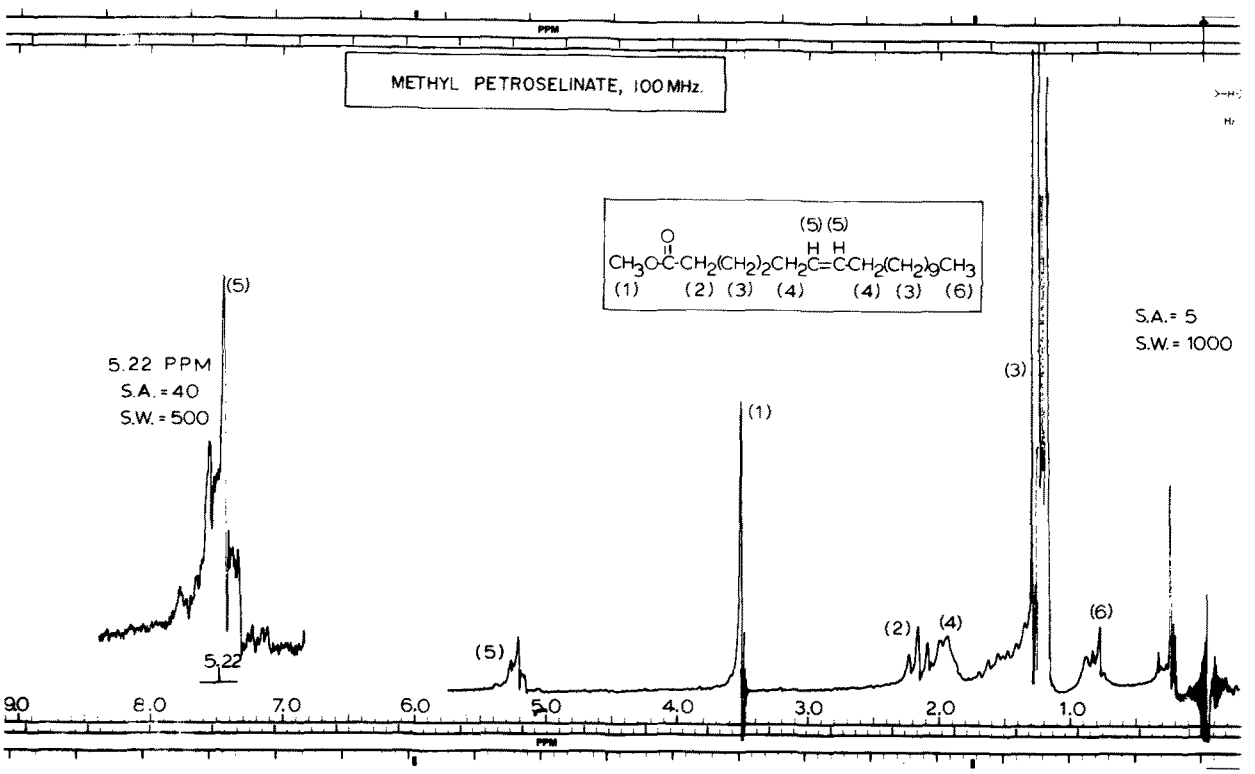

FIG. 1. 100-MHz NMR spectrum of methyl petroselinate in CCl_4.

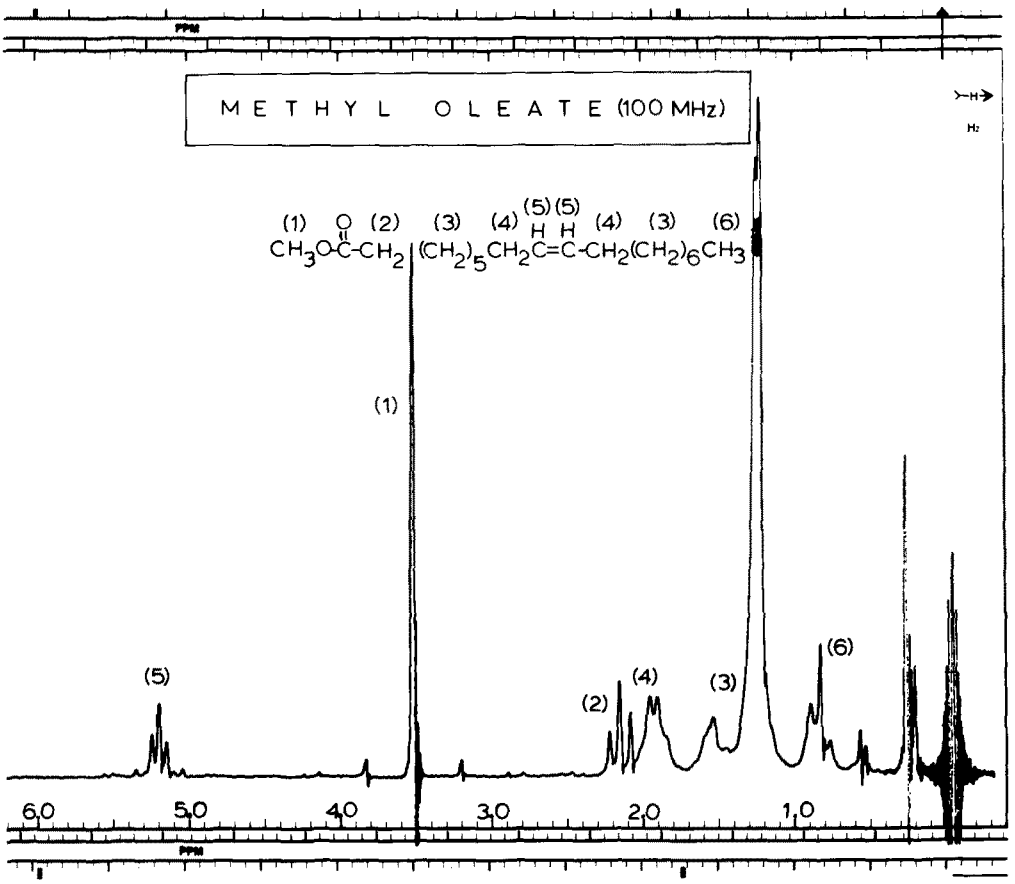

FIG. 2. 100-MHz NMR spectrum of methyl oleate in CCl_4.

tion or chain branching at C-3 or C-17 in either case. With the exception of the singlet for the methoxy protons (protons 1) the multiplicity of other signals is poorly defined due to peak overlap, so no other specific structural information can be obtained. Although olefinic proton signals (protons 5) are present in both spectra the positions of the double bonds are not determinable; moreover, since the majority of the chain methylene protons are magnetically indistinguishable, it is impossible to confirm spectrally the presence or absence of chain substituents or chain branching.

We have previously reported that NMR chemical shift reagents (csr) can augment considerably the amount of structural information obtainable from NMR studies of lipid derivatives (1). More recently we discussed the use of csr to determine the position of double bonds, chain branching and substituents in long chain compounds (2). In this paper, using methyl petroselinate and methyl oleate as examples, we wish to elaborate further on the scope and limitations of using csr in NMR studies of unsaturated fatty esters and to present some new useful experimental and interpretive techniques.

EXPERIMENTAL PROCEDURES

Spectra were determined using a Varian XL-100, 100-MHz NMR spectrometer and 5 mm diam. NMR tubes (Wilmad Royal Imperial NMR tubes, cat. no. 528-PP, Wilmad Glass Co., Inc. Buena, N.J., 08316).

The csr used was tris-(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedionato)europium (III) which has been more conveniently designated $Eu(fod)_3$ (Norell Chemical Co., Landing, N.J., 07850). The reagent was used as received but because it decomposes when exposed to water or acids it was stored in a desiccator over indicating Drierite.

Carbon tetrachloride (Allied Chemicals, Morristown, N.J.) was distilled to remove possible traces of water or acid and stored over molecular sieve Linde 4A.

Methyl petroselinate and methyl oleate were obtained from Applied Science Laboratories, State College, Pa. 16801 (purity >99%).

Incremental Addition Studies

An NMR tube was tared on a Mettler balance and 20-30 mg (± 0.1 mg) of ester (methyl petroselinate or methyl oleate) were placed in the tube. Carbon tetrachloride (0.5 ml) was then added and the tube was capped and shaken until the ester dissolved. Several drops of tetramethylsilane (TMS) were added to the tube as an internal standard and the 100-MHz NMR spectrum of the ester was recorded.

A 20 mg (± 0.1 mg) increment of $Eu(fod)_3$ was added to the NMR tube containing the ester-CCl_4-TMS solution and another NMR spectrum was recorded. This procedure was repeated until the addition of further 20 mg increments of $Eu(fod)_3$ failed to produce a significant change in the NMR spectrum. $Eu(fod)_3$ has the advantage of being readily soluble in CCl_4 at room temperature and no heating is required to dissolve it.

RESULTS AND DISCUSSION

When a csr such as $Eu(fod)_3$ is used in NMR studies of lipid derivatives both the quantity and quality of spectral data are dramatically increased. Figure 3 shows the 100-MHz NMR spectrum of methyl petroselinate in the presence of $Eu(fod)_3$. A discrete signal, with the expected peak area and first order multiplicity, is observed for the magnetically nonequivalent protons in the molecule as far down the chain as C-8. Figure 4 shows the 100-MHz NMR spectrum of methyl oleate in the presence of $Eu(fod)_3$. A discrete signal, with the expected peak area and first order

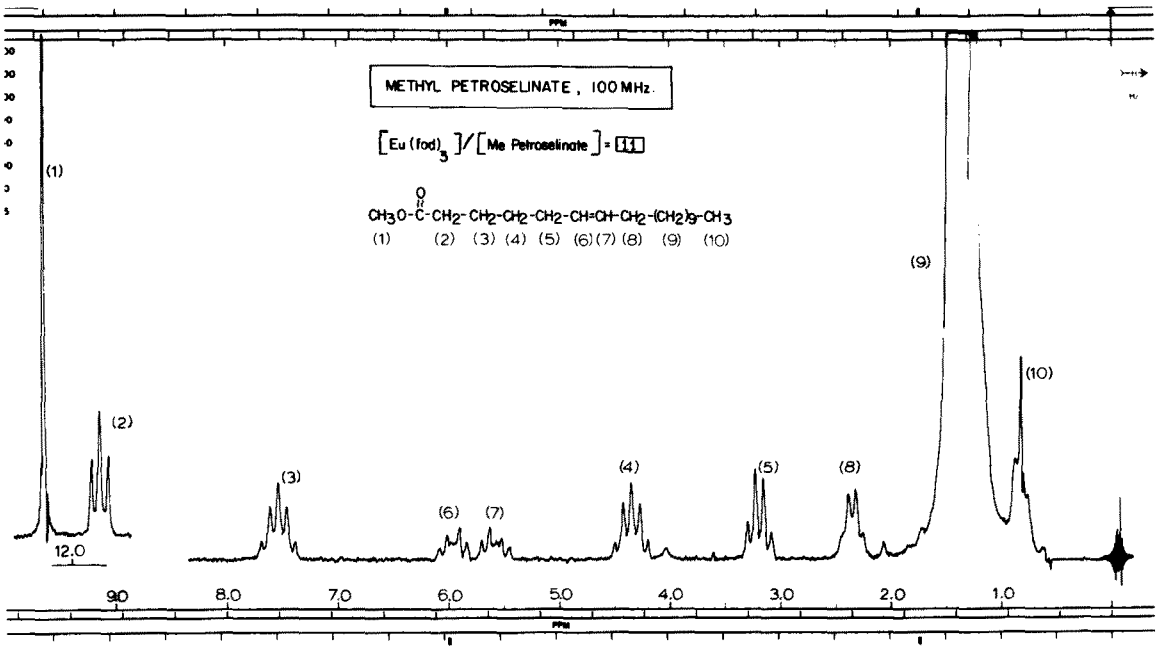

FIG. 3. 100-MHz NMR spectrum of methyl petroselinate in CCl_4. Molar ratio [Eu(fod)$_3$]-[methyl petroselinate] = 1.1.

multiplicity, is observed for the olefinic protons and other magnetically nonequivalent protons as far down the chain as C-5. The interpretation of these spectra (Figs. 3,4) as well as preferred techniques for obtaining structural information from csr studies is discussed below.

Mechanism of CSR Interaction–Induced Shift

The precise mechanism by which csr induce these changes in chemical shift is under continuing debate at the present time (3,4). Fortunately, in most csr applications only a general understanding of the interaction is necessary. If a molecule contains a functional group having sufficient Lewis basicity it can form a complex with csr. The bonding in csr complexes is considered to be mainly, if not exclusively, dipolar (5) and it has been reported to decrease in strength as the Lewis basicity of the functional group decreases: amines>alcohols>ketones≥aldehydes≥ethers> esters>nitriles; halides, indoles, and double bonds are inactive (6). Thiols, thioethers and aryl phosphines form csr complexes but, consistent with differences in Lewis basicity, coordinate much less strongly than do their oxygen and nitrogen analogs (7). Csr induce changes in the NMR chemical shift of proton signals because the magnetic environment of protons in a complexed molecule differs from the magnetic environment of protons in an uncomplexed molecule.

Downfield shifts are usually induced in protons affected by functional group complexation with Eu(fod)$_3$. Induced shift is defined by equation 1:

$$\text{Induced shift} = \delta_{Eu} - \delta_{O} \qquad [1]$$

where δ_{Eu} represents the chemical shift of protons in the presence of Eu(fod)$_3$ and δ_O represents the chemical shift of the same protons in the absence of Eu(fod)$_3$. Induced shift values are meaningless unless they are correlated to a

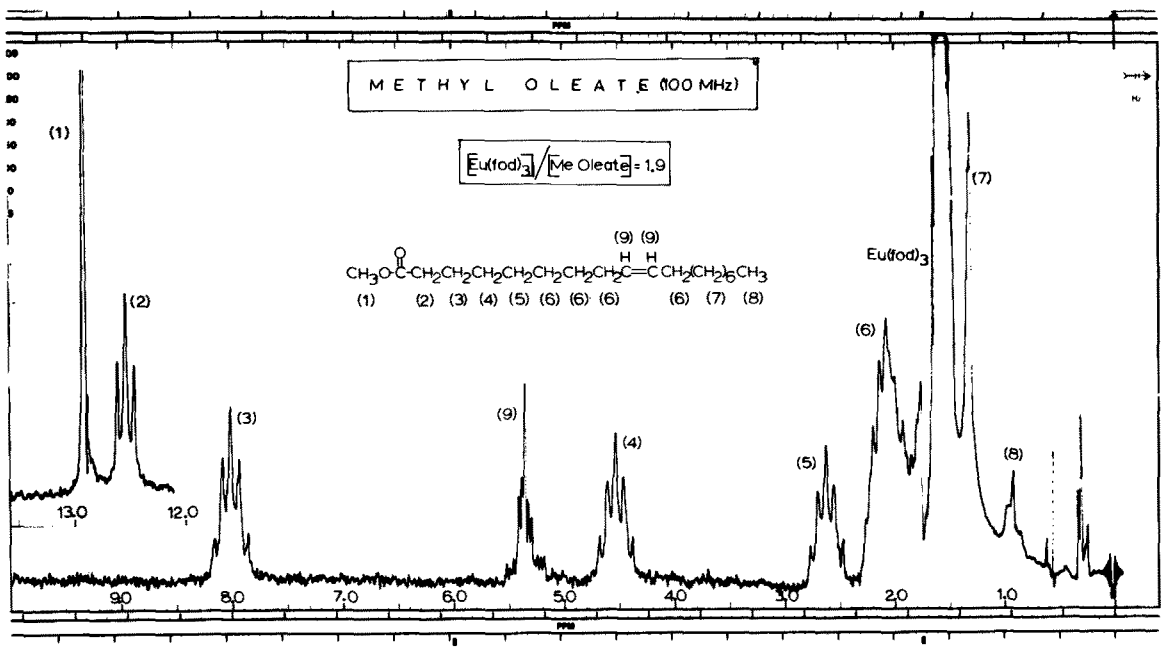

FIG. 4. 100-MHz NMR spectrum of methyl oleate in CCl_4. Molar ratio [Eu(fod)$_3$]-[methyl oleate] = 1.9.

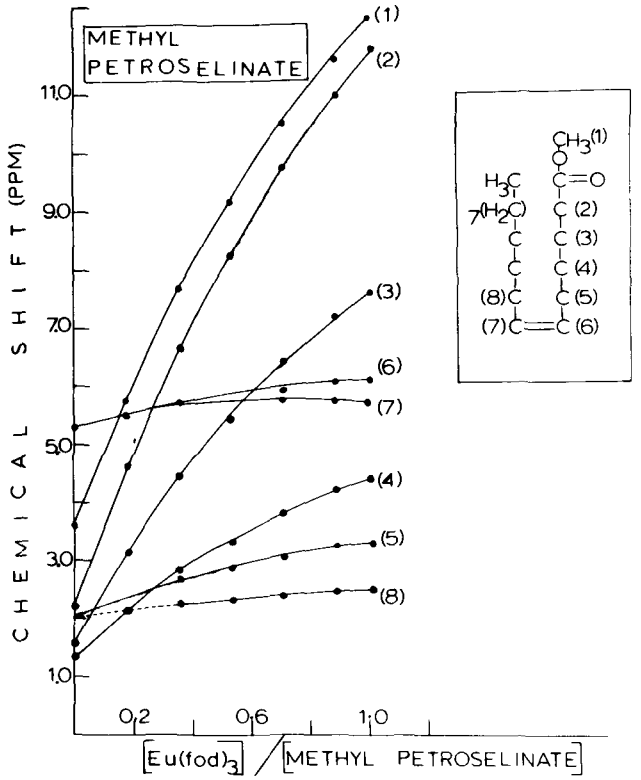

FIG. 5. Chemical shift plots obtained from incremental addition of $Eu(fod)_3$ to methyl petroselinate.

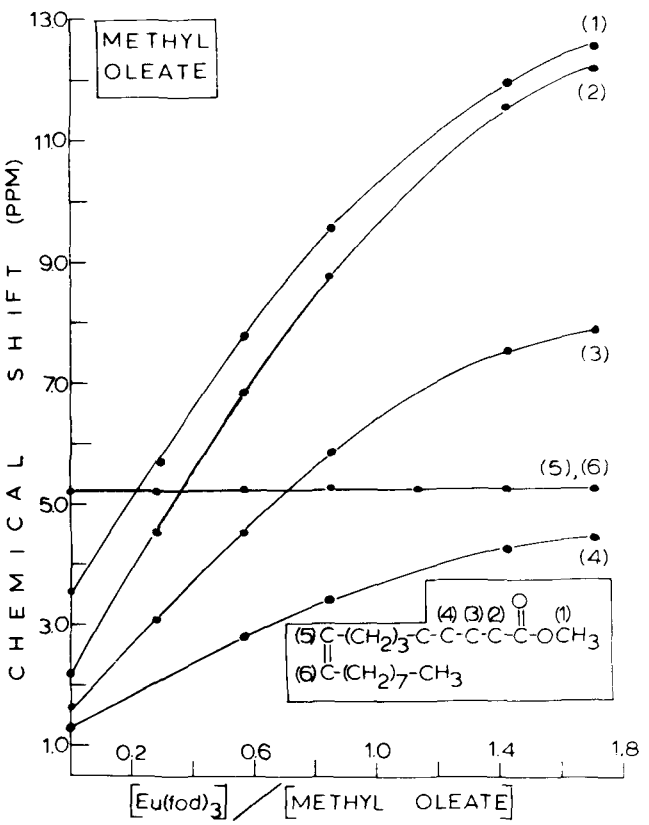

FIG. 6. Chemical shift plots obtained from incremental addition of $Eu(fod)_3$ to methyl oleate.

specific [$Eu(fod)_3$]-[substrate] molar ratio since the magnitude of the induced shift increases with increasing concentration of csr until finally a limiting point is reached beyond which further increases in $Eu(fod)_3$ concentration effect no further changes in the magnitude of the induced shift. This concentration dependence indicates that the following rapid exchange takes place (8):

csr + uncomplexed molecule $\rightleftharpoons$ complexed molecule

The exchange is so rapid that NMR signals for both complexed and uncomplexed species are not observed. The observed proton signals absorb at chemical shift positions that are an average of the theoretical but unobservable chemical shift positions that correspond to complexed and uncomplexed substrate protons.

Attempts have been made, in csr studies of rigid molecules, to correlate mathematically the magnitude of the induced shifts observed for different protons in the complexed substrate (at specific [$Eu(fod)_3$]-[substrate] ratios) to parameters of both internuclear csr-proton distance and csr-proton angle which define the geometry of the complexed molecule (4). However, mathematical correlations are neither applicable nor necessary for compounds with variable geometry such as nonrigid long chain compounds that can assume countless rotameric conformations in solution. In such cases NMR signals can be accurately assigned on the simple basis that protons that undergo the greatest induced shift—that is, those protons that undergo the greatest change in magnetic environment—are the protons that are closest to the coordination site. The magnitude of the induced shifts of protons effected by csr-functional group complexation decreases as their distance from the coordination site increases. When overlapping resonances are separated by csr into discrete peaks, coupling constants and peak multiplicity become visible and define the structural environment of assigned protons; first order analysis of the spectra often becomes possible. For example, from the first order multiplicities observed for the distinguishable proton signals in Figures 3 and 4, it can be determined that there is no chain substituent or branching in methyl petroselinate as far down the chain as C-8, nor is there any chain substituent or branching in methyl oleate as far down the chain as C-5.

The magnitude of the induced shift of specific protons in the complexed substrate (at specific [$Eu(fod)_3$]-substrate] ratios) varies according to the strength of the complex. One of the factors that defines the strength of the complex is the Lewis basicity of the functional group, and accounts in part for the advantage in using $Eu(fod)_3$, a stronger Lewis acid than many other csr. However, the structural geometry of substrate molecules can often sterically hinder complexation so induced shift magnitudes will not necessarily follow the idealized order of csr-functional group complex strengths listed above. Similarly, any other factor that affects the stability of the complexed substrate species, such as changes in solvent, temperature or substrate concentration will affect the magnitude of the induced shift; the effect of changing csr concentration has already been discussed. All these variables make it difficult to obtain reproducible induced shift values.

Incremental CSR Addition—Proton Chemical Shift Plots

Csr spectral interpretations are aided by the fact that the magnitude of the induced shift increases with increasing csr concentration. By using incremental additions of csr (see Experimental Procedures) one obtains a series of NMR spectra corresponding to various [$Eu(fod)_3$]-[substrate] ratios from zero to optimum. Data from this series of spectra are most useful when the chemical shift positions (in ppm) of all the distinguishable, magnetically nonequivalent protons in the molecule are plotted against the corresponding [$Eu(fod)_3$]-[substrate] ratios. Figure 5 shows the proton chemical shift plots constructed from spectral data obtained during incremental addition of $Eu(fod)_3$ to methyl petroselinate. Chemical shift values for [$Eu(fod)_3$]-[methyl petroselinate] = 0 were obtained from

Figure 1; chemical shift values plotted for [Eu(fod)$_3$]-[methyl petroselinate] = 1:1 were obtained from Figure 3. The chemical shift values plotted for other [Eu(fod)$_3$]-[methyl petroselinate] ratios were similarly obtained from NMR spectra at other molar ratios by the incremental csr addition studies. Figure 6 shows the proton chemical shift plots constructed from spectral data obtained during incremental addition of Eu(fod)$_3$ to methyl oleate. Chemical shift values plotted for [Eu(fod)$_3$]-methyl oleate] = 0 were obtained from Figure 2; chemical shift values plotted for [Eu(fod)$_3$]-[methyl oleate] = 1:9 were obtained from Figure 4. The chemical shift values plotted for other [Eu(fod)$_3$]-[methyl oleate] ratios were similarly obtained from NMR spectra at other csr molar ratios.

Since at the optimum [Eu(fod)$_3$]-[methyl oleate] ratio it is possible to obtain discrete proton signals only as far down the chain as C-5, the specific position of the double bond can not be determined. However, by comparison of the proton chemical shift plots in Figures 5 and 6, the position of the double bond in methyl oleate is revealed to be at or beyond C-9. Thus, Figure 5 demonstrates that measurable induced shifts are observed as far down the ester chain as C-8. Since Figure 6 demonstrates that no induced shift is observed for the olefinic protons of methyl oleate, these protons must be beyond C-8 in the chain.

Also, by demonstrating that measurable induced shifts are not observed for olefinic protons in methyl oleate, Figure 6 confirms a previous report that double bonds are inert toward csr complexation (6). The olefinic protons in methyl petroselinate must undergo induced shifts solely because of their proximity to the coordination site.

Plotting the spectral data obtained during incremental addition studies has several other advantages. Otherwise unobtainable δ_O values (no csr present) can be estimated by extrapolation of proton plots to [Eu(fod)$_3$]-[substrate] of 0 (7,9). In fact, δ_O values for methyl petroselinate can probably be determined more accurately from the proton plots of Figure 5 than from the NMR spectrum shown in Figure 1, since the precise chemical shift positions of some of the proton signals in Figure 1 are uncertain due to signal overlap.

NMR signals of magnetically nonequivalent protons often overlap at certain [Eu(fod)$_3$]-[methyl petroselinate] ratios, that is, those ratios at which the proton plots intersect (Fig. 5). If shifted proton signals overlap it is impossible to determine their structural environment because peak multiplicity and coupling constants cannot be obtained. However, if further increments of Eu(fod)$_3$ are added, peak overlap can be eliminated and NMR spectra are often obtained that consist of discrete proton signals unobscured by overlapping peaks.

By plotting the spectral data obtained during incremental Eu(fod)$_3$ additions it is possible to determine additional [Eu(fod)$_3$]-[methyl petroselinate] ratios where proton signals do not overlap, that is, ratios where the proton plots do not intersect.

Proton plots also reveal that shifted signals often "crossover" one another. Figure 5 demonstrates that these crossovers furnish information concerning the relative proximity of various protons to the coordination site. For example, the manner in which the plot of proton 2 crosses over the plot of proton 6 defines proton 2 as being closer to the functional group. This is a trivial illustration of the use of signal crossover phenomena, since protons 2 undergo the greatest induced shift in the presence of csr and on that basis alone can be assigned as the protons closest to the functional group. However, the interpretive importance of the signal crossover technique will be more effectively demonstrated in a subsequent paper in which analyses of more complicated, polyfunctional molecules such as methyl ricinoleate, epoxyoctadecanol and methyl epoxystearate will be discussed.

TABLE I

Shift Gradients (ΔEu Values) and Induced Shift Ratios for Some Magnetically Nonequivalent Protons in Methyl Oleate and Methyl Petroselinate

Part A

[Eu(fod)$_3$][a] / [Methyl oleate]	ΔEu, Chain positions						Induced shift ratios, Chain positions					
	α	β	γ	δ	ϵ	Methoxy	α	β	γ	δ	ϵ	Methoxy
0.29	7.6	5.1	2.8	---	---	8.2	1.0	0.62	0.34	---	---	0.92
0.57	7.5	5.1	2.8	---	---	8.2	1.0	0.62	0.34	---	---	0.92
0.86	7.1	4.9	2.6	---	---	7.7	1.0	0.64	0.33	---	---	0.92
1.42	5.9	4.5	2.1	---	---	6.6	1.0	---	0.33	---	---	0.90
1.91	5.3	4.2	1.9	---	---	5.8	1.0	0.63	0.32	---	---	0.91

Part B

[Eu(fod)$_3$][a] / [Methyl petroselinate]	ΔEu, Chain positions						Induced shift ratios, Chain positions					
	α	β	γ	δ	ϵ	Methoxy	α	β	γ	δ	ϵ	Methoxy
0.18	13.6	8.7	4.5	1.9	1.0	12.4	1.0	0.64	0.33	0.14	0.08	0.92
0.36	12.4	7.9	4.1	1.8	0.8	11.4	1.0	0.64	0.33	0.15	0.08	0.92
0.53	11.3	7.1	3.7	1.6	0.8	10.4	1.0	0.63	0.32	0.13	0.07	0.92
0.71	10.6	6.7	3.4	1.4	0.7	9.7	1.0	0.63	0.32	0.14	0.06	0.92
0.89	9.8	6.3	3.2	1.4	---	8.9	1.0	0.64	0.33	0.14	---	0.91
1.1	9.0	5.6	2.9	1.2	---	8.2	1.0	0.62	0.32	0.13	---	0.91

[a]Molar ratio, CCl_4 solution.

TABLE II

Average Induced Shift Ratios of Methyl Esters and Alcohols

Chain position	Induced shift ratios			
	Methyl esters	Methyl tetradecanedioate[a]	Alkanols[b]	Methyl ricinoleate[c]
Methoxy	0.93[d]	0.92	---	---
α	1.0[d]	1.0	1.0	1.0
β	0.63[d]	0.69	0.60	0.58
γ	0.34[d]	0.35	0.42	0.43
δ	0.14[d]	0.14	0.20	0.17
ϵ	0.07[e]	0.07	0.14	0.09
ζ	---	---	0.08	0.05
η	---	---	0.02	0.03

[a]Induced shift ratios were calculated from the published nuclear magnetic resonance (NMR) spectrum of methyl tetradecanedioate (6).

[b]Literature values (6).

[c]Induced shift ratios are averages of the values calculated during incremental $Eu(fod)_3$ addition studies of methyl ricinoleate; the induced shift ratios recorded are those that were obtained for chain positions C-12 to C-18.

[d]Induced shift ratio is an average of those calculated for methyl oleate, methyl petroselinate, and methyl-12-hydroxystearate.

[e]Induced shift ratio is an average of those calculated for this chain position in methyl petroselinate (see Table I, Part B).

Shift Gradient (ΔEu)

Shift gradient is defined by equation 2:

$$\text{Shift gradient} = \Delta Eu = \frac{\text{induced shift}}{[Eu(fod)_3]/[\text{substrate}]} = \frac{\delta_{Eu} - \delta_o}{[Eu(fod)_3]/[\text{substrate}]} \qquad [2]$$

If Figures 5 and 6 are compared with equation 2 it can be seen that shift gradient is simply another name for the slope of a proton plot. Previous workers have implied that ΔEu values are constant and proton plots are linear and can be used to characterize magnetically nonequivalent protons in complexed substrate molecules (6). However, Figures 5 and 6 demonstrate that the slopes of proton plots vary with changing $[Eu(fod)_3]$-[ester] ratios, except at low $[Eu(fod)_3]$-[ester] ratios. Table I illustrates typical ΔEu variations for some of the magnetically nonequivalent protons of methyl petroselinate. Table II illustrates typical ΔEu variations for some of the magnetically nonequivalent protons of methyl oleate.

Shift gradient values are meaningful at low $[Eu(fod)_3]$-[ester] ratios where proton plots are linear but they are useful only insofar as they are reproducible. The difficulties involved in reproducing induced shift values at specific $[Eu(fod)_3]$-[substrate] ratios have already been discussed. Since equation 2 directly relates ΔEu values and induced shift values it is obvious that only very precise experimental control can lead to meaningful ΔEu values. Because of the limitations just mentioned, the determination of ΔEu values appears to be a questionable technique for making proton assignments in csr studies. However, shift gradient values are a useful way to communicate qualitative approximations of induced shift magnitudes that might be expected for protons near different functional groups and in different structural environments. Characteristic ranges of ΔEu values have been reported in the literature for protons adjacent to a variety of functional groups (6,10).

Sometimes previous workers have obtained ΔEu values by extrapolating induced shift values that were determined at known $[Eu(fod)_3]$-[substrate] ratios to a $[Eu(fod)_3]$-[substrate] ratio of unity, at which point the induced shift and shift gradient are identical. This is simply a graphical alternative to the use of equation 2. However, neither approach is an acceptable way to obtain induced shift values. Equation 2 suggests that it should be possible to use ΔEu values that are experimentally determined at a single $[Eu(fod)_3]$-[substrate] ratio to calculate induced shift values at other $[Eu(fod)_3]$-[substrate] ratios. Unfortunately, ΔEu values are constant only at low $[Eu(fod)_3]$-[substrate] ratios and are reproducible only under carefully controlled experimental conditions. Therefore calculations based on a single experiment are unreliable. Induced shift values can not be extrapolated in the manner just described. They can only be obtained by direct experiment or from proton plots obtained by incremental addition studies.

Induced Shift Ratios (Shift Attenuation Factors)

We prefer to use induced shift ratios or shift attenuation factors, as described shortly, rather than shift gradients. When esters are complexed by $Eu(fod)_3$, the α-methylene protons undergo the greatest induced shift. The ratios obtained by dividing the induced shifts of the other ester protons by the induced shift of the α-methylene protons are called shift attenuation factors or induced shift ratios. For example, the induced shift ratio corresponding to the β-methylene protons in an ester is defined at any specific $[Eu(fod)_3]$-[ester] ratio by equation 3:

$$\text{Induced shift ratio} = \frac{(\delta_{Eu}-\delta_o)_\beta}{(\delta_{Eu}-\delta_o)_\alpha} \qquad [3]$$

Where $(\delta_{Eu}-\delta_o)\beta$ is induced shift of the β-methylene protons and $(\delta_{Eu}-\delta_o)\alpha$ is the induced shift of the α-methylene protons. The fact that experimental variables make absolute *induced shift values* difficult to reproduce becomes unimportant when one deals with *induced shift ratios*. Since variables effect similar changes in the absolute induced shift values of all the protons in a molecule, induced shift ratios are reproducible even though absolute induced shift values are not. Table I shows that reproducible induced shift ratios are obtained for the same magnetically nonequivalent protons in methyl petroselinate and methyl oleate that have variable ΔEu values.

Since ΔEu values are obtained from induced shift values, ΔEu ratios must be identical with induced shift ratios. A comparison of equation 3 and equation 2 shows that this is indeed the case:

$$\text{Induced shift ratio} = \frac{\Delta Eu\,\beta}{\Delta Eu\,\alpha} = \frac{(\delta_{Eu}-\delta_o)_\beta}{(\delta_{Eu}-\delta_o)_\alpha} \qquad [4]$$

Induced shift ratios are far superior to ΔEu values for making proton assignments. Tables I and II demonstrate that, in contrast to ΔEu values, induced shift ratios are

characteristic of chain position. The position of the double bond in methyl petroselinate can thereby be determined as C-6 since the induced shift ratio of the olefinic proton (signal 6 in Fig. 3) matches the induced shift ratio in Table II that is characteristic of chain position C-6.

Induced shift ratios can be used to follow proton shifts in incremental csr addition where peaks overlap. When discrete peaks are observed, induced shift ratios can be correlated with peak multiplicity to determine the position of branching in a chain.

Table II also shows that identical induced shift ratios are not obtained for protons occupying analogous chain positions in both alcohols and esters. Apparently induced shift ratios can be used to identify different functional groups. This point will be more fully discussed in a paper now in preparation.

SCOPE AND LIMITATIONS

Although methyl esters of fatty acids have been used as examples, the same experimental and interpretive techniques can be applied to saturated, unsaturated, chain branched and substituted hydrocarbons, fatty acids, alcohols, amines, and every other class of long chain compounds containing methylene protons that are formally magnetically nonequivalent but which are indistinguishable at 100-MHz.

In csr studies of methyl petroselinate, discrete NMR signals are observed for protons as far down the chain as C-8; discrete signals with methyl oleate are observed as far down the chain as C-5. Obviously csr studies give information only about protons near a coordination site. The amount of information obtainable through csr studies of unsaturated methyl esters should be tremendously increased by chemically introducing a second coordination site into the molecule through derivatization of the double bond. We are currently completing csr studies of methyl-9,10-epoxystearate and methyl-9,10-dihydroxystearate to determine which derivative of methyl oleate affords the greatest amount of structural information.

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