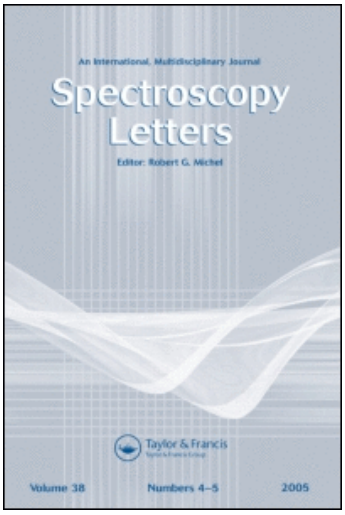

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NMR Studies of Drugs. Applications of Achiral and Chiral Lanthanide Shift Reagents to the Analgesic, Famprofazone: "Anomalous Shifts"

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NMR STUDIES OF DRUGS. APPLICATIONS OF ACHIRAL AND CHIRAL LANTHANIDE SHIFT REAGENTS TO THE ANALGESIC, FAMPROFAZONE: "ANOMALOUS SHIFTS".

Key Words: 4-Isopropyl-2-methyl-3-[N-methyl-N-(α-methylphenylethyl)aminomethyl]-1-phenyl-3-pyrazolin-5-one, 1,2-Dihydro-1-methyl-4-(1-methylethyl)-5-[[methyl(1-methyl-2-phenylethyl)amino]methyl]-2-phenyl-3H-pyrazol-3-one, Europium, LSR, $Eu(FOD)_3$, $Eu(HFC)_3$, Analysis, Enantiomeric excess, Stereoisomer, Methamphetamine, Dynamic NMR.

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ABSTRACT

The 200 MHz ^1H NMR spectra of the analgesic, famprofazone, 1, have been studied in $CDCl_3$ solution at ambient temperatures with the achiral lanthanide shift reagent (LSR) tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium(III), $Eu(FOD)_3$, 2, for spectral simplification, and with the chiral LSR, tris[3-(heptafluoropropylhydroxymethylene)-(+)-

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camphorato]europium(III), $Eu(HFC)_3$, 3. Lanthanide induced shift (LIS) magnitudes suggested predominant LSR binding at the carbonyl oxygen. Substantial enantiomeric shift differences were observed for several nuclei of 1 with added 3 which could permit direct determinations of enantiomeric excess.

INTRODUCTION

Famprofazone, 1, known as 4-isopropyl-2-methyl-3-[N-methyl-N-(α-methylphenylethyl)aminomethyl]-1-phenyl-3-pyrazolin-5-one or 1,2-dihydro-1-methyl-4-(1-methylethyl)-5-[[methyl(1-methyl-2-phenylethyl)amino]methyl]-2-phenyl-3H-pyrazol-3-one, is considered a moderately strong analgesic and antipyretic (1,2). Structurally, 1 can be seen to possess the methamphetamine moiety together with the important five-membered heterocyclic ring structure of the 3-pyrazolin-5-one (referred to as a 1,2-dihydro-3H-pyrazol-3-one in Chemical Abstracts 12th Collective Index 1987-91 Index Guide). This structure was intriguing to us for several reasons. The methamphetamine portion contains a chiral center so that 1 exists as a pair of enantiomers. The broad class of aryl isopropylamines and related amino alcohols has enormous importance as pharmaceuticals.

One important method for direct determination of enantiomeric excess (% e.e.) involves the use of chiral lanthanide shift reagents (LSR). Applications and principles of both chiral and achiral LSR have been reviewed (3-6). The structure of 1 also includes a heterocyclic ring, of interest to us because of our ongoing LSR studies on many heterocyclic systems, especially five- and six-membered ring systems. Several recent examples to the five-membered ring compounds are provided (7-16). Since 1 is a multifunctional compound with more than one potential LSR binding site, we were interested in its NMR behavior with the achiral LSR, tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium(III), $Eu(FOD)_3$, 2, and the chiral tris[3-heptafluoropropylhydroxymethylene)-(+)-camphorato]europium(III), 3, known as $Eu(HFC)_3$ or $Eu(HFBC)_3$.

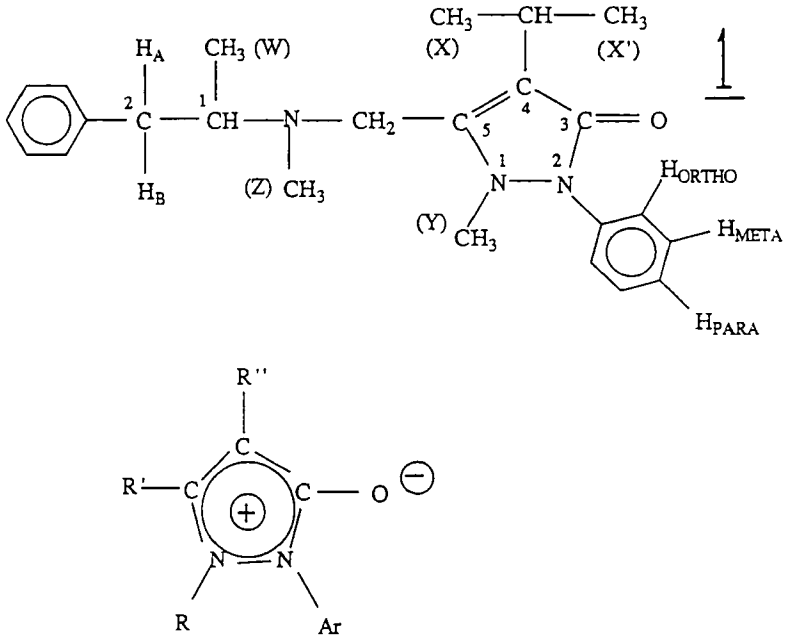

Figure 1. Structure of famprofazone, 1, as canonical forms (resonance structures).

EXPERIMENTAL

Racemic 1 was obtained from Sigma Chemical Co. (St. Louis MO 63178). $CDCl_3$ (99.8 at % D) and shift reagents were obtained from Aldrich Chemical Corp. (Milwaukee WI 53201). The $CDCl_3$ was dried and stored over 3Å molecular sieves. Drug and LSRs were stored in a desiccator over P_2O_5 and were used as supplied. Chemical shifts are reported in δ (ppm) relative to tetramethylsilane (TMS), used as internal reference at 0.00 ppm. In runs with LSR, increments of solid shift reagent were accurately weighed directly into a 5mm thin-walled oven-dried NMR tube containing an aliquot of a standard solution of the drug; drug concentrations were ca. 0.05 M. The LSR was dissolved by shaking and the spectra were then acquired. NMR studies were performed with a Bruker

AC200-F Fourier transform (FT) NMR spectrometer with Aspect 3000 data system operating at a ^1H observe frequency of 200.13 MHz. Spectra were obtained in the FT mode at ambient temperatures using a switchable $^1H/^{13}C$ probe. Chemical shifts were generally obtained from spectrometer printouts or by inspection from spectral expansions for uncalled, severely broadened peaks, and are believed accurate to $\pm$0.02 ppm. Coupling constants and enantiomeric shift differences were calculated by subtraction of values from peak frequency lists and are believed accurate to $\pm$ 0.2 Hz; where possible, multiple values between multiplets were obtained and averaged. Typical FT-NMR parameters were as follows: 4 KHz spectral width (about -4 to +16 ppm) over 64K complex data points collected in the quadrature detection mode for a digital resolution of ca. 0.12 Hz per point, pulse width 3.0 μs, ca. 8s acquisition time, 1s relaxation delay; 32 FIDs were accumulated. No line broadening or resolution enhancement was applied. In runs with chiral LSR where enantiomeric shift differences ($\Delta\Delta\delta$) were observed for selected resonances, reported chemical shifts are the average values for the two enantiomers.

RESULTS AND DISCUSSION

For 0.0583 M racemic 1 at ambient temperature in $CDCl_3$, the 200 MHz ^1H spectrum showed several regions of considerable complexity due to signal overlaps. For some resonances, our assigned chemical shifts must be considered approximate, i.e., $\pm$0.03 ppm, specifically for the isopropyl methine [Me_2CH], the methine at the chiral center [MeCHN], and the lower field benzylic proton [designated H_B]. The reported coupling constants are observed values based on peak frequency separations, assuming a simple first-order analysis. For the incompletely resolved multiplets noted above, the J values may be $\pm$0.3 Hz. For multiplets that were free from overlaps, i.e., the higher field benzylic proton [designated H_A], the diastereotopic isopropyl methyls (X and X') and the methyl at the chiral center [MeCHN], both chemical shifts and reported couplings are probably more

accurate, i.e., ±0.01-.02 ppm for shifts and ±0.1-.2 Hz for J values. Where possible, replicate observed couplings were obtained and averaged for the reported values. Rationales for the assignments will be presented below. Our summary assignments, δ in ppm relative to TMS, are as follows: 7.14-7.47 (10H, complex multiplet, aryl H); 3.51 (2H, s, accidentally equivalent diastereotopic protons of CH_2 on heterocyclic ring); 3.06 (1H, m, incompletely resolved from signals ca. 2.75-2.97 ppm, Me<u>CH</u>N, five(?) lines, see <u>Discussion</u>, average observed ^3J ca. 6.9 Hz); ca. 2.89 (observed ^2J ca. 13.6 Hz, ^3J 7.5 Hz, $C_6H_5C\underline{H_B}$, overlapped with the following multiplet), see <u>Discussion</u>; 2.83 (2H total including $C_6H_5C\underline{H_B}$, $Me_2\underline{CH}$, six (?) lines, average observed ^3J ca. 7.0 Hz); 2.75 (3H, s, MeNN); 2.57 (1H, dd, ^2J = 13.23 Hz, ^3J = 6.93 Hz, $C_6H_5C\underline{H_A}$; 2.31 (3H, s, $MeNR_2$); 1.30 (3H, d, ^3J=6.99 Hz) and 1.29 (3H, d, ^3J = 6.98 Hz) diastereotopic methyls of the isopropyl; 1.05 (3H, d, ^3J = 6.46 Hz, <u>Me</u>CHN). Increments of the achiral LSR, <u>2</u>, were added to solutions of racemic <u>1</u> for spectral simplification. One of the key assignments in the unshifted spectrum of <u>1</u> is the pair of doublets attributed to the <u>geminal</u> methyls, X and X', diastereotopic by virtue of the chiral center in <u>1</u>, and anisochronous despite their protons being separated from the chiral center by seven bonds. While the <u>gem</u> methyl protons are anisochronous, differing in chemical shifts by 0.01 ppm, they are isogamous (equally coupled) with respect to their <u>vicinal</u> coupling to the isopropyl methine $Me_2\underline{CH}$, since the doublets centered at 1.30 and 1.29 ppm exhibit observed splittings of 6.99 and 6.98 Hz, respectively. These splitting values differ by appreciably more than the experimental error from the splitting in the highest field doublet at 1.05 ppm (^3J = 6.46 Hz), and permits our assignment of the 1.30 and 1.29 ppm doublets to the isopropyl and the 1.05 ppm doublet to the methyl at the chiral center, <u>Me</u>CHN, [Me(W)]. At extremely low [LSR]/[drug] molar ratios, [<u>2</u>]/[<u>1</u>], the isopropyl methyls undergo a remarkable degree of broadening, appearing as a 6H intensity lump at the first

increment of 2, (a 2/1 ratio of 0.0236) with a width at half-height of about 23 Hz for the 6H absorption, while the higher field doublet remains sharp. In addition, the complex region of multiplets ca. 2.8-3.1 ppm changes significantly in appearance and in the observed (approximate) integrations. The isopropyl methine, Me_2CH, could be expected to be a septet if the spectra were first order. We believe that this wide multiplet undergoes severe broadening comparable to the gem dimethyl signals, resulting in the virtual disappearance of fine structure for the Me_2CH multiplet and such broadening that integration does not readily allow unambiguous assignment of the shifts of this methine at lower [2]/[1] ratios.

With the added 2, significant changes occur in the aryl spectral region. A severely broadened 2H intensity peak moves rapidly to low field, with sharper 2H and 1H intensity triplets also separating from the aryl absorption envelope. We interpret these aryl signals exhibiting large downfield lanthanide-induced shift (LIS) magnitudes as being the aryl ortho, meta (2H, t) and para (1H, t) signals of a phenyl. At very high 2/1 molar ratios (ca. 0.4) the broad 2H lump sharpens and exhibits doublet structure consistent with ortho protons possessing one vicinal neighbor.

Thus, extremely severe broadening is seen for the signals assigned to the isopropyl group and to one set of aryl ortho protons. At the outset, we considered two major potential LSR binding sites in 1, at the tertiary aliphatic sidechain amino group, $MeNR_2$, or at the carbonyl oxygen. Significant LSR binding at the sidechain alkylamino nitrogen should lead to larger LIS magnitudes and gross line broadening for proximal proton signals. However, this is clearly not observed, since fine structured double doublets (dd) are maintained, consistent with the diastereotopic benzylic protons $C_6H_5CH_AH_B$ [H_A refers to the higher field dd, and H_B to the lower field dd] at the highest 2/1 ratios used. Also, the methyl doublet with the smaller ^3J = 6.46 Hz, assigned to the methyl (W) at the chiral center, stays sharp.

Only slight broadening was apparent in the (accidental) 2H singlet assigned to the diastereotopic protons NCH_2, although these protons are alpha to the sidechain nitrogen. Substantial LSR-induced broadening would be expected for these proton signals if major LSR complexation were occurring at the (acyclic) sidechain nitrogen. Together with comparative LIS magnitude data, discussed below, these results led us to conclude that 2 overwhelmingly binds to the carbonyl oxygen of the pyrazolinone ring of 1. This could account for the exceptional broadening (and large LIS values) for protons proximal to the carbonyl: the isopropyl and the N-phenyl ortho protons. Proximity considerations would dictate that the aryl protons displaying large downfield LIS and severe broadening could self-consistently be assigned to the N-phenyl. Such behavior for the other phenyl protons can not be rationalized in the absence of significant broadening or large LIS values for the signals of protons close to the sidechain nitrogen (for the rigorously assignable dd benzylic protons, Me(W), and the 2H singlet CH_2).

With the first added increment of $Eu(FOD)_3$, 2/1 molar ratio of 0.0236, extreme signal broadening essentially removes the expected septet for the $Me_2\underline{CH}$ resonance from the 2.5-3.2 ppm region, allowing clear direct observation of the higher field dd near 2.58 ppm, $C_6H_5C\underline{H}_A$, with observed line spacings of 6.906 and 6.953 Hz (aver. 6.93 Hz) assigned to the vicinal ^3J, $\underline{H}_ACC\underline{H}Me$; spacings of 13.213 and 13.260 Hz (aver. 13.24 Hz) are consistent with the geminal ^2J H_ACH_B coupling between the diastereotopic benzylic protons. The lower field dd at 2.90 ppm, assigned to $C_6H_5C\underline{H}_B$, showed line spacings of 7.434 and 7.481 Hz (aver, 7.46 Hz) consistent with a vicinal ^3J, $\underline{H}_BCC\underline{H}Me$; spacings of 13.301 and 13.348 Hz (aver. 13.32 Hz) are in excellent agreement with the geminal H_ACH_B ^2J value extracted from the H_A signal, and suggests good experimental reproducibility for these coupling measurements. The different vicinal couplings from the methine to each of the benzylic protons, H_A and H_B, imply different dihedral angles of contributing rotamers. Peak intensities of the H_B

dd ("leaning") indicate the larger coupling to an upfield resonance, i.e., H_A, and the smaller coupling to the neighboring downfield resonance centered at ca. 3.08 ppm, assigned to the methine at the chiral center, MeCHN. Six peaks may be recognized by visual inspection, of which five were "called" by the spectrometer. The intensities of these five or six peaks are grossly in accordance with a Pascal triangle sextet, but the four measured line spacings of 7.003, 6.621, 6.914 and 6.319 Hz show much more scatter than, e.g., spacings in the dd resonances. Since the methine is coupled to three nonequivalent sets of neighbors, CH_3(W), H_A and H_B, with different vicinal couplings involved, a true (N+1) perfect sextet with equal line spacings would not be expected. Evidently the three relevant ^3J values are similar enough so that no more than six lines are ever clearly exhibited for this resonance (at higher levels of 2, as well) in contrast to the (N + 1) equally-spaced septet lines predicted for the isopropyl CH.

Assignments of the two methyl singlets, Me(Y) at lower field and Me(Z) at higher field, were made as follows. A chemical shift rationalization might predict lower field position for the heterocyclic N-methyl based on expected inductive electron withdrawal of the second nitrogen in the ring. Further deshielding should be associated with an aromatic ring current in the pyrazolinone ring and electronic effects of mesomeric electron donation from the heterocyclic ring nitrogens to the carbonyl oxygen leading to partial positive charge character delocalized over the pyrazolinone ring (Figure 1). The lower field signal of Me(Y) exhibits greater broadening and LIS magnitudes than the Me(Z) signal when 2 is added. We have therefore assigned Me(Y) as the MeNN resonance and Me(Z) as the alkylamino $MeNR_2$ signal since these latter effects are consistent with greater proximity to lanthanide bound at the carbonyl. A more quantitative discussion of results of least squares line fitting for variation of chemical shifts with LSR levels will be presented below.

With 2/1 molar ratios ca. 0.15-0.2 or more, a very broad signal can be seen downfield of the 2H CH_2 singlet signal, moving from ca. 4 to 6 ppm as the 2/1 ratio is increased from about 0.15-0.5. We have tentatively assigned this as the isopropyl methine, Me_2CH based on integration, LIS magnitudes and the extrapolated chemical shift for 1 without LSR, based on the signal at higher levels of 2. At the highest 2/1 ratio used, 0.607, the signal broadening has decreased to the point where multiplet fine structure is revealed. Five lines (of the potential septet) appear definable.

With 2/1 molar ratios from ca. 0.02-0.25, the gem dimethyl absorption appears as a single broad 6H intensity signal (although adventitious overlaps occur with other peaks), but at a 2/1 ratio of 0.3, two broad peaks near 3.0 and 2.9 ppm are resolvable with a valley height between them of about 60%. This suggests sharpening of the gem methyl signals, X and X'. The higher field peak exhibits incipient doublet character. With a 2/1 ratio of 0.35 or more, signal sharpening continues so that the diastereomeric methyls each show up as clear doublets. Signal sharpening is also notable for the N-C_6H_5 ortho signal at the highest levels of 2, with a distinct doublet appearing.

Increments of the chiral LSR, $Eu(HFC)_3$, 3, were added to 0.056 M 1 in order to compare effects of LIS and broadening, in addition to expected induction of enantiomeric shift differences, $\Delta\Delta\delta$. There were some general similarities between results with added 2 and 3, especially with respect to large LIS magnitudes and severe broadening for the signals of the isopropyl group. The lower field methyl singlet assigned to the heterocyclic CH_3NN, Y, broadened faster than the other methyl singlet, Z, assigned to the acyclic nitrogen CH_3NR_2, with both 2 and 3. However, the aryl spectral appearance with added 3 developed in a dramatically different way than with 2. For 3/1 molar ratios ca. 0.4 or more, it is clear that there are some "anomalous" (upfield) shifts occurring for selected aryl proton signals, integrating to 3H. By the highest 3/1 ratio used, 1.25, these signals have

resolved into approximate triplets of 2H and 1H intensity, with the 1H triplet at highest field. On the low field (high frequency) side of the aryl region, two 1H broad doublets separate. We assign the low field doublets to the ortho N-phenyl H-2',6' protons, separated by $\Delta\Delta\delta$. The anomalously shifted triplets are assigned to the N-phenyl meta protons, H-3',5' (2H signal) and the N-phenyl para proton, H-4' (1H signal). Since tris-beta-diketonate LSRs derived from Eu(III) are generally regarded as "downfield" reagents (3-5), upfield induced shifts with these reagents have been regarded as "anomalous" or "wrong-way" shifts. They have been explained (17,18) as resulting from the angular part of the simplified McConnell-Robertson equation (19). We had previously reported analogous upfield shifts in two structurally similar compounds (20,21). The upfield shifts in 1 with 3 are consistent with major LSR binding at the oxygen, but not with LSR binding at the sidechain amino nitrogen. Major LSR binding at the oxygen of 1 with 2 and 3 could be reasonable if binding at the acyclic nitrogen is sterically suppressed, since the carbonyl might be more accessible to LSR despite hindrance from the isopropyl and N-phenyl groups. LSRs are considered very sensitive to steric hindrance (3-5). Rackham (22), using $Eu(DPM)_3$ in $CDCl_3$ at 27°, reported a binding constant of 1360 for piperidine (pK_a 11.0) and a binding constant of only 16.9 for N-methylpiperidine (pK_a 10.1). Alpha substitution also retarded LSR binding, with a binding constant of 314 for 2-methylpiperidine (pK_a 10.9). The hindered tertiary alkyl amino group of 1 could well be only a minor binding site compared to the polarized carbonyl oxygen. It is less clear why 2 and 3 should differ in causing anomalous shifts. A very speculative explanation might be a longer Eu-O bond in the bound complex of 1 with 2 than in 1 with 3. A longer bond length would decrease the size of the angle, α, defined in the angular term $(3\cos^2\alpha-1)$ of the simplified expression for the pseudocontact LIS (19). If this angle is less than 54.7°, "normal" downfield shifts should be seen from the

Eu(III); somewhat higher values than 54.7° cause a reversal of the direction of shift.

The NCH_2 protons of 1 are diastereotopic but appeared to be essentially isochronous (coincidentally equivalent in chemical shift) with added 2, the modest broadening perhaps suggesting some imminent separation between the signals of these protons. With higher 3/1 ratios, however, the resonance assigned to the NCH_2 protons was much more complex, with five distinct peaks. The pattern was consistent with a tightly coupled AB quartet attributed to the NCH_2 of one enantiomer of 1, with an apparent singlet due to isochronous signals from the NCH_2 of the other enantiomer overlapping the AB quartet. The observed geminal coupling measured from the AB quartet lines with 3/1 ratios ca. 0.5-1.05 was about 14.1 Hz, consistent with an NCH_2. Thus, the chiral 3 has induced modest $\Delta\Delta\delta$ for each proton of this methylene but causes anisochronicity between the diastereotopic nuclei for only one of the enantiomers. Signal separation leading to the assignable peaks occurred only with high molar ratios of 3/1, in a region where considerable curvature was evident in the plots of chemical shift versus 3/1 ratio; we have, therefore, not attempted to calculate separate line equations for these diastereotopic nuclei of the enantiomers of 1. At lower molar ratios of 3/1 the signal separations did not permit clear distinct shift assignments. A representative spectral trace is shown in Figure 2(a). Small differences in bound complex geometries of 1 with 2 or 3 (rather than lanthanide-oxygen bond length changes) or contributions from bound complexes with LSR at the alkylamino sidechain nitrogen of 1 mayl also account for these differences. Such differences may partly reflect differing steric requirements of the different LSRs.

We believe that there are some justifications for attributing the "doubling" of some signals of 1 with added chiral LSR to be enantiomeric shift differences. The "doublings" designated as $\Delta\Delta\delta$ were seen only with the chiral 3, and not with the achiral 2. In addition, where the

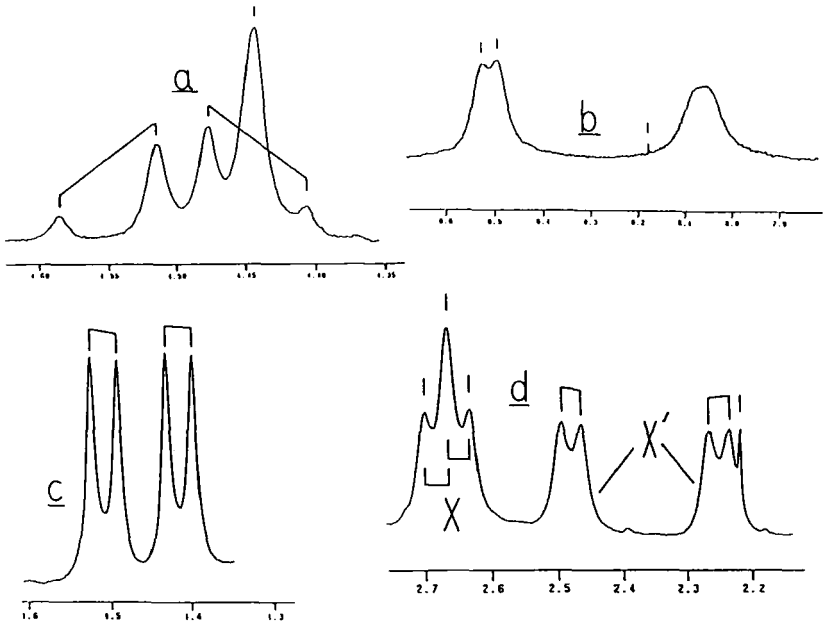

Figure 2. Spectral expansions of 200 MHz ^{1}H NMR spectra of 1 with added LSR: (a) NCH_2 with 3/1 ratio 0.603, showing isochronous signals for one enantiomer (large peak) with the AB quartet for the anisochronous signals from the other enantiomer (geminal couplings shown with brackets). The low field doublet is "NCH_2(A)." (b) N-phenyl H(ortho), 3/1 ratio 0.897; note less line broadening on lower field doublet (observed J ca. 6.8 Hz) and $\Delta\Delta\delta$ of 91 Hz; (c) MeCHN with 3/1 ratio 0.897, showing ^3J = 6.47 Hz and $\Delta\Delta\delta$ = 18.6 Hz, with valley height ca. 8%; (d) isopropyl methyls with 3/1 ratio 0.897. The low field "triplet" is Me(X), with apparent ^3J and $\Delta\Delta\delta$ ca. 6.8 Hz; the pair of doublets are assigned to Me(X'), with average apparent ^3J ca. 6.1 Hz and $\Delta\Delta\delta$ ca. 45.8 Hz, essentially baseline resolved. The sharp peak is attributed to an impurity.

doubled signals could be approximately measured (i.e., by integrations), they appeared essentially equal in area. This is consistent with $\Delta\Delta\delta$ for racemic 1 but would be coincidental if the signals reflected diastereomeric rotamers (unless a hindered rotation interconverted degenerate structures). Thus, slow N-phenyl rotation could lead to separate but equal intensity signals for the H-2',6' ortho protons. But slow rotation of the isopropyl group, exchanging nondegenerate conformational isomers, might be expected to produce unequal intensity signals if the absorptions were the result of conformers of differing energies.

With respect to some chemical shift assignments of 1 with added LSR, we make these comments. 1) We cannot rule out the possiblility of a "crossover" of the gem dimethyl signals, X and X', as the LSRs 2 or 3 are added. In unshifted 1, these diastereotopic methyls are anisochronous by ca. 0.01 ppm. With the severe broadening induced by initial low levels of either LSR, it is possible that the methyl initially at higher field (designated X') may actually move downfield faster than the other methyl, X, as LSR is added, leading to a "crossover". For simplicity, we have assumed that this does not happen, and the slope calculations presented below reflect our assignments that the higher field (least shifted) methyl signal in the presence of added LSR was also the higher field methyl without LSR.

2) The extremely complex aryl proton absorption region does not simplify to permit unambiguous assignments of individual protons (except for the N-phenyl ortho proton signals) until substantial LSR has been added; this is particularly true for added 3. We have assigned a single approximate "weighted average" chemical shift to all of the unresolved aryl protons until a sufficient LSR level has been reached to elicit well separated signals. This results in an artifactual plateau or step in the plots of chemical shift versus [LSR]/[1] molar ratios. The line equations subsequently calculated and shown below, particularly for 3, are based on the portions of these

plots at higher LSR levels where assignments were clearer, even though some nonlinearity and curvature may be present in the plots over these regions. 3) Even at the highest levels of 2 or 3, we have not attempted to separately assign the C-phenyl ortho, meta or para resonances; only a single average chemical shift for this phenyl group was employed.

Table 1 presents the line equations derived from a linear least squares line fitting based on plots of the chemical shifts of the protons of 1 versus molar ratios of [LSR]/[1] for each LSR. The equations show the intercept, corresponding to the chemical shifts of each nucleus extrapolated to zero levels of added LSR. Usually these intercepts agree rather closely ca. ±0.02 ppm with the assigned chemical shifts for 1 in the absence of LSR. Larger deviations are noted for nuclei which gave severely broadened signals with initial increments of LSRs, such as the gem methyls and the isopropyl methine; some aryl signals show large deviations, probably reflecting our inability to make unambiguous assignments at low levels of LSR. Correlation coefficients, R, for the lines were usually acceptable, 0.99-1.00. Poorer correlations for the CC_6H_5 signals, and with 3, for the N-phenyl H(meta) signals, reflect errors associated with low LIS magnitudes and inability to assign signals at very low LSR levels. As noted above, separate assignable signals for the NCH_2 of one enantiomer of 1 with added 3 were apparent only at molar ratios ca. 0.4 or higher, in which regions substantial line curvature was apparent; tabulated slopes from lower LSR levels reflect essentially a single, unresolved absorption for these diastereotopic protons. The nearly identical slopes shown for the diastereotopic gem methyls of the isopropyl group with added 2 is almost certainly artifactual, since severe line broadening of these signals with 2 resulted in a single broad singlet up to a 2/1 ratio of ca. 0.25. At 2/1 ratios of ca. 0.3 and higher, separate signals could clearly be assigned and significantly different LIS magnitudes were apparent.

Table 1. Equations from least squares line fittings of chemical shifts vs. [LSR]/[1] molar ratios.

	Eu(FOD)$_3$, 2			Eu(HFC)$_3$, 3		
Nucleus		(R)[b]	Normalized slopes[c]		(R)[b]	Normalized slopes[c]
H(ortho)NPh	y =7.250 + 8.555x		3.299	y = 7.282 + 1.564x	(0.99)	0.967
H(meta)NPh	7.416 + 2.025x		0.781	7.417 - 0.595x	(0.91)	-0.368
H(para)NPh	7.301 + 0.988x	(0.99)	0.381	7.360 - 0.667x	(0.99)	-0.412
CC_6H_5	7.228 + 0.406x	(0.98)	0.157	7.162 + 0.532x	(0.97)	0.329
NCH_2	3.522 + 2.593x	(0.99)	1.0	3.532 + 1.618x		1.0
MeCHN	3.062 + 1.289x	(0.99)	0.497	3.065 + 0.945x	(0.99)	0.584
C_6H_5CH(B)	2.892 + 0.702x		0.271	2.893 + 0.496x	(0.99)	0.307
$HC(Me)_2$	2.832 + 8.172x	(0.99)	3.152	2.872 + 5.445x		3.365
Me(Y) [MeNN]	2.747 + 2.213x		0.853	2.764 + 1.030x	(0.99)	0.637
C_6H_5CH(A)	2.567 + 0.768x	(0.99)	0.296	2.571 + 0.526x	(0.99)	0.325
Me(Z) [$MeNR_2$]	2.308 + 1.153x		0.445	2.317 + 0.767x		0.474
Me(X) [i-Pr]	1.279 + 6.827x		2.633	1.305 + 1.978x		1.222
Me(X') [i-Pr]	1.275 + 6.857x		2.644	1.314 + 1.558x		0.963
MeCHN	1.053 + 0.958x	(0.99)	0.369	1.059 + 0.566x		0.350

Notes: (a) Line equations are based on least squares line-fittings for linear, lower molar ratio regions of plots of chemical shift vs. molar ratios. See **Results and Discussion**. (b) Correlation coefficients, R, were equal to 1.00 unless noted otherwise (in parentheses). (c) Normalized slope values are given relative to a value of 1.0 for the slope of the line for the signals assigned to the NCH_2 resonance for each LSR. See **Results and Discussion**.

For each nucleus, the plot of chemical shift versus [LSR]/[1] ratio was examined over the entire range, up to 0.607 for 2 and 1.25 for 3. The region selected for line equation calculation was chosen visually for good linearity. With 2, molar ratios from 0.0 to 0.2 (seven experimental points) were usually used. With 3, the usual molar ratios were from 0.0 to 0.31 (ten experimental points). Somewhat higher molar ratio points (or fewer points) had to be used for some aryl proton signals and, for 3, the $\underline{HC}(Me)_2$ signal, since assignments were uncertain with lower levels of LSR. Nonlinearities were apparent with 3 for the CC_6H_5 and N-phenyl H(para) plots.

The slopes in these line equations are considered relative indices of the LIS magnitudes. If the induced shifts are largely dipolar pseudocontact in origin, these slopes should reflect distance and geometric aspects of the McConnell-Robertson equation (19). Deviations from linearity in the plotted curves, especially apparent at [LSR]/[1] ratios above 0.5, may imply contributions from bound complexes of differing stoichiometry or geometry. Our Table 1 includes not only the line equations with the "raw" slope values, but also normalized slope values. We selected the NCH_2 signal with each LSR as the reference for this normalization for several reasons. The signals were always easily assignable and accurately measurable. The NCH_2 moiety is directly attached to the relatively rigid pyrazolinone system rather than being part of the "floppy" sidechain. Being well removed from the presumed main lanthanide binding site on oxygen, Fermi contact contributions were not expected to be important for the NCH_2 resonances. With this normalization, the relative LIS magnitudes for many of the nuclei of 1 are seen to be in surprisingly good agreement for 2 and 3. The alkylamino sidechain resonances, including the benzylic H_A and H_B, methyl(Z) [$MeNR_2$], $\underline{Me}CHN$, and even the isopropyl methine, have normalized slopes which agree within 10%. The values for the $Me\underline{CH}N$ agree within 20%. Values for the methyl(Y) on the heterocyclic ring differ by about 30%;

perhaps some contact shift may be transmitted through the conjugated system here. But the largest differences in normalized slopes are certainly most apparent for the N-phenyl resonances and the isopropyl methyls. (We suspect that a substantial portion of the differences in normalized slopes for the CC_6H_5 simply reflects experimental errors in assigning an "average" chemical shift to the different proton signals, and to the relatively small LIS magnitudes for these signals.) These N-phenyl and gem methyls would be closest to a lanthanide bound at oxygen. Especially large slopes are seen for the N-phenyl ortho protons with 2; the gem methyl normalized slopes are more than twice as large with 2 than with 3. With both 2 and 3, normalized slopes generally decrease as one moves from the carbonyl past the sidechain nitrogen, which supports our proposed major binding site. It is somewhat surprising that normalized slopes for the isopropyl methine are so similar with 2 and 3 whereas the isopropyl methyls and N-phenyl protons differ greatly. This may be interpreted as the result of the europium being critically positioned in the bound complexes of 2 and 3. If the angles, α, involved are close to 54.7° (19) between the magnetic symmetry axis of the complex (approximated by the Eu--O bond) and the distance vector from metal to the nucleus in question, then fairly small changes in distances or angles could have large effects on LIS magnitudes. This also could produce the "anomalous" upfield shifts found with 3. The chemical shift variations with 2 and 3 are presented in Figures 3 and 4, respectively. [In Figure 4, NCH_2(A) refers to the lower field half of the AB quartet; the difference in chemical shift between the upfield half of the AB quartet and the singlet due to accidentally isochronous NCH_2 signals in the other enantiomer was too small to show, and the NCH_2 singlet is simply called "NCH_2" in this graph.]

Figure 5 presents the variations in $\Delta\Delta\delta$ for the nuclei of 1 with added 3. By far the largest $\Delta\Delta\delta$ magnitudes are seen for the N-phenyl H(ortho) signal, followed by methine at the chiral center (MeCHN) and the higher field methyl (X′) of

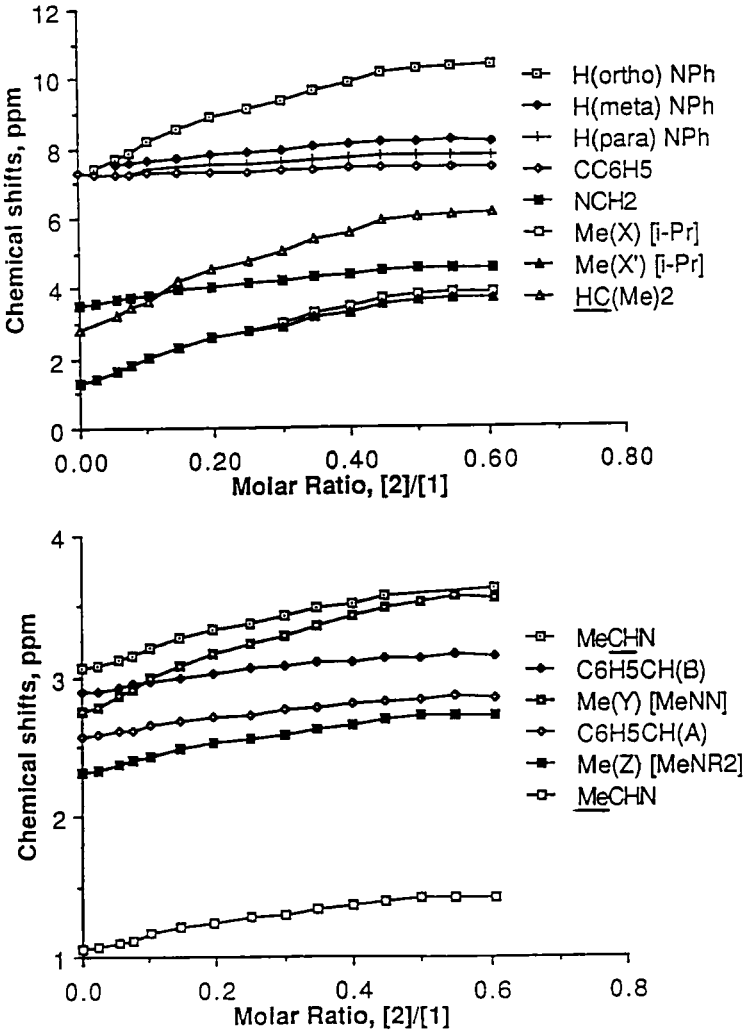

Figure 3. Variation of chemical shifts with molar ratio of $[Eu(FOD)_3]/[1]$; for clarity, this is shown in two separate graphs.

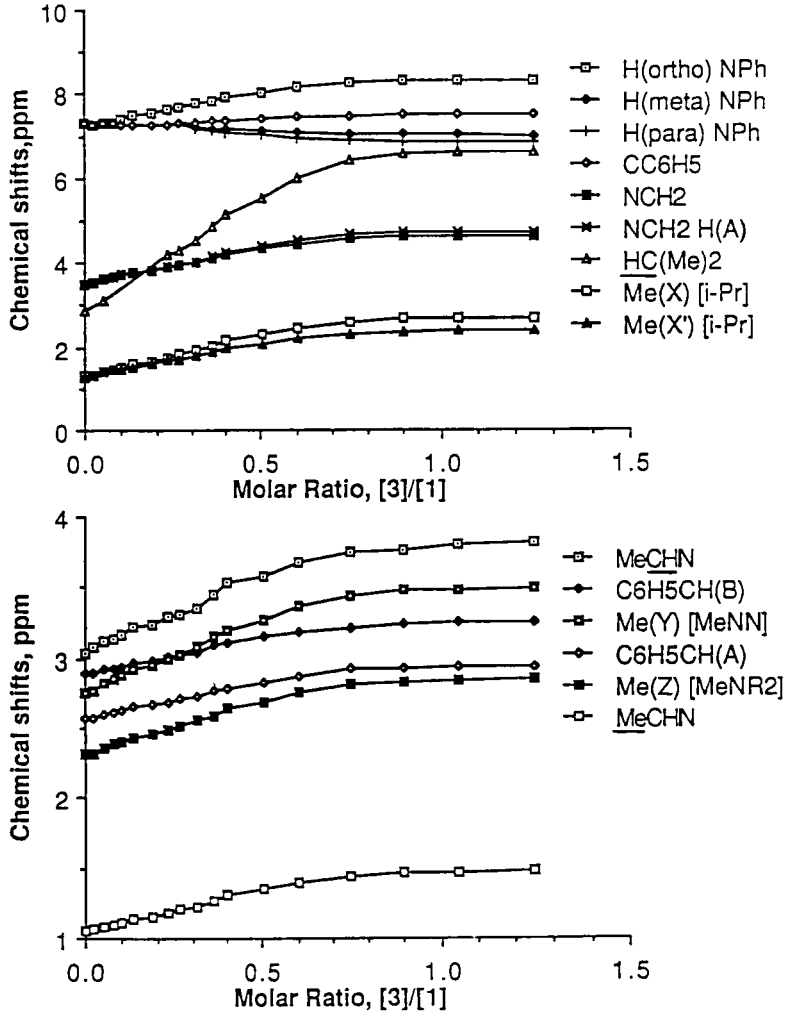

Figure 4. Variation of chemical shifts with molar ratio of $[Eu(HFC)_3]/[1]$; for clarity, this is shown in two separate graphs.

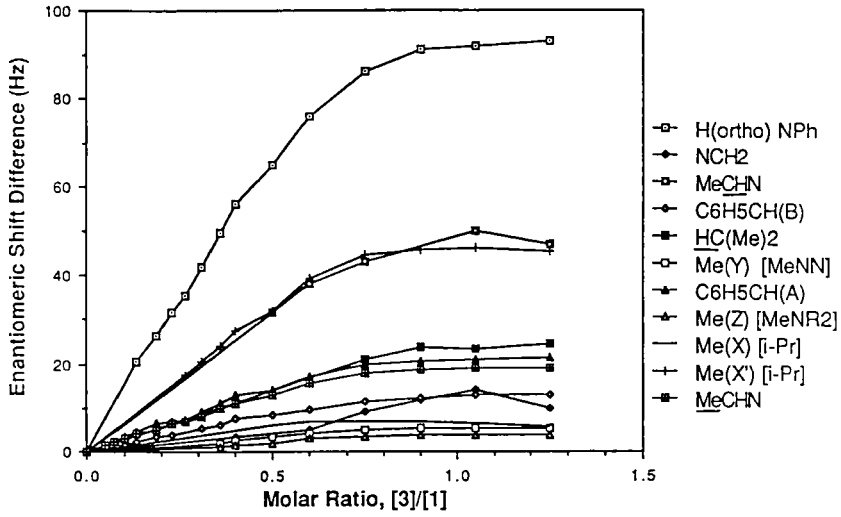

Figure 5. Variation of $\Delta\Delta\delta$ (in Hz) with molar ratio of $[Eu(HFC)_3]/[\underline{1}]$.

the isopropyl. The benzylic protons H(A,B) and the methyl MeCHN, all vicinal to the chiral center, have larger $\Delta\Delta\delta$ than the Me(Z) [$MeNR_2$], further from the chiral center. The isopropyl methine shows large $\Delta\Delta\delta$ although it is far from the chiral center, presumably due to proximity to chiral 3 bound at oxygen. Thus, nuclei near the chiral center or the LSR binding site exhibit bigger $\Delta\Delta\delta$. The signals with greatest potential analytical utility for direct determination of % e.e. for samples of 1 appear to be the N-phenyl H(ortho), nearly baseline resolved at 3/1 ratios ca. 0.4 or more. Of secondary utility might be the MeCHN or Me(X') isopropyl signals with 3/1 ratios of ca. 0.9-1.05. Representative spectral traces are included in Figure 2. It is interesting that the $\Delta\Delta\delta$ seen for the isopropyl methyls and the N-phenyl H(ortho) signals reflect magnetic nonequivalences of nuclei seven or eight bonds, respectively, from the chiral center.

CONCLUSIONS

The 300 MHz ^{1}H NMR spectra of racemic famprofazone have been studied at ambient temperatures in $CDCl_3$ solution in the presence of the achiral LSR, $Eu(FOD)_3$, and the chiral $Eu(HFC)_3$. Both reagents induced substantial shifts interpreted as consistent with predominant LSR binding at the carbonyl oxygen of 1. Substantial line broadening with 2 was seen for the N-phenyl H(ortho) and isopropyl resonances of 1, but these signals became sharper with 2/1 ratios above 0.35. The chiral 3 also caused severe line broadening of the isopropyl absorptions at 3/1 ratios below 0.4, but much less broadening of the N-phenyl signals, with 3 causing "anomalous" upfield shifts for the H(meta) and H(para) proton signals. Significant enantiomeric shift differences were elicited with 3, with near baseline resolution for the N-phenyl H(ortho) resonances over a range of 3/1 molar ratios from ca. 0.3-1.25 that should offer some analytical utility for % e.e. determinations of samples of 1. Both LSRs enhanced the anisochronous nature of the gem isopropyl methyls but only 3 produced anisochronous signals (for one enantiomer of 1) with the diastereotopic NCH_2 protons.

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