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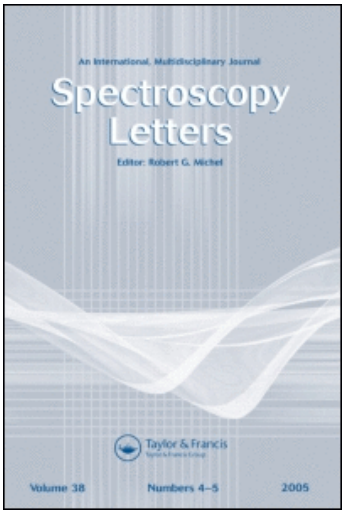

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^{1}H NMR Spectral Simplification with Achiral and Chiral Lanthanide Shift Reagents. Fenimide. Direct Simultaneous Determination of Four Stereoisomers

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^{1}H NMR SPECTRAL SIMPLIFICATION WITH ACHIRAL AND CHIRAL LANTHANIDE SHIFT REAGENTS. FENIMIDE. DIRECT SIMULTANEOUS DETERMINATION OF FOUR STEREOISOMERS.

Key Words: Lanthanide, NMR Shift Reagent, Fenimide, Europium, Chiral, Optical Purity, Enantiomer, Diastereomer, 4-ethyl-3-methyl-3-phenyl-2,5-pyrrolidine-dione

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ABSTRACT:

The 60 MHz ^{1}H NMR spectra of racemic commercial fenimide, 4-ethyl-3-methyl-3-phenyl-2,5-pyrrolidine-dione, **1**, have been studied in $CDCl_3$ solution at 28°

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with the achiral shift reagent, tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium-(III), 2, and the chiral reagent, tris[3-(heptafluoropropylhydroxymethylene)-d-camphorato]europium(III), 3. Use of 2 substantially increased the chemical shift differences between resonances of corresponding nuclei in the alpha and beta diastereomers, simplifying mixture analyses. Use of the chiral 3 produced enantiomeric shift differences, $\Delta\Delta\delta$, for several signals of 1. In particular, with a 0.309 molal solution of 1 and a 3:1 molar ratio near 0.4, four distinct singlets can be seen for the quaternary methyl signal. Direct simultaneous analysis for all four stereoisomers (two pairs of enantiomers) should be feasible.

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INTRODUCTION:

Fenimide, 4-ethyl-3-methyl-3-phenyl-2,5-pyrrolidinedione, 1, was first reported in 1963 (1). It has been used as an antipsychotic. Fenimide had shown sedative, anticonvulsant and anesthetic activity in the mouse, with anticonvulsant activity comparable to that of phenobarbital or meprobamate; fenimide had also prevented formation of stress-induced gastric lesions in the rat (2). Later studies examined drug effects on electrically induced seizures (3).

The compound has two chiral centers at C-3 and C-4 of the pyrrolidinedione ring and can exist as two pairs

of enantiomers, known as the alpha and beta modifications. A racemic sample of 1 could therefore consist of a mixture of four stereoisomers. The precise stereochemistry of a drug has increasingly been a matter of concern since diastereomers, and even enantiomers, may differ significantly in pharmacological action and physiological effects. Such stereoisomers may differ in potency or toxic side effects.

Studies in our laboratories have aimed at the use of achiral and chiral lanthanide NMR shift reagents for spectral simplification and for direct optical purity determinations. Basic methods in use of lanthanide shift reagents (LSR) have been reviewed (4-10). In particular, we have studied a number of compounds structurally related to 1, including glutethimide (11), ethotoin (12), mephenytoin (13), ethosuximide and analogs (14), vinclozolin (15,16), metaxalone (17) and aminoglutethimide (18), which involve five- or six-membered ring compounds possessing amide, imide or related functionality. Since the LSR has proven useful in this series, within certain limitations (14), we were most interested in extending the technique to 1.

The LSR methods have also been applied to related heterocycles, including hydantoins, barbiturates and oxazolidinediones. In the related case of 7-chloro-3,3a-dihydro-2-methyl-2H,9H-isoxazolo[3,2-b][1,3]benz-

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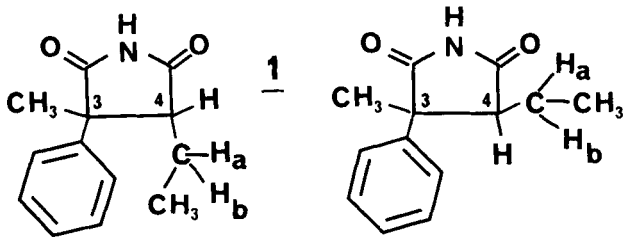
oxazin-9-one, an achiral LSR had allowed distinguishing two diastereomeric racemic pairs of enantiomers (19) and a chiral LSR had distinguished the enantiomers (20).



1

EXPERIMENTAL:

A sample of racemic fenimide, 1 [PD/(CI) No. 24,925, lot T] was kindly provided by Warner Lambert Co., Pharmaceutical Research Div., Ann Arbor MI 48105, U.S.A., and was used as received. The manufacturer stated the composition of this batch to be 39% alpha and 61% beta isomers. Chloroform-d, (99.8 atom % D), obtained from Aldrich Chemical Corp., Milwaukee WI 53201, U.S.A, or from Norell, Inc., Landisville NJ 08326, U.S.A., was dried over 3A molecular sieves. Shift reagents were obtained from Aldrich and were stored in a desiccator over P_2O_5. Materials were used as supplied except as noted.

In general, an accurately weighed portion of drug (about 35 mg) was added to about 500 mg of $CDCl_3$

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[containing about 0.4% tetramethylsilane (TMS) as internal standard] in an NMR sample tube and dissolved by shaking; increments of shift reagent were added, dissolved by shaking, and the spectra immediately run.

All spectra were run on a Varian EM360A 60 MHz ^1H NMR spectrometer at a probe temperature of 28°. Chemical shifts are reported in parts per million (δ) relative to TMS as internal standard and are believed accurate to $\pm$ 0.05 ppm. In spectra where TMS was obscured by shift reagent peaks, $CHCl_3$ (present as an impurity in the solvent) was used as internal standard. In cases with chiral 3 when enantiomeric shift differences, $\Delta\Delta\delta$, were observed, reported chemical shifts refer to the average values for the two enantiomers.

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RESULTS AND DISCUSSION:

The 60 MHz ^1H NMR spectrum of racemic 1 as a 0.310 molal solution in $CDCl_3$ at 28° showed resonances consistent with the sample being a mixture of two diastereomeric pairs of enantiomers. For example, two distinct triplets were observed for the methine proton, at 2.67 ppm (minor isomer) and 2.97 ppm (major isomer) and two singlets were seen for the quaternary methyl, at 1.80 ppm (minor) and 1.63 ppm (major). Overlap of the triplet signals expected for the CH_3 of the ethyl groups led to an approximate upfield quartet centered

near 0.98 ppm. A broad multiplet for the methylene proton signals was seen from 1.5-2.3 ppm. The aryl protons appeared roughly as two closely-spaced peaks at 7.4 ppm, and the broad imide signal appeared at 9.2 ppm. Incremental additions of the achiral LSR, tris[6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato]europium(III), 2, known as $Eu(FOD)_3$, led to spectral simplification, with separation of the triplets of the $\underline{CH_3}CH_2$ protons using a 2:1 molar ratio near 0.5, the signal of the major diastereomers being downfield. The lanthanide-induced shifts, $\Delta\delta$, were greater for the major isomers based on the methine and methyl triplets, and for the minor isomers based on the quaternary CH_3 singlet. (The $\Delta\delta$ value for a particular nucleus is the change in chemical shift observed in the presence of LSR relative to the chemical shift in the absence of LSR.) Facile analysis for the alpha and beta isomers would best be based on the CH_3 triplets, free from overlaps, with 2:1 ratios from about 0.5-0.8. Such molar ratios also caused the aryl ortho hydrogen signals to move well downfield of the remaining aryl signals. Results with 2 are summarized in Figure 1.

Using the chiral LSR, tris[3-(heptafluoropropylhydroxymethylene)-d-camphorato]europium(III), 3, known as $Eu(HFC)_3$ or $Eu(HFBC)_3$, enantiomeric shift differences, $\Delta\Delta\delta$, were clearly seen for the quaternary methyl

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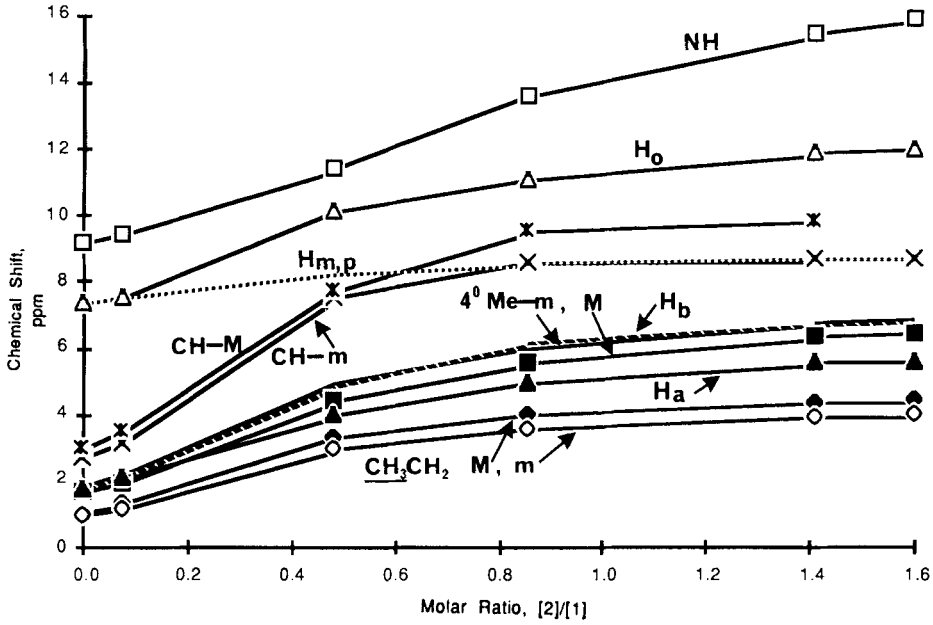

FIG. 1. Variation of chemical shift with molar ratio of [2]/[1]. Note 1: Data for the major and minor isomers are indicated by M and m, respectively. Note 2: Data for the aromatic ortho hydrogens and for the meta and para hydrogens of the phenyl group are indicated by H_o and $H_{m,p}$, respectively.

signals with a 3:1 molar ratio of 0.179 using a 0.309 molal solution of 1. (The enantiomeric shift difference is the difference in chemical shift for a given nucleus in two enantiomers produced by the chiral reagent.) Smaller $\Delta\Delta\delta$ is also seen for the major isomer's (downfield) methine signal at this same low 3:1 ratio. For this racemic sample of 1, $\Delta\Delta\delta$ values for the quaternary methyl signals appear to reach a

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maximum (3:1 ratio near 0.4), to decrease with further 3 (3:1 ratio near 0.8) and to increase again (3:1 ratio near 1.4, the highest level studied.) This behavior suggests the possibility of a change in the sense of magnetic nonequivalence for this methyl, with the signal of one enantiomer being at higher field for low levels of 3 and at lower field for high levels of 3. Observed $\Delta\Delta\delta$ would be zero at the "crossover point;" confirmation could be obtained by LSR studies using non-racemic mixtures of the enantiomers of 1. Such behavior can suggest changes in geometry or stoichiometry of the LSR:substrate complex as the molar ratio of the two components is changed. Some uncertainty exists in these $\Delta\Delta\delta$ values for the quaternary CH_3 signals because of the alternative possibility (which we could not rule out) that adventitious overlaps of the two peaks from the major and minor enantiomeric pairs might also account for the observed results. Direct assignment of these peaks to "major" or "minor" isomers simply based on peak heights or areas was not possible because of overlap with the exceedingly complex multiplets corresponding to the CH_2 signals; these latter signals formed an envelope several ppm in width at 3:1 ratios that achieved substantial $\Delta\Delta\delta$ values.

Distinct $\Delta\Delta\delta$ for the triplet CH_3CH_2 could also be observed. For example, 0.244 molal 1 with a 0.949 3:1

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ratio resulted in the major isomers' signal being downfield, with $\Delta\Delta\delta$ about 13 Hz, and the minor isomer (upfield) with $\Delta\Delta\delta$ about 3.5 HZ. Under these same conditions, the methine signal of the major isomer (downfield) shows $\Delta\Delta\delta$ of 21.5 Hz. The height of the valley separating each enantiomer's triplet signal is only 27.5% of the average height of each triplet's center branch; good analytical utility is thus available for optical purity determinations of the major isomer. The minor isomers result in a methine resonance at higher field, appearing as a quartet because of $\Delta\Delta\delta \approx {}^{3}J \approx 6$ Hz.

Importantly, high 3:1 levels result in distinct separation of NH signals for all four stereoisomers, free from any interfering overlaps. This contrasts with the observation for 2 that no separate NH signals are seen for the diastereomers. Using 3, the minor pair of enantiomers produces NH signals downfield from those of the major isomers. For NH of the minor isomers, $\Delta\Delta\delta$ is about 16 Hz, and for the major, about 35Hz, with a 3:1 ratio of 1.12. With these conditions, the valley height between the signals of the major isomer's enantiomers is only 21.4% of the enantiomer peak heights. Although separation between the minor isomer's enantiomer's peaks is not as good (about 58.6% by the valley height criterion), $\Delta\Delta\delta$ values were seen to

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increase at higher 3:1 ratios, to 22 and 37 Hz for the minor and major isomers' NH signals, respectively, with a 3:1 ratio of 1.42. These conditions would allow satisfactory simultaneous determination of all four stereoisomers of 1, without overlap on other signals as would be the case using the quaternary methyl signals. (Presumably, a substantial contribution to the broadness of the NH resonances results from the nuclear quadrupole of nitrogen-14. Heteronuclear double resonance techniques employing broadband irradiation and decoupling of the imide nitrogens should be expected to result in sharpened imide signals in the ^1H NMR spectrum, which could improve resolution of each enantiomer's peaks and increase analytical utility.) A high 3:1 ratio also results in good separation of the aryl ortho protons, with the minor diastereomers downfield; no $\Delta\Delta\delta$ was seen for the signals of these protons.

Although observation of $\Delta\Delta\delta$ for the NH signal of amides when using chiral LSR is not commonplace, it is not unprecedented (12). That the alpha and beta isomers of 1 have different chemical shifts for the NH signal on treatment with 3 versus 2 could be consistent with different geometries in the complex of 1 with shift reagent. In particular, the fact that not all resonances for the protons in either the alpha (or

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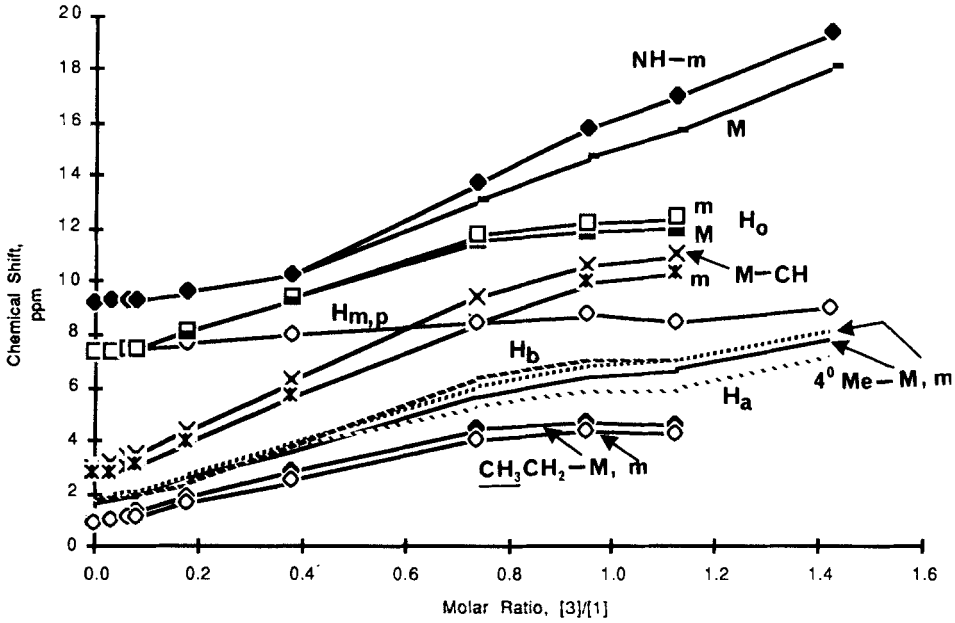

FIG. 2. Variation of chemical shift with molar ratio of [3]/[1]. Where $\Delta\Delta\delta$ was observed for a given resonance, average chemical shifts for the two enantiomers are presented. Some chemical shift values at higher levels of shift reagent may reflect assignment uncertainties. See Notes 1 and 2 for Figure 1. Note 3: Presented data include results for solutions of 1 either 0.244 molal or 0.319 molal.

beta) isomers are consistently upfield (or downfield) of the signals for corresponding nuclei in the diastereomer argues strongly for these chemical shift differences being derived from changes in geometry in the LSR-substrate complex. If the relative $\Delta\delta$ values simply reflected different binding constants between LSR and the alpha or beta isomers, the resonances of a

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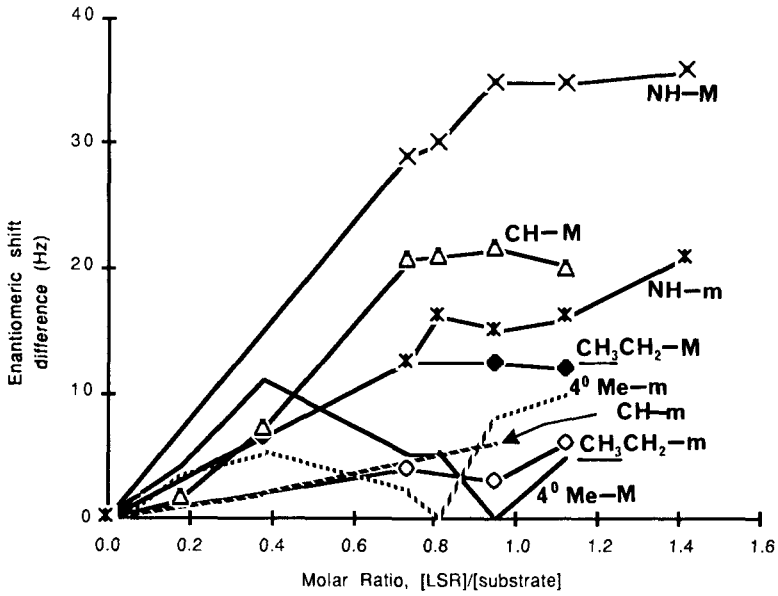

FIG. 3.
Variation of enantiomeric shift differences (in Hz) with molar ratio of [3]/[1]. See Note 1 for Figure 1 and Note 3 for Figure 2.

given diastereomer would be expected to be uniformly upfield (or downfield) of the other diastereomer. Results with 3 are summarized in Figures 2 and 3.

In summary, the lanthanide shift reagents 2 and 3 have been shown to be quite useful for ^{1}H NMR spectral simplification of fenimide and for direct observation of all four stereoisomers. Observation of substantial $\Delta\Delta\delta$ for selected resonances of 1 is consistent with general principles established for related compounds (14). In particular, 1 is a relatively rigid, cyclic

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system, with chiral centers close to the LSR's expected carbonyl binding sites. Absence of alkyl substitution on nitrogen leaves the oxygens relatively unhindered as Lewis base sites for the europium. The major isomer in our sample of 1 displayed larger $\Delta\Delta\delta$ magnitudes for all resonances with observed $\Delta\Delta\delta$ values. The major isomers' signals were downfield of those of the minor for the $\underline{CH_3}CH_2$ and CH methine protons, and upfield for the aryl ortho, NH and quaternary CH_3 protons.

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