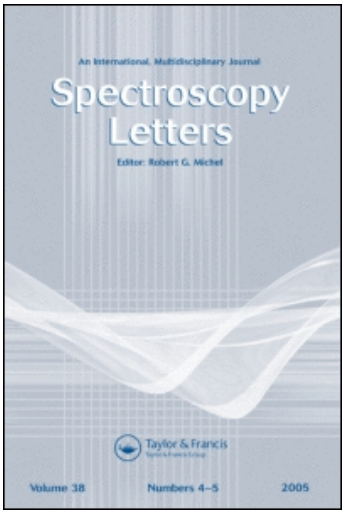

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

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To cite this Article Reifsneider Jr., Robert J. , Hoffman, Bernard H. , Venkatasubban, Kunisi S. , Rothchild, Robert and Martin, Rolf(1991) 'NMR Studies of Drugs. Application of a Chiral Lanthanide Shift Reagent for Potential Direct Determination of Enantiomeric Excess of Methsuximide', Spectroscopy Letters, 24: 10, 1373 — 1394
To link to this Article: DOI: 10.1080/00387019108021768
URL: http://dx.doi.org/10.1080/00387019108021768

SPECTROSCOPY LETTERS, 24(10), 1373-1394 (1991)

NMR STUDIES OF DRUGS. APPLICATION OF A CHIRAL LANTHANIDE SHIFT REAGENT FOR POTENTIAL DIRECT DETERMINATION OF ENANTIOMERIC EXCESS OF METHSUXIMIDE.

Key Words: Europium, $Eu(HFC)_3$, Optical Purity, Analysis, Anticonvulsant, ^1H NMR, Succinimides.

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ABSTRACT

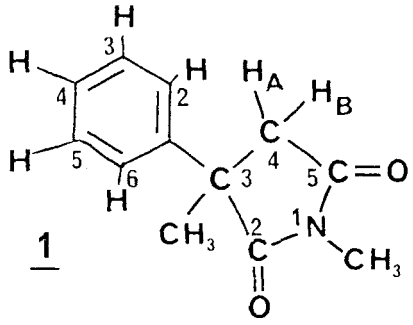
The 200 MHz ^1H NMR spectra of methsuximide, 1,3-dimethyl-3-phenyl-2,5-pyrrolidinedione, 1, have been studied in $CDCl_3$ solution with the chiral reagent, tris[3-(heptafluoropropyl-hydroxymethylene)-d-camphorato]europium(III), 2, $Eu(HFC)_3$. Substantial lanthanide-induced shifts are seen. In addition, significant enantiomeric shift differences are observed for several of the nuclei of 1. With a 2:1 molar ratio near 1.0, the CCH_3 and NCH_3 signals show nearly baseline resolution between the peaks from each enantiomer, providing excellent potential for direct determinations of enantiomeric excess of samples of 1. As little as 2% of a minor enantiomer should be readily detectable.

INTRODUCTION

The determination of enantiomeric excess has grown in importance in the last twenty years. Such determinations are crucial in studies of enantioselective synthetic techniques and for any examinations of the interactions of molecules with chiral centers. In the field of pharmaceuticals,

determination of stereochemistry and enantiomeric excess of drug samples has become of great concern, since substances may differ dramatically in their pharmacology, potency, toxicity or legal classification depending on the stereochemical configuration. Some important analytical methods using NMR spectroscopic approaches have been based upon chiral shift reagents (1), chiral solvating agents or solvents (2), and chiral derivatizing reagents (3). Chromatographic methods have increasingly included chiral separations by gas chromatography or high performance liquid chromatography, often with chiral stationary phases (4). The available techniques should be regarded as complementary.

One group of pharmaceuticals that has been frequently examined for enantiomeric excess determinations by the various techniques has included succinimides, glutarimides, barbiturates, oxazolidinediones and analogs. These compounds include numerous anticonvulsants and sedative hypnotics of considerable pharmacological importance. Methsuximide, 1, 1,3-dimethyl-3-



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phenyl-2,5-pyrrolidinedione, is an example of this class, finding use as an anticonvulsant in treating some kinds of epilepsy. Liquid chromatographic resolutions on chiral stationary phases have been reported (5). Synthesis of the enantiomers of 1 was achieved (6) and absolute configurations were assigned to the enantiomers (7). Proton NMR data for 1 in $CDCl_3$ (at 100 MHz) have appeared (8). Recently, 1 was included in a study of related compounds (9) using achiral and chiral lanthanide shift reagents (LSR). The results of the latter work did not appear to offer analytical utility for direct enantiomeric excess determinations of 1 with the chiral LSR employed (9) using the 60 MHz NMR spectrometer available earlier. This present investigation employed a

200 MHz spectrometer frequency for ^1H NMR and a different chiral LSR, tris[3-(heptafluoropropylhydroxymethylene)-d-camphorato]-europium(III), 2.

EXPERIMENTAL

Samples of racemic 1 were obtained from Warner-Lambert Co. Pharmaceutical Research Div., Ann Arbor MI, as PD8,670. Chloroform-d (99.8 atom % D), obtained from Aldrich Chemical Corp., Milwaukee WI 53201 or from Wilmad Glass Co., Buena NJ 08310, was dried and stored over 3A Molecular Sieves. Shift reagents were obtained from Aldrich and were stored in a desiccator over anhydrous $CaSO_4$. Materials were used as received except as noted.

Stock solutions of 1 and the shift reagent, 2, were separately prepared in $CDCl_3$ and solutions of 1 with 2 were then prepared volumetrically. Solutions were stored in a freezer at about -13° after preparation and warmed to ambient temperatures prior to spectral acquisition, or spectra were acquired immediately after solution preparation. A Bruker AC200-F Fourier transform

NMR spectrometer equipped with ASPECT 3000 data system was employed for a ^{1}H observe frequency of 200.13 MHz. All these spectra were obtained in the FT mode at ambient probe temperature in a thin-walled 5 mm sample tube. Chemical shifts are reported in δ (ppm) relative to tetramethylsilane (TMS) at 0.00 ppm. TMS was used as an external standard in a sealed capillary. Spectra were acquired using the dual $^{1}H/^{13}C$ probe. Where enantiomeric shift differences were observed (in runs with added shift reagent), the reported chemical shifts are the average values for the two enantiomers. Chemical shifts were obtained from spectral peak tables. Coupling constants and enantiomeric shift differences were determined by subtraction from peak frequency printouts and are believed accurate to $\pm$ 0.1 Hz. Typical FT-NMR parameters were as follows: 3206 Hz spectral width (about -4 to +12 ppm) over 64K data points collected in the quadrature detection mode for a digital resolution of 0.098 Hz per point, pulse width 3.0 μs, 10.22 s acquisition time, 1.0 s relaxation delay; 64 FIDs were accumulated. No

line broadening or resolution enhancement was applied.

DISCUSSION

The 200.132 MHz ^1H spectrum for racemic 1 (0.123 molar in $CDCl_3$) showed signals as follows (δ, ppm): 7.35 (m, 5H, aryl H), 3.13 (d, J=18.3 Hz, 1H, $C\underline{H}_aH_b$), 2.86 (d, J=18.3 Hz, 1H, $CH_a\underline{H}_b$), 3.07 (s, 3H, NCH_3), 1.73 (s, 3H, CCH_3). The methylene protons produce an AB quartet; reported chemical shifts are the algebraic average (not the weighted average) for each doublet's pair of lines. Compared to Nuhn et al. (8), our chemical shifts are all about 0.07 ppm at lower field for the non-aryl protons. Nuhn and coworkers reported geminal coupling of 18.5 Hz which is in reasonable agreement with our value. Some of the differences in shift may be concentration-related; the concentration was not specified in the earlier paper (8).

The effects of added 2 on the ^1H NMR spectra of 1 are summarized in Figures 1 and 2. The LSR functions as a Lewis acid and is expected to preferentially bind to the carbonyl oxygen of an

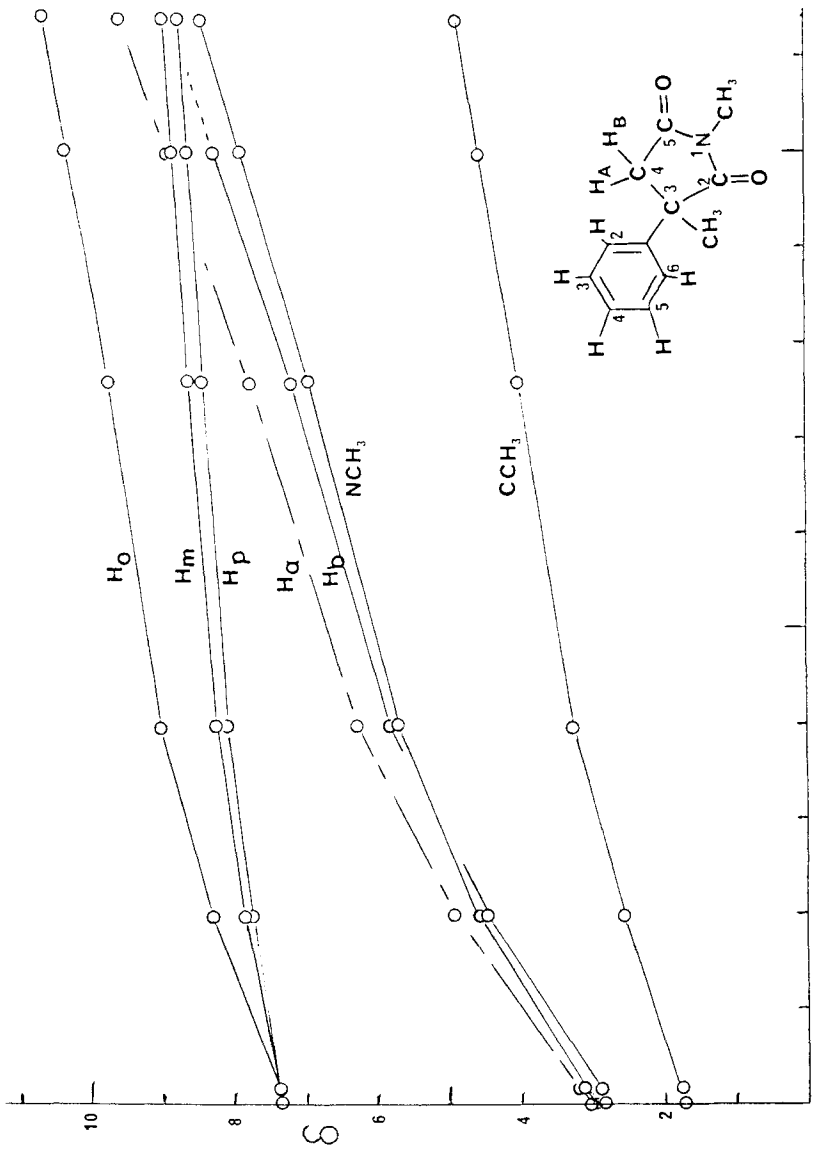

Fig. 1. Variation of chemical shift with molar ratio of 2:1.

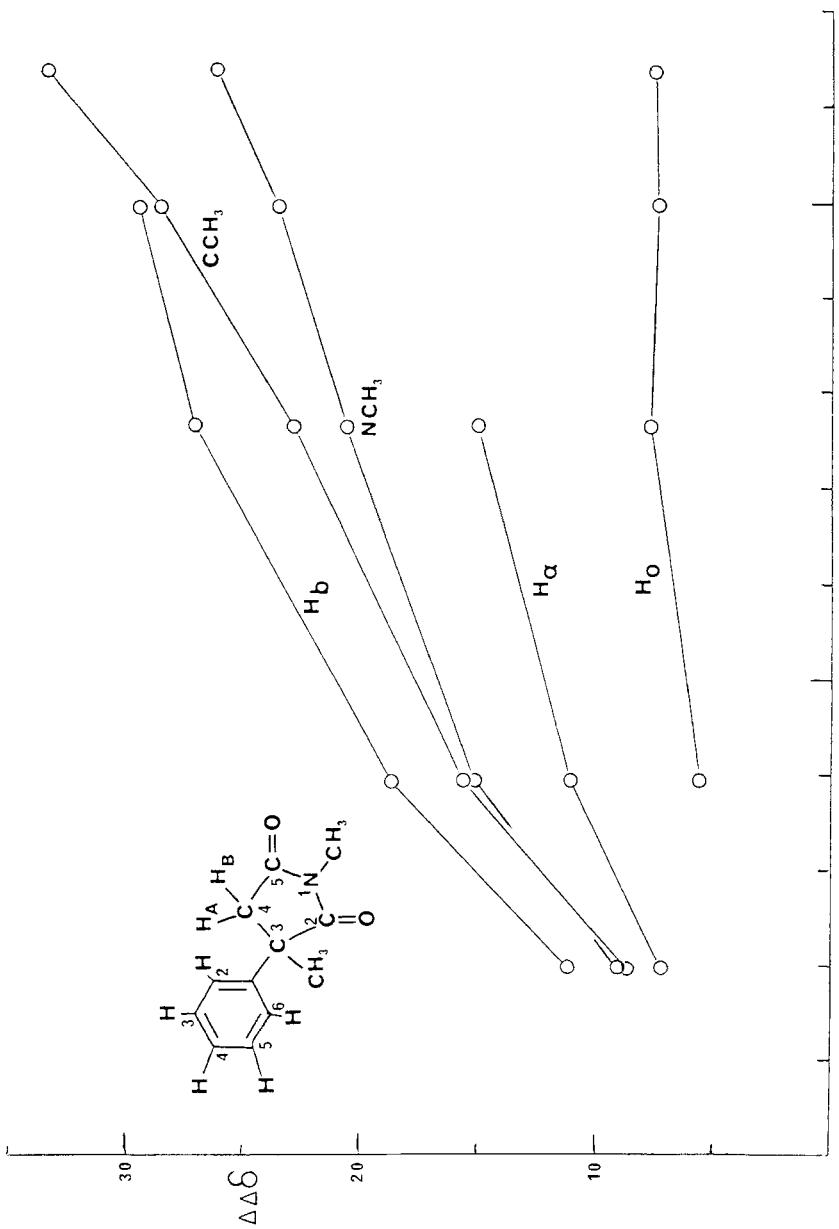

Fig. 2. Variation of enantiomeric shift difference (in Hz) with molar ratio of 2:1.

amide. For imides, fast equilibria should result in LSR binding to both carbonyl oxygens, and observed spectra will reflect fast-exchange limit averaging of free 1 and the different bound complexes of 1 with 2, weighted according to the populations (10). The results presented here show substantial lanthanide-induced shifts (LIS) of 1 by 2 (Fig. 1) as well as enantiomeric shift differences ($\Delta\Delta\delta$, Fig. 2).

The enantiomeric shift differences observed for samples of 1 approximately equimolar with 2 indicate considerable analytical potential for direct determinations of enantiomeric excess of 1, as shown in Figure 3. While both the NCH_3 and CCH_3 protons display nearly baseline resolution between the peaks of each enantiomer, the latter signal is more free from overlaps with other peaks. Both methyl groups are good analytical marker signals (sensor or reporter nuclei) since they are high intensity sharp singlets. The CCH_3 is directly attached to the chiral center while the NCH_3 is two bonds further away, so that the CCH_3 might be expected to exhibit greater magnetic

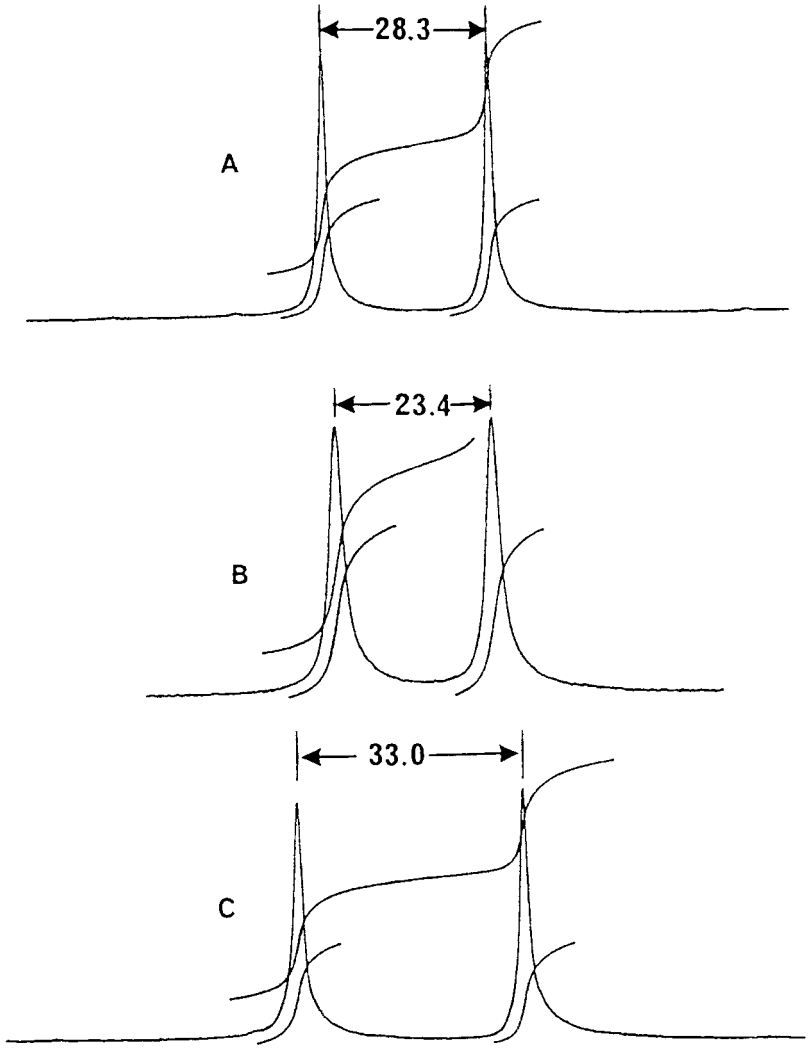

Fig. 3. Spectral expansions at 200 MHz of selected signals of 1 with added 2. For each trace, the observed nucleus, drug molarity, 2:1 molar ratio, and average chemical shift are specified. The enantiomeric shift differences (in Hz) are shown. (a) CCH_3, 0.0600 M, 0.996, 4.65 ppm; (b) NCH_3, 0.0600M, 0.996, 8.01 ppm; (c) CCH_3, 0.0637 M, 1.138, 4.96 ppm.

nonequivalence in the short-lived diastereomeric solvates produced by binding with 2. However, on average, the NCH_3 is closer to the lanthanide at its carbonyl binding sites. The less hindered carbonyl, in fact, constitutes a binding site that is actually rather remote from the methyl at C-3. Larger LIS values are seen for the NCH_3 consistent with closer "average proximity" to the bound lanthanide, but $\Delta\Delta\delta$ magnitudes are qualitatively similar. Suitable cautions should be observed in interpretations that are overly simplistic based on distance arguments, since geometric factors must also be considered (11), and $\Delta\Delta\delta$ magnitudes do not always parallel LIS magnitudes. With the experimental conditions used here, the peaks for the CCH_3 signals of the enantiomers of 1 are sufficiently well-resolved to allow detection of as little as 2% of the minor enantiomer in samples of 1.

Earlier LSR studies of 1 used the achiral reagent, tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium(III), 3, $Eu(FOD)_3$, and the chiral tris[3-trifluoro-

methylhydroxymethylene)-d-camphorato]-europium(III), 4, known as $Eu(TFC)_3$ or $Eu(FACAM)_3$ (9). Higher substrate concentrations and a 60 MHz ^1H NMR were employed (9). With the earlier studies using 4:1 ratios as high as 1.32, no $\Delta\Delta\delta$ was seen for the NCH_3 and the best resolution for the CCH_3 reflected a valley height of ca. 70%. The CCH_3 signal was partly obscured by a peak from 4 at the higher 4:1 ratios (0.5 or more) and peak broadening was a problem. In the present work, instrument sensitivity allowed the use of lower substrate and LSR concentrations, thus reducing viscosity-related line broadening. The use of 2 at the lower concentrations resulted in a CCH_3 signal of 1 completely free from overlaps with peaks of 2 (see below), and the enhanced dispersion of the 200 versus the 60 MHz spectrometer improved the separation between each enantiomer's signal. Finally, LIS values seen here with 2 were roughly twice those reported for 4 (although the other changes in conditions do not allow direct comparisons of these values to be made) (12). The longer perfluoroalkyl group in 2

compared to 4 ($CF_2CF_2CF_3$ versus CF_3) is thought to render 2 a stronger Lewis acid. Other things being equal, this should result in higher binding constants for substrates when 2 is used. With strongly basic substrates, such as amines, reagent 4 may be preferred, but for substrates with lower basicity, such as amides or imides, 2 may be more successful in eliciting useful $\Delta\Delta\delta$ values.

With 2:1 ratios greater than about 0.2, the 200 MHz spectra clearly indicated enantiomeric shift differences for all nuclei except the phenyl meta protons (H_m, aryl H-3,5) and the para proton (H_p, aryl H-4). These protons are relatively remote from the expected europium binding sites. (But note that $\Delta\Delta\delta$ became apparent for even H_m and H_p in runs performed with a 300 MHz spectrometer and a 2:1 ratio near 0.9; see below.) In contrast, the phenyl ortho protons (H_o, aryl H-2,6) are closer to the chiral center and the lanthanide. These latter protons show modest $\Delta\Delta\delta$ which begins to level off for 2:1 ratios above 0.5. It is not unexpected for LIS and $\Delta\Delta\delta$ values to level off with these higher amounts of LSR,

since bound complexes of differing stoichiometries may increasingly occur.

In particular, if 1 is regarded as a bifunctional substrate in which the C-5 carbonyl is a preferred (less hindered) LSR binding site, one might speculate that higher LSR levels would lead to increased LSR occupation of the more hindered C-2 carbonyl site. With an increasing fraction of bound complex in which LSR is near the crowded chiral center, it might be predicted that $\Delta\Delta\delta$ for the CCH_3 would increase (rather than level off) at elevated 2:1 ratios. This appears consistent with our observations (Fig. 2) that show $\Delta\Delta\delta$ leveling off for $H_{a,b}$ and increasing for the CCH_3. However, H_o does not seem to follow the trend for the C-3 methyl.

To establish the "robustness" of this method for potential analytical utility, racemic samples of 1 were examined at different concentrations in $CDCl_3$ with different batches of the LSR 2. Some of these runs were performed with a different spectrometer (300 MHz ^1H frequency, ambient probe temperature) to thoroughly examine the effects of

modest variation of analysis conditions. (Full details are not included here). In all cases, consistent large enantiomeric shift differences were observed for the CCH_3 reporter nuclei which would readily allow enantiomeric excess determinations. However, different samples of 2 resulted in some variation in the location of spectral peaks assigned to the LSR reagent itself. Thus, an LSR peak near 6.5 ppm for one sample of 2 with 1 appeared near 5.1 ppm using a different sample of 2 with similar concentrations and 2:1 ratios. Changing concentration by more than a factor of two, and varying 2:1 ratio by more than 20% (in the region of 2:1 ratio ca. 1) would vary the LSR peak position of a given sample by only a few tenths of a ppm. This suggests that the LSR peak position was most likely batch-related. However, the lanthanide-induced shifts for the CCH_3 are highly dependent on 2:1 ratio, so that modest adjustment of this ratio could readily position the CCH_3 marker signal adequately clear of LSR peaks. The two peaks of the 1 enantiomers' CCH_3 absorption are thus essentially baseline

resolvable over a wide 2:1 ratio (see Figs. 2 and 3) either with a 300 MHz or a lower dispersion 200 MHz spectrometer.

We label the H_a signal as the low field portion of the AB quartet for unshifted 1 which moved downfield faster (displays larger LIS magnitudes) with added LSR, relative to H_b. These observations are consistent with H_a assigned to the proton anti to phenyl and syn to the C-3 methyl. Anisotropic shielding by the phenyl should be greater for H_b, syn to the aryl ring. Different LIS values can be accounted for by an averaged LSR location out of the succinimide ring plane; if the lanthanide favors the side of the ring away from the bulkier (phenyl) group at C-3, the LSR would be closer to H_a and produce greater LIS. These assignments are consistent with those made by Nuhn et al. (8). Enantiomeric shift difference and LIS magnitudes are not always parallel, since the former includes contributions to magnetic nonequivalence due to local chirality and interactions with the chiral LSR; a recent example was illustrated for a lactam with 4 (13)

in which a nucleus exhibiting larger LIS showed smaller enantiomeric shift difference, as is the case here for $H_{a,b}$.

CONCLUSIONS

Methsuximide, in the presence of added chiral LSR, $Eu(HFC)_3$, in $CDCl_3$ solution, exhibits nearly baseline resolution for the CCH_3 signals of the enantiomers in the 200 MHz ^1H NMR spectrum. This degree of resolution indicates the potential for facile direct determinations of enantiomeric excess of 1 with detection of as little as 2% of the minor enantiomer. Analytical utility appears practical over a wide range of 2:1 ratios, from 0.4 - 1.1.

ACKNOWLEDGMENTS

Samples of methsuximide were kindly provided by Warner-Lambert Co. Pharmaceutical Research Div., Ann Arbor MI 48105. Partial support for this work has been provided by the National Science Foundation Instrumentation and Laboratory Improvement Program grant no. USE-8851684, U.S. Department of Education Minority Science Improvement Program grant no. G008641165, Hewlett-

Packard Co. grant no. 0017-80769, U.S. Department of Energy ERLE grants nos. AL-89-169 and AL-90-092, Hoffmann-La Roche Inc., Berlex Labs, Inc., and The Forty-Five Foundation (to R.R.). Additional support was obtained from the Petroleum Research Fund of the American Chemical Society and the Research Corporation (to K.S.V.) and from the University of North Florida for purchase of the Bruker 200 MHz NMR spectrometer.

REFERENCES

1. a) Wenzel T.J. NMR Shift Reagents. FL: CRC Press, 1987.

   b) Morrill T.C. ed. Lanthanide Shift Reagents in Stereochemical Analysis, Methods in Stereochemical Analysis, vol. 5. New York: VCH Publ., 1986.

2. a) Pirkle W.H., Hoover D.J. NMR Chiral Solvating Agents. In: Allinger N.L., Eliel E.L., Wilen S.H. eds. Topics in Stereochemistry, vol. 13. New York: John Wiley - Interscience 1982: 263-331.

   b) Weisman G.R. Nuclear magnetic resonance analysis using chiral solvating agents. In: Morrison J.D. ed. Asymmetric Synthesis, vol. 1. New York: Academic Press 1983: 153-171.

   c) Casy A.F., Mercer A.D. Application of cyclodextrins to chiral analysis by ^{1}H NMR spectroscopy. Magn. Reson. Chem. 1988; 26(9): 765-774.

3. a) Dale J.A., Dull D.L., Mosher H.S. α-Methoxy-α-trifluoromethylphenylacetic acid, a versatile reagent for the determination of enantiomeric composition of alcohols and amines. J. Org. Chem. 1969; 34(9): 2543-2549.

b) Alexakis A., Mutti S., Normant J.F., Mangeney P. A new reagent for a very simple and efficient determination of enantiomeric purity of alcohols by ^{31}P NMR. Tetrahedron:Asym. 1990; 1(7): 437-440.

c) Silks, III, L.A., Dunlap R.B., Odom J.D. Quantitative detection of remotely disposed chiral centers using selenium-77 nuclear magnetic resonance spectroscopy. J. Am. Chem. Soc. 1990; 112(12): 4979-4982.

d) Shapiro M.J., Archinal A.E., Jarema M.A. Chiral purity determination by ^{1}H NMR spectroscopy. Novel use of 1,1'-Binaphthyl-2,2'-diylphosphoric acid. J. Org. Chem. 1989; 54(24): 5826-5828.

4. a) Zief M., Crane L.J., eds. Chromatographic Chiral Separations. Chromatographic Science Series 40. New York and Basel: Marcel Dekker, 1988.

b) Hara S., Cazes J., eds. Optical Resolution by Liquid Chromatography. J. Liq. Chromatogr. 1986; 9(2/3): special double issue.

c) Lough W.J. ed. Chiral Liquid Chromatography Glasgow U.K.: Blackie, 1989.

d) König W.A. The Practice of Enantiomer Separation by Gas Chromatography. Heidelberg: Hüthig, 1987.

5. a) Yang Z.-Y., Barkan S., Brunner C., Weber J.D., Doyle T.D., Wainer I.W. Application of high-performance liquid chromatographic chiral stationary phases to pharmaceutical

analysis. Resolution of enantiomeric barbiturates, succinimides and related molecules on four commercially available chiral stationary phases. J. Chromatogr. 1985; 324: 444-449.

b) Schulze J., König W. Enantiomer separation by high-performance liquid chromatography on silica gel with covalently bound monosaccharides. J. Chromatogr. 1986; 355: 165-175.

6. Knabe J., Koch W. Synthesis of the enantiomers of some disubstituted succinimides. Archiv der Pharmazie 1972; 305(10): 757-765.

7. Knabe J., Koch W. Configuration of some disubstituted chiral succinimides. Archiv der Pharmazie 1972; 305(11): 849-854.

8. Nuhn P., Zschunke A., Woitkowitz P. NMR spectroscopy of drugs. 4. Proton NMR spectroscopic study of some antiepileptic preparations. Pharmazie 1973; 28(7): 484-485.

9. Avolio J., Thomson Eberhart S., Rothchild R., Simons P. ^{1}H NMR spectral simplification with achiral and chiral lanthanide shift reagents. Pt. II: Ethosuximide, 3-Ethyl-3-methyl-2,5-pyrrolidinedione, and comparisons with analogs. Appl. Spectrosc. 1986; 40(4): 531-537.

10. Cockerill A.F., Davies G.L.O., Harden R.C., Rackham D.M. Lanthanide shift reagents for nuclear magnetic resonance spectroscopy. Chem. Rev. 1973; 73(6): 553-588.

11. McConnell H.M., Robertson R.E. Isotropic nuclear resonance shifts. J. Chem. Phys. 1958; 29(6): 1361-1365.

12. Tomić L., Majerski Z., Tomić M., Sunko D.E. Temperature and concentration dependence of the paramagnetic induced shifts in proton

magnetic spectroscopy. J. Chem. Soc., Chem. Commun. 1971; 719-720.

13. Belleney J., Bui C., Carrière F.J. Determination of the enantiomeric purity of disubstituted β-lactams by NMR spectroscopy using a chiral shift reagent. Magn. Reson. Chem. 1990; 28: 606-611.

Date Received: 08/01/91
Date Accepted: 09/04/91