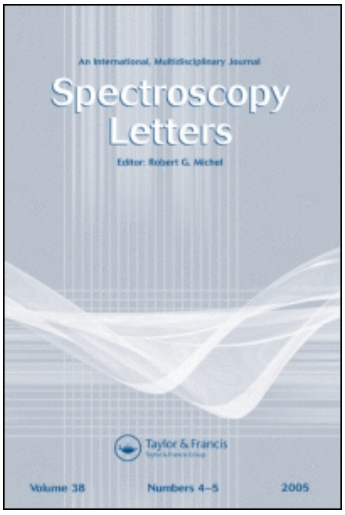

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NMR STUDIES OF DRUGS. ACHIRAL AND CHIRAL LANTHANIDE SHIFT REAGENTS WITH MEPHENOXALONE, A SUBSTITUTED 2-OXAZOLIDINONE.

Key Words: ^{1}H NMR, Eu(FOD)$_3$, Eu(HFC)$_3$, Stereochemistry, Enantiomers, Europium, Muscle relaxant, Optical purity, Analysis, 5-[(2-Methoxyphenoxy)methyl]-2-oxazolidinone.

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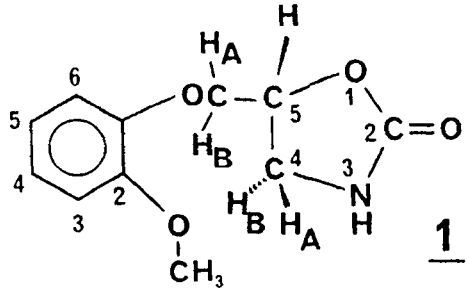
ABSTRACT

The ^1H NMR spectra of the skeletal muscle relaxant, mephenoxalone, 1, 5-[(2-methoxyphenoxy)-methyl]-2-oxazolidinone, have been studied at 200 MHz in $CDCl_3$ solution at 20° in the presence of the achiral lanthanide 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)-(+)-camphorato]europium(III), 3. Both reagents 2 or 3 produced significant lanthanide induced shifts for spectral simplification suggesting major lanthanide binding at the carbonyl. With 3, enantiomeric shift differences were observed for the signals of the NH, OCH_3, methine CHO, both protons of the CH_2O and one proton of the CH_2N, and the aryl proton H-6. Direct determinations of enantiomeric excess of samples of 1 appear feasible by use of 3.

INTRODUCTION

Mephenoxalone, 1, 5-[(2-methoxyphenoxy)methyl]-2-oxazolidinone (1), has been of some interest as a skeletal muscle relaxant (2-4). Studies of metabolites (5) and the mass spectra of metabolites of 1 (6) have been reported.

Achiral and chiral lanthanide shift reagents (LSR) have been employed for ^1H NMR spectral simplification and potential direct determination of enantiomeric excess (ee) of metaxalone (7), which (like 1) is also a 5-substituted 2-oxazolidinone.



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There has been much interest in LSR techniques applied to the broad class of pharmaceuticals that possess five- or six-membered rings possessing amide, imide, urea, carbamate or ester types of functionality within the ring (8). Among such cyclic systems are the drug classes of barbiturates, hydantoins, gluetethimides, succinimides, etc. We wanted to extend these LSR studies to the 2-oxazolidinone ring system of the skeletal muscle relaxant, 1. Examination of this ring system was encouraged by recent concerns regarding the abuse potential of ephedroxane (9),

cis-3,4-dimethyl-5-phenyl-2-oxazolidinone. Basic principles and techniques of LSR use have been described (10-12). In recent years there has been greatly increased interest in techniques for ee determination of pharmaceuticals since drug enantiomers may differ in physiological effects, potencies, toxicities and legal classification. For these investigations, we employed the achiral LSR, tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium(III), 2, known as $Eu(FOD)_3$, and the chiral LSR, tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium(III), 3, known as $Eu(HFC)_3$ or $Eu(HFBC)_3$.

EXPERIMENTAL

Samples of racemic mephenoxalone, 1, were obtained from A.H. Robins Co., Richmond VA 23220 as AHR-233, disp. #85-468. Chloroform-d (99.8 atom % D), obtained from Aldrich Chemical Co., Milwaukee WI 53201, 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. Chemical shifts are reported in δ (ppm) relative to internal tetramethylsilane (TMS) at

0.00 ppm. For typical runs with LSR, an accurately weighed portion of drug was added to $CDCl_3$ (containing a trace of TMS as internal standard) in an oven-dried thin wall 5 mm sample tube and dissolved by shaking; increments of solid shift reagent were added directly to the sample, dissolved by shaking, and the spectra immediately obtained. In runs with chiral LSR where enantiomeric shift differences were observed for selected resonances, reported chemical shifts are the average values for the two enantiomers unless otherwise noted. $CHCl_3$ (present as an impurity in the solvent) served as a secondary internal standard. Spectra were obtained with a Bruker AC200-F Fourier transform NMR spectrometer with ASPECT 3000 data system and a ^1H observe frequency of 200.13 MHz, operating in the FT mode with quadrature detection using the dual $^1H/^{13}C$ probe at ambient temperatures (ca. 20°). Chemical shifts were obtained from spectral peak tables. Observed coupling constants and enantiomeric shift differences were determined by difference from peak frequency listings (unless noted) and are believed accurate to ± 0.2 Hz. Typical FT-NMR parameters were as follows: 4032 Hz spectral width (about -4

to +16 ppm) over 64K data points for a digital resolution of 0.123 Hz per point, pulse width 3.0 μs, 8.13 s acquisition time, 1.0 s relaxation delay; 16 FIDs were accumulated. For enhanced signal-to-noise ratio in some runs with 3, 256 FIDs were accumulated using 4K data points (0.51 s acquisition time, 1.97 Hz per point digital resolution). No line broadening or resolution enhancement was applied.

RESULTS AND DISCUSSION

The ^1H reference spectrum of 1 was recorded as a solution 0.65 % (w/v) in $CDCl_3$ and showed signals as follows (δ, ppm): 6.91 complex mult., 4H (aryl H); 5.47 br. s, 1H (NH); 4.97 m, 1H (oxazolidinone H-5, CHO); 4.21 approx. dd, J (observed) ca. 10.4, 4.7 Hz, 1H (OCH_AH_B); 4.13 approx. dd, J (observed) 10.4, 5.9 Hz, 1H (OCH_AH_B); 3.83 s, 3H (CH_3O); 3.77 approx. t, 1H (oxazolidinone H-4, NCH_A); 3.67 approx. dd, 1H (oxazolidinone H-4, NCH_B). The upfield spectral region is very complex due to a strongly coupled system of five nonequivalent protons of the $OCH_2CH(O)CH_2N$ moiety. The two H-4 protons of the oxazolidinone ring are diastereotopic and anisochronous, being cis or trans with respect to the aryloxymethyl substituent

at the heterocyclic ring 5-position. In addition, the two protons of the exocyclic methylene group are diastereotopic and anisochronous because of the chiral center at C-5 of the oxazolidinone ring. The approximate observed couplings for the CH_2O protons are consistent with a geminal coupling (10.4 Hz) and vicinal couplings to H-5. We have labeled the higher field proton H_A of the CH_2O with the larger vicinal coupling and the downfield H_B with the smaller coupling. For the NCH_2 group, H-$4_{A,B}$, the lower field multiplet for unshifted 1 is designated H_A. The results of adding the achiral $Eu(FOD)_3$ to 1 are summarized in Figure 1.

Although the CH_2N resonance for unshifted 1 is a complex multiplet, the signal appears as a deceptively simple doublet (observed J 7.6 Hz) with a 2:1 molar ratio near 0.75. Higher 2:1 ratios resulted in a high field approximate triplet (observed J ca. 8.8 Hz) and a lower field approximate double doublet (observed J ca. 8.3, 6.7 Hz) at a 2:1 ratio near 1.5. We interpret this as suggesting that the diastereotopic CH_2N protons "cross over" in chemical shifts as LSR is added, with the hydrogen NCH_A at lower field for unshifted 1 and at higher field with 2:1 ratios above 0.75.

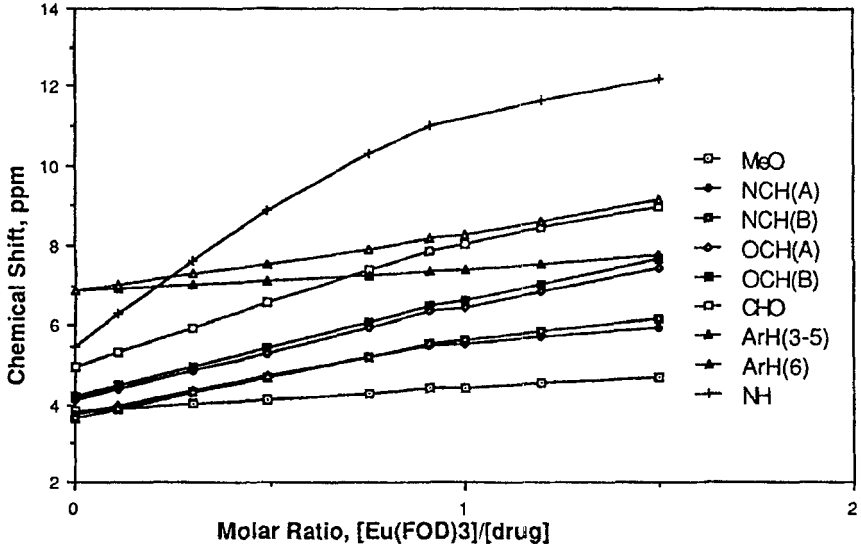

Fig. 1. Variation of chemical shift (in ppm) with molar ratio of 2:1.

When the two protons become isochronous at this molar ratio of 0.75, the simple doublet is observed. Assuming that the cis vicinal coupling constant, H-4 to H-5, is greater than the trans coupling in the five-membered ring heterocycle, we tentatively assign H-4A as cis to H-5 (with roughly equal vicinal and geminal coupling constants). H-4B, at higher field than H-4A for unshifted 1 and lower field with high 2:1 ratios, appears as a gross dd since it is trans to H-5 and the trans vicinal coupling is appreciably smaller than the

geminal coupling to H-4A. The observed J values cited here must certainly deviate from the true values since the system is not first order.

Quite different results are observed for the diastereotopic OCH_2 protons. Each proton appears as a dd for unshifted 1 and with added 2. Rather than crossing over, the two protons OCH_AH_B steadily increase their chemical shift differences with increasing LSR levels. Extraction of apparent (observed) average geminal couplings from 2:1 ratio ratios from 0 to 1.0 gave values ranging from 10.42 (2:1 ratio of 0) to 10.23 Hz (2:1 ratio of 1.0) but the values did not vary monotonically. Variation may simply reflect experimental error and unwarranted first order approximations. The observed vicinal couplings showed greater fluctuation but, again, not monotonic variation. The lower field dd, OCH_AH_B, exhibited average observed vicinal couplings ranging from 4.71 to 5.08 Hz. The higher field dd, OCH_AH_B, displayed average vicinal couplings ranging from 5.66 to 6.01 Hz. Thus, there does not appear to be significant coupling constant variation for 1 with added 2 for these nuclei.

A corresponding series of spectra of 1 were acquired using increments of the chiral reagent 3,

$Eu(HFC)_3$. A nominal concentration of 1 of 0.65% (w/v) in $CDCl_3$ was again used. Variation of chemical shifts for the nuclei of 1 with 3 are summarized in Figure 2. In addition to the lanthanide induced shifts (LIS), 3 also elicited enantiomeric shift differences ($\Delta\Delta\delta$) for several nuclei, as shown in Figure 3. While the gross spectral changes with respect to LIS magnitudes were fairly similar with both 2 and 3, the presence of enantiomeric shift differences with 3 could result in some added spectral complexity due to the potential "doubling" of some signals. In the case of the exocyclic CH_2O group, sixteen lines could be observable corresponding to dd (four line) patterns for each diastereotopic nucleus, OCH_AH_B, of each enantiomer. Nevertheless, we were able to extract both average observed coupling constants (based on first order approximations) as well as $\Delta\Delta\delta$ values for each nucleus, at each 3:1 molar ratio up to 1.0. At the highest 3:1 ratios examined (up to 1.5), LSR-induced line broadening led to less favorable signal-to-noise ratio making it less practical to accurately determine positions of smaller peaks. Somewhat greater variation in observed J values for 3 versus 2 probably reflects

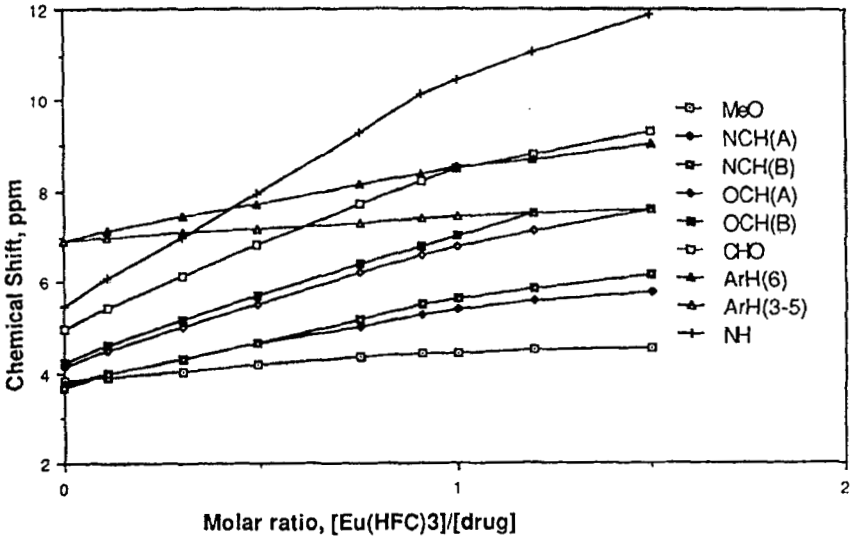

Fig. 2. Variation of chemical shift (in ppm) with molar ratio of 3:1. See text of Discussion for estimation of shifts of NCH_A nucleus.

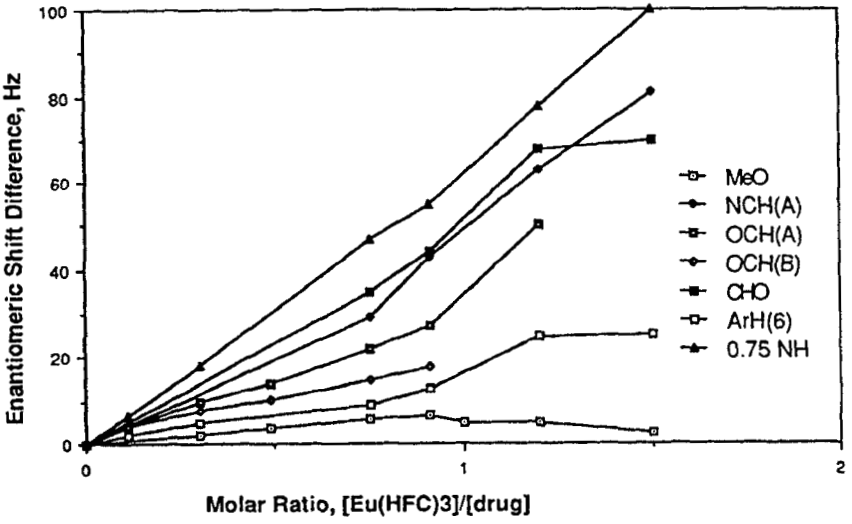

Fig. 3. Variation of enantiomeric shift differences (in Hz, at 200.1 MHz) with molar ratio of 3:1. Note: The actual observed enantiomeric shift differences for the NH signal have been reduced by multiplication with a scaling factor of 0.75 to keep the plot on scale. The $\Delta\Delta\delta$ values for the NCH_A nucleus have been estimated as described in the text. See Discussion section.

this. Our average observed geminal couplings for the CH_2O group with 3 ranged from 10.00 to 10.48 Hz with 3:1 ratios from 0.0 to 1.0. The higher field dd signals, OCH_A, exhibited observed vicinal couplings ranging from 5.76 to 6.32 Hz. The lower field signals, OCH_B, showed vicinal couplings from 4.24 to 5.31 Hz over the same range of 3:1 ratios. In no case did the observed couplings of the CH_2O vary monotonically with LSR level and we ascribe these variations, as noted above with 2, to experimental error due to digital resolution limits and peak position uncertainties (as well as to deviations from first order spectra). The higher field nucleus, OCH_A, with larger vicinal coupling to the methine OCH (H-5), displayed appreciably larger $\Delta\Delta\delta$ magnitudes than the diastereotopic OCH_B.

For the H-4 protons, NCH_AH_B, the absorptions were less well resolved. But at 3:1 ratios from 0.75 to 1.5, an approximate triplet was seen at the higher field portion of the NCH_2 region, integrating to 0.5H. We tentatively assign this to NCH_A of one of the optical antipodes of 1. The diastereotopic NCH_B remains incompletely resolved in a complex multiplet with the NCH_AH_B signals of the other enantiomer. For the NCH_A "triplet," the

chemical shift values plotted in Fig. 2 are those for the higher field enantiomer's signal, rather than the average of shifts for both enantiomers. The $\Delta\Delta\delta$ values of Fig. 3 for the NCH_A are estimates as measured from the resolved 0.5H upfield triplet of one enantiomer to the center of the 1.5H intensity multiplet of the remaining NCH_AH_B absorption. Due to extra signal complexity from enantiomeric shift differences with 3, the crossing-over of chemical shifts for the diastereotopic NCH_2 protons is less clearcut than with 2. With 3, the apparent observed couplings of the resolved enantiomer's triplet NCH_A signal ranged from 8.72 to 9.23 Hz; as above, the variations were not monotonic and are assumed to result from experimental error and second order effects.

Most important, from the standpoint of analytical utility for determination of % ee, are the significant $\Delta\Delta\delta$ values for several of the nuclei of 1 with added 3. Clear $\Delta\Delta\delta$ is seen for the NH, methine HCO (H-5), NCH_A (H-4), both protons of the OCH_2, CH_3O, and for one of the aryl protons (assigned as aryl H-6). For several nuclei, near-baseline resolution is achieved (Figure 4)

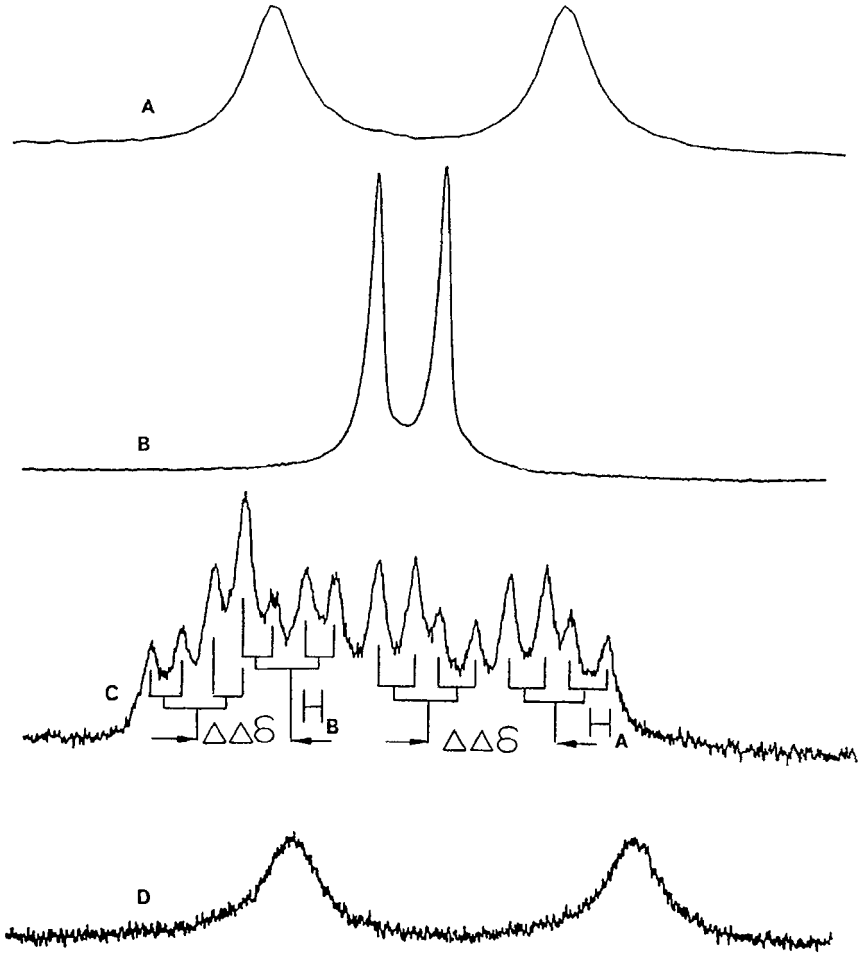

Fig. 4. Spectral expansions showing enantiomeric shift differences for selected protons in 1 with added 3 under specified conditions: observed nucleus, average chemical shift in ppm [enantiomeric shift difference in Hz], (3:1 molar ratio); a) NH, 9.26 [62.5 Hz] (0.75); b) CH_3O, 4.40 [6.4 Hz] (0.91); c) OCH_AH_B: 6.21 [21.7 Hz] for H_A; 6.41 [15.0 Hz] for H_B; (0.75); d) NH, 10.12 [73.2 Hz] (0.91). For trace (a), 256 FIDs were acquired at a digital resolution of 1.97 Hz/point; for (b), (c) and (d) 16 FIDs with a resolution of 0.123 Hz/point were used.

suggesting excellent analytical potential for % ee measurements. For the NH signal, $\Delta\Delta\delta$ of 133 Hz was seen with a 3:1 molar ratio of 1.5. (Because of these very large values, a scaling factor of 0.75 has been applied to the plotted $\Delta\Delta\delta$ values of the NH signal in Fig. 3 to keep them on scale, as noted by the label "0.75 NH.") Despite the broadness of the NH peaks, presumably due to ^{14}N quadrupole broadening and LSR-induced line broadening, this signal is free from interferences and should be analytically useful for 3:1 molar ratios ca. 0.91. Signal-to-noise ratio improvement is readily achieved in a reasonable time period by acquiring additional FIDs at somewhat coarser digital resolution (Fig. 4). The methoxy signal is a sharp high intensity singlet with excellent potential for % ee analyses for sub-milligram samples of 1 because of favorable signal-to-noise ratio, but the enantiomer signals are not fully resolved by 3, with a minimum valley height of about 17.6% between the peaks of each enantiomer for 3:1 ratios ca. 0.9-1.0. Nevertheless, the observed $\Delta\Delta\delta$ for the methoxy is striking since these protons are seven bonds from the chiral center at C-5 of the heterocyclic ring and ten bonds from the presumed

major LSR binding site on the carbonyl oxygen. With adequate amounts of 1 (a few mg), the NH signal is most suitable for % ee measurements because of low valley heights separating the enantiomer signals and freedom from interfering peaks.

Table 1 presents the relative slopes of the lanthanide-induced shifts versus LSR:1 molar ratios for the nuclei of 1 based on the data of Figs. 1 and 2, using a linear least squares line fitting. The values were calculated for the linear portions of the plots from a molar ratio of 0.0 up to 0.49 or more, so that 4-6 experimental points could be used for each nucleus. In all cases, a value for R=1.00 was obtained, indicating quite good linearity. Some leveling off was seen in the plots at LSR:1 ratios above 1. In addition to the unnormalized values of slope, values are also shown that are normalized to the value of the slope for the methoxy group. This group was chosen as the reference since its chemical shift is measurable with good accuracy (because it is a sharp singlet) and it is remote from the expected major LSR binding site at the carbonyl (10,12). Comparisons of the normalized slopes for LSRs 2 and 3 show

Table 1. Slopes[a] of Lanthanide-Induced shifts (LIS) versus Molar Ratios of [LSR]/[drug] for Nuclei of Mephenoxalone

	$Eu(FOD)_3$ data		$Eu(HFC)_3$ data	
Nucleus	Unnormalized	Normalized, note (e)	Unnormalized	Normalized, note (e)
CH_3O	[d]0.603	1.0	[b]0.694	1.0
NCH_A	[c]1.899	3.15	[b]1.789	2.58
NCH_B	[d]1.995	3.31	[d]1.955	2.82
OCH_A	[d]2.391	3.97	[c]2.756	3.97
OCH_B	[d]2.489	4.13	[c]2.911	4.19
CHO	[d]3.194	5.30	[b]3.762	5.42
ArH(3-5)	[c]0.464	0.77	[b]0.573	0.83
ArH(6)	[c]1.343	2.23	[d]1.609	2.32
NH	[b]6.961	11.54	[d]5.067	7.30

Notes: a) Slopes based on linear least-squares line fitting. R=1.00 for all calculated lines. See text in Discussion.
b) Slopes based on four experimental points, molar ratios 0-0.49.
c) Slopes based on five experimental points, molar ratios 0-0.75.
d) Slopes based on six experimental points, molar ratios 0-0.91.
e) Normalized valves based on a value of 1.0 for the slope of the CH_3O signals for each LSR.

remarkably good agreement for all nuclei of 1 except for the NCH_2 protons (oxazolidinone H-4) and the NH. For the NCH_2 protons, LSR 2 gives normalized slopes no more than 22% greater than those with 3, but the NH value is much greater with 2. We suggest that these differences for 2 versus 3 may reflect Fermi contact shift contributions to the induced shifts due to the proximity of the NH and NCH_2 nuclei to the presumed major LSR binding site on the carbonyl oxygen (13,14). That the bound complexes of 1 with either 2 or 3 are essentially isostructural is suggested by two observations: a) normalized slopes for all nuclei except NH and NCH_2 agree very closely for the two LSRs (Table 1), and b) major monotonic variations of observed coupling constants for the exocyclic OCH_2 protons are not seen with addition of either LSR. The latter observation would tend to rule out LSR-induced conformational changes involving the OCH_2 portion of 1 and suggests that bidentate chelation of LSR at the OCH_2 moiety is probably not significant for 1. The relative (normalized) LIS magnitudes for each LSR decrease in the order NH > H-5 (CHO) > OCH_2 > NCH_2 > aryl H-6 > OCH_3 > aryl H-3,4,5 which is qualitatively consistent with the

carbonyl oxygen as the major LSR binding site (10,12).

CONCLUSIONS

The 200.1 MHz ^1H NMR spectra of mephenoxalone, 1, have been studied in dilute $CDCl_3$ solution at ca. 20° with the achiral LSR, $Eu(FOD)_3$, 2, and the chiral LSR, $Eu(HFC)_3$, 3. Both LSRs elicited significant lanthanide-induced shifts. The LIS values are interpreted as consistent with major LSR binding at the carbonyl oxygen and with bound complexes of 1 with either 2 or 3 that were essentially isostructural. Enantiomeric shift differences produced with 3 added to 1 w observed for several nuclei, with nearly baseline-resolved NH signals for each enantiomer of 1 using 3:1 molar ratios near 0.9. This should offer considerable potential for direct % ee determination of 1.

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REFERENCES

1. Lunsford C.D., Mays R.P., Richman, Jr. J.A., Murphey R.S. 5-Aryloxymethyl-2-oxazolidinones. J. Amer. Chem. Soc. 1960; 82: 1166-1171.

2. Bottari F., Saettone M.F., Serafini M.F., Tellini N. The preparation and preliminary pharmacological evaluation of some oxazolones related to mephenesin carbamate. Farmaco, Ed. Sci. 1969; 24(7): 672-684.

3. Savel J. New medicines. Prod. Probl. Pharm. 1964; 19: 610-612.

4. Parés J., Bultó I., Parés R., Villarroya T., Martí M., Langer M. Structure of mephenoxalone (OM518). Chim. Ind. (Paris) 1963; 89(3): 293-298.

5. Eckhardt G., Goenechea S., Gielsdorf W. Isolation and identification of some metabolites of mephenoxalone (control-OM) from human urine. J. Clin. Chem. Clin. Biochem. 1977; 15(3): 115-120.

6. Gielsdorf W. Mass spectrometric characteristic data for some drugs, their biotransformation products, as well as intermediate products in illegal narcotic syntheses. Arch. Kriminol. 1983; 172(1-2): 41-51.

7. Hatzis A., Rothchild R. ^{1}H NMR spectral simplification with achiral and chiral lanthanide shift reagents. IV. Metaxalone, 5-(3,5-dimethylphenoxymethyl)-2-oxazolidinone; Evidence for multidentate chelation. Spectrosc. Lett. 1986; 19(8): 939-951.

8. Aboul-Enein H.Y. NMR methods for optical purity determination of pharmaceuticals. Analytical Lett. 1988; 21(12): 2155-2163.

9. Spurlock L., Kopec R., Morris W.A. Identification of ephedroxane: a new drug of abuse? Private communication, U.S. Drug Enforcement Administration, Forensic Sciences Section, March 1992.

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. Morrill T.C. ed. Lanthanide Shift Reagents in Stereochemical Analysis New York: VCH, 1986.

12. Wenzel T.J. NMR Shift Reagents. Boca Raton FL: CRC Press, 1987.

13. Peters J.A., Nieuwenhuizen M.S., Raber D.J. Analysis of multinuclear lanthanide-induced shifts. 1. Investigations of some approximations in the procedure for separation of diamagnetic, contact, and pseudocontact shifts. J. Magn. Reson. 1985; 65: 417-428.

14. Raber D.J., Peters J.A., Nieuwenhuizen M.S. Analysis of multinuclear lanthanide-induced shifts. Part 2. The geometry of ketone binding to lanthanides. J. Chem. Soc. Perkin Trans. II 1986; 853-859.

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