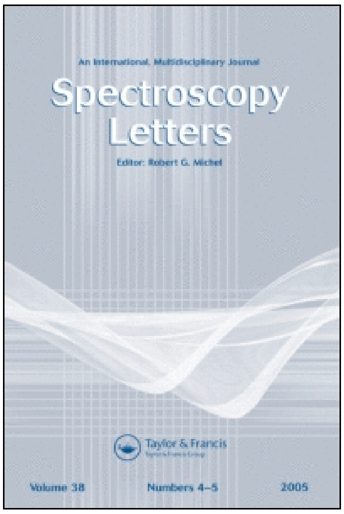

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NMR Studies of Drugs. Applications of Achiral and Chiral Lanthanide Shift Reagents to Medetomidine. Observations of "Anomalous Shifts."

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NMR STUDIES OF DRUGS. APPLICATIONS OF ACHIRAL AND CHIRAL LANTHANIDE SHIFT REAGENTS TO MEDETOMIDINE. OBSERVATIONS OF "ANOMALOUS SHIFTS."

Key Words: LSR, Europium, Eu(FOD)$_3$, Eu(HFC)$_3$, Eu(FACAM)$_3$, 4-[1-(2,3-Dimethylphenyl)ethyl]-1H-imidazole, α_2-Adrenoceptor agonist, Sedative, Analgesic, Anxiolytic, Enantiomers, Analysis, Upfield shifts, Lanthanide-induced shifts.

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ABSTRACT

The 60 MHz ^{1}H NMR spectra of the potent, selective and specific α_2-adrenoceptor agonist, medetomidine, have been studied in CDCl$_3$ at 28±1° for the racemic free base, 1, with the added achiral lanthanide shift reagent (LSR), tris(6,6,7,7,8,8,8-

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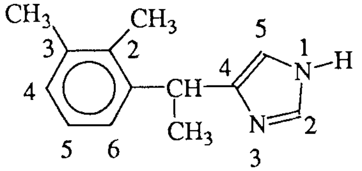
heptafluoro-2,2-dimethyl-3,5-octanedionato)europium(III), 2, for spectral simplification, and with the chiral LSRs, tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium(III), 3, and tris[3-(trifluoromethylhydroxymethylene)-(+)-camphorato]europium(III), 4, for potential enantiomeric shift differences. Substantial lanthanide-induced shifts (LIS) were observed with each added LSR, consistent with LSR binding at the basic imidazole nitrogen. The three LSRs gave distinctly different LIS results for the NH signal, with substantial "anomalous" (upfield) shifts observed with 2 and smaller anomalous shifts produced with 3, at low [LSR]:[1] ratios; normal downfield shifts resulted at higher LSR levels of 2 or 3. With LSR 4, only consistent "normal" downfield shifts were seen for the NH signal.

INTRODUCTION

The compound medetomidine appears to be a novel, potent, specific and selective α_2-adrenoceptor agonist with activity as a sedative, analgesic, or anxiolytic in various animal species (1-6). The racemic compound, 1, has been resolved into the enantiomers (1,7,8) and the (+)-*dextro* enantiomer appears to possess nearly all of the α_2-agonistic activity (1,5). Crystal structures were determined for the hydrochloride salt of racemic 1 (2) as the hydrate or anhydrous forms, and the crystal structure and absolute configurations for dexmedetomidine (the *dextro* enantiomer of 1) and its tosyl derivative were determined (8). The (+)-enantiomer of 1 was assigned the *S* configuration (8).



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For some time, we have been interested in the use of lanthanide shift reagents (LSR) for NMR spectral simplification, and, with chiral LSR, for potential direct determinations of enantiomeric excess (% e.e.) of chiral compounds. These subjects have been reviewed (9-12). Since 1 provided the opportunity to study LSR binding in an intriguing substrate with the 4-arylalkyl imidazole heterocyclic structure, we proceeded to examine the 60 MHz ^{1}H NMR of 1 with 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 with the chiral LSRs, tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium(III), 3, known as $Eu(HFC)_3$ or $Eu(HFBC)_3$, and tris[3-(trifluoromethylhydroxymethylene)-(+)-camphorato]europium(III), 4, known as $Eu(FACAM)_3$ or $Eu(TFC)_3$.

EXPERIMENTAL

Samples of the racemic free base of 1 were kindly provided by Orion Corporation, Orion-Farmos, FIN-90650 Oulu, Finland. Chloroform-d and shift reagents were obtained from Aldrich Chemical Corp., Milwaukee WI 53201. Materials were used as received except as noted. $CDCl_3$ was dried and stored over 3Å molecular sieves; LSR reagents were stored in a desiccator over P_2O_5. For runs with shift reagents, accurately weighed portions of drug were added to weighed solvent in an NMR sample tube and dissolved by shaking. Increments of solid LSR were added directly to the sample, dissolved by shaking, and the spectra immediately obtained using a Varian EM360A 60 MHz NMR spectrometer with EM3630 lock/spin decoupler accessory. Probe temperature was 28±1°. Tetramethylsilane (TMS, ca. 1%) was used as internal reference at 0.00 ppm. Chemical shifts are believed accurate to ±0.05 ppm and coupling constants to ±0.2 Hz.

RESULTS AND DISCUSSION

The racemic free base medetomidine, 1, 0.364 molal in $CDCl_3$, displayed the following ^1H spectrum (δ in ppm relative to TMS at 0.00 ppm): 1.53, 3H, d ($^3J \sim 6.7$ Hz), $\underline{CH_3}CH$; 2.21, 3H, s, aryl 3-methyl; 2.14, 3H, s, aryl 2-methyl; 4.33, 1H, q ($^3J \sim 6.7$ Hz), methine $\underline{CH}CH_3$; 6.97, 3H, approx. s, aryl H-4,5,6; 6.62, 1H, s, imidazole H-5; 7.21, 1H, s, imidazole H-2; 12.33, 1H, slightly broad s, NH. The imidazole protons H-2 and H-5 are assigned based on expected lower field shifts for H-2, flanked by two electronegative nitrogen atoms, than for H-5, adjacent to only one nitrogen, N-1 (13). The two methyl groups at C-2 and C-3 of the benzene ring are assigned based on lanthanide-induced shift (LIS) magnitudes with added LSR (see below). The lowest field resonance, of the imidazole N-1 hydrogen, appeared slightly concentration dependent. Thus, a less concentrated solution of 1, 0.296 molal, displayed the NH absorption at higher field, i.e., 11.70 ppm. This is consistent with the expectation of a more deshielded signal resulting from more hydrogen-bonding with higher concentration of 1 (14). Addition of increments of Eu(FOD)$_3$, 2, to 0.364 molal 1 resulted in the induced shifts summarized in Figure 1. The NH absorption exhibited an "anomalous" (upfield) shift over the 2:1 molar ratio range from zero to 0.177, with the NH appearing at 11.21 ppm at the latter molar ratio. This represents a substantial $\Delta\delta$ of 1.1 ppm to *higher* field for the NH signal. All the remaining (carbon-bound) proton resonances exhibited the normal downfield shifts expected with tris-ß-diketonate LSRs derived from europium(III) in low-polarity organic solvents (15). With 2:1 ratios above 0.177, the NH signal begins to move rapidly downfield and broadens dramatically. At the highest 2:1 ratios examined, 0.658, the three benzene ring protons, H-4,5,6, which are essentially isochronous for unshifted 1 (at 60 MHz), are resolved, with H-6 moving downfield fastest.

With additions of the chiral Eu(HFC)$_3$, 3, to 0.296 molal 1, LIS values were observed, summarized in Figure 2. The NH signal displayed small anomalous shifts over the 3:1 molar ratio range from zero (11.70 ppm) to 0.0648 (11.50 ppm), an upfield shift of only ca. 0.2 ppm. With 3:1 ratios of 0.115 or more, normal downfield shifts were seen for the NH. No discernible enantiomeric shift differences, $\Delta\Delta\delta$, were seen for the doublet $\underline{CH_3}$CH signal (directly attached to the chiral center of 1) or for the imidazole protons (close to the expected lanthanide binding site). Under the experimental conditions employed here, $\Delta\Delta\delta$ for the aryl H-6 signal could not be confirmed due to limitations imposed by signal-to-

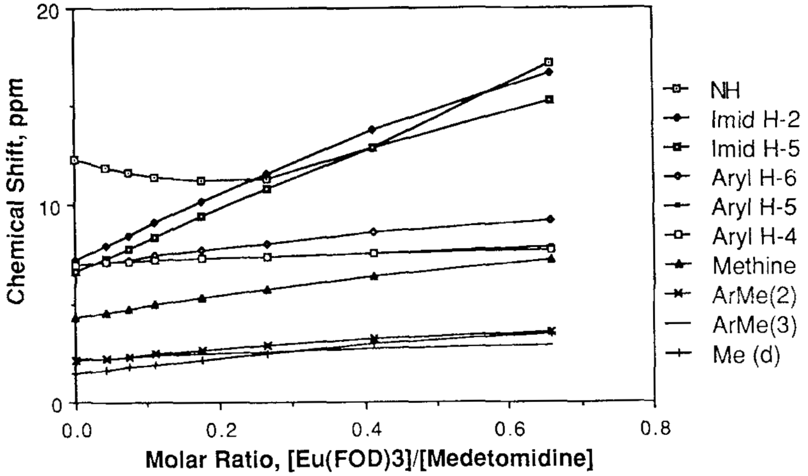

Figure 1. Variation of chemical shifts (δ, ppm) for nuclei of 1 with molar ratio of $[Eu(FOD)_3]:[1]$.

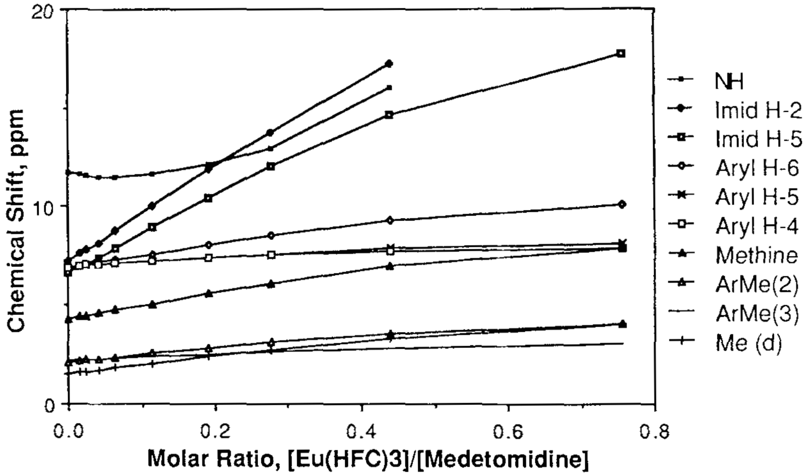

Figure 2. Variation of chemical shifts (δ, ppm) for nuclei of 1 with molar ratio of $[Eu(HFC)_3]:[1]$.

noise ratio (S/N) and lanthanide-induced line broadening.

With the addition of the chiral LSR, 4, Eu(FACAM)$_3$, to 0.306 molal 1, induced shifts were elicited as summarized in Figure 3. No $\Delta\Delta\delta$ was observed with 4 under our conditions. In contrast to results with 2 or 3, no anomalous shifts were seen for the NH resonance with added 4. Thus, significantly different behavior appears for the NH signal with each of these three LSRs, ranging from appreciable anomalous shift magnitudes (with 2), to modest magnitudes (with 3), to exclusive "normal" shifts (with 4). Anomalous shifts may be accounted for by geometric factors, for example, reflecting the angular part of the pseudocontact (dipolar) term in the simplified McConnell-Robertson equation (16), depending on the geometry in the bound complex of substrate with lanthanide. The changeover from anomalous upfield shifts to normal downfield shifts at higher LSR levels may reflect changing contributions from bound complexes of differing stoichiometry or geometries (e.g., different conformations) with the different LSRs; such effects may be subtle. Alternatively or additionally, some Fermi contact (through-bond) contribution (15,17) may be invoked, particularly in the case of the quadrupolar ^{14}N nuclei in the substrate, 1, with the nucleus displaying the anomalous shift being attached to nitrogen and proximal to the presumed LSR binding site. The differences in the NH anomalous shifts with three superficially similar europium(III) LSRs was unexpected. We also note that the carbon-bound imidazole protons, H-2 and H-5, broaden considerably

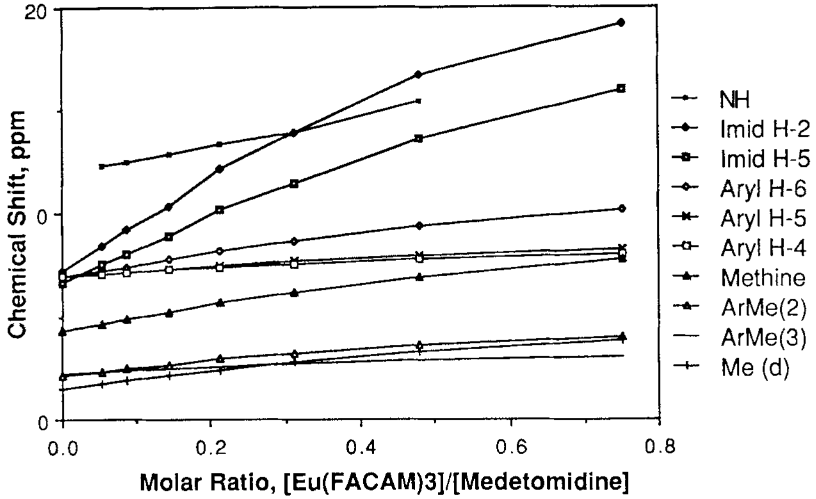

Figure 3. Variation of chemical shifts (δ, ppm) for nuclei of 1 with molar ratio of [Eu(FACAM)$_3$]:[1].

even at lower levels of LSR, while the NH absorption exhibits sudden and substantial broadening only at the higher LSR levels. In part, these results may reflect exchange-broadening effects due to NH exchange and tautomerization of the imidazole ring, as well as the possible contributions of two different bound complexes with LSR bound either to N-1 or N-3 in the two possible tautomers of 1. Some potential effects of exchange rate of ammonium NH protons were considered for the NMR of etidocaine hydrochloride in CDCl$_3$ (18), and for exchange/tautomerization in the 4,5-dihydro-1H-imidazole, lofexidine (19) (with added LSR).

In Table 1 we have shown the unnormalized ("raw") slope values for the different protons of 1

Table 1. Slopes of lanthanide-induced shifts vs. molar ratios of [LSR]:[medetomidine] for nuclei of 1 with added 2,3 or 4. (See note 1.)

	$Eu(FOD)_3$ data		$Eu(HFC)_3$ data		$Eu(FACAM)_3$ data	
Nucleus	Unnorm.	Normalized	Unnorm.	Normalized	Unnorm.	Normalized
NH	-9.052	-1.700	-7.576	-1.136	6.908	1.111
(note 2)	15.31	2.875	16.00	2.400	-	-
Imid. H-2	16.57	3.111	23.64	3.545	23.02	3.704
Imid. H-5	15.95	2.995	19.13	2.869	16.63	2.676
Aryl H-6	4.162	0.781	5.688	0.853	5.716	0.919
Aryl H-5	2.120	0.398	3.118	0.468	2.114	0.340
Aryl H-4	2.120	0.398	3.118	0.468	1.848	0.297
Methine	5.326	1.0	6.669	1.0	6.217	1.0
ArMe(2)	3.017	0.566	3.605	0.541	3.687	0.593
ArMe(3)	1.711	0.321	1.763	0.264	1.690	0.272
Methyl(d)	3.788	0.711	4.676	0.701	4.250	0.684

Notes: (1) Slopes are based on least-squares line fitting from data of Figs. 1,2, and 3. Normalized values are given relative to a value of 1.0 for the slope of the line for the signals assigned tc the methine CH resonance. See Results and Discussion for numbers of experimental points used in determining line equations and the correlation coefficients, R. (2) The two sets of values shown for NH with LSRs 2 and 3 reflect slopes calculated at low LSR levels (negative slope values) and high LSR levels (positive slope values) for the two LSRs which elicited "anomalous" (upfield) shifts for this nucleus. Only the slope values for low LSR levels are indicated for 4 since no anomalous shifts were observed with this reagent.

with 2, 3 or 4, in addition to normalized slope values relative to slopes of 1.0 assigned for the lines calculated from resonances assigned to the methine CH. These slopes reflect the data of Figs. 1-3. The methine signal was selected for referencing for two reasons: a) the LIS magnitudes for the CH proton were the largest for any proton in 1 (with the exception of the imidazole ring protons) so that experimental errors due to chemical shift uncertainties should be relatively small; and b) since the methine CH is not directly on the imidazole ring and is not conjugated with the ring, it can probably be safely assumed that the LIS for the methine results almost exclusively from pseudocontact (dipolar) shifts with negligible contributions to the LIS from any Fermi contact shifts (17,20,21). Except for the NH signal, even the unnormalized slope values for the nuclei of 1 are qualitatively similar with all three LSRs, consistent with a strongly coordinating substrate that exhibits little selectivity in binding to the different LSRs, as expected for a basic imidazole functionality. The large slope magnitudes for the imidazole ring's carbon-bound protons confirm lanthanide coordination at this heterocyclic ring and support high binding constants between 1 and each LSR. If the normalized slope values are compared, agreement appears excellent between all three LSRs for all protons (other than imidazole ring protons) with the normalized slopes varying by less than ±0.1 from 2 to 3 to 4. On a relative basis, excellent agreement is also obtained for the imidazole H-5; only the imidazole H-2 shows moderate variations of the carbon-bound protons. Possible Fermi contact shift contributions and geometric (angular) factors for the NH and imidazole H-2 seem to be the most likely explanation (11,17). If proton tautomerization occurs between N-1 and N-3, rendering both nitrogens available for lanthanide binding, the imidazole H-2 would be proximal to coordinated europium in both of the possible bound complexes, whereas H-5 is similarly close only if europium binds at N-1. The slightly greater normalized slopes seen for imidazole H-2 versus H-5 may reflect preferential LSR binding at N-3 or may be consistent with comparable binding at both nitrogens.

Assignments for the aryl H-4,5,6 protons and for the aryl 2-methyl vs. 3-methyl were based on larger normalized slopes expected for the aryl H-6 and 2-methyl protons, based on proximity to LSR bound on the imidazole moiety.

For the NH resonance, slopes were calculated with both low and high LSR levels to evaluate regions of anomalous (upfield) shifts with _2_ or _3_ (where slopes were negative) as well as regions of normal downfield shift; only the low molar ratio levels were used for slope calculations with _4_ since anomalous shifts were not seen in this case. Rather good correlation coefficients (R values) were generally obtained using 3-6 experimental points for the line equations. Thus, for _2_, R=1.00 was obtained for all nuclei except NH at high LSR levels (R = 0.99, _2_:_1_ ratios from 0.27-0.66) and aryl H-6 (R=0.99). With _3_, R values were 1.00 except for NH (R=0.97 for _3_:_1_ ratios from 0-0.02; R=0.99 for ratios 0.19-0.44); imidazole H-2 and aryl 2-methyl (R=0.99); aryl H-4,5 and aryl 3-methyl (R=0.98). Smaller LIS magnitudes leading to greater experimental errors presumably cause the poorer correlations of the aryl H-4,5 and aryl methyls, reflecting their remote locations, distal to bound lanthanide. R values of 1.00 were attained for all slopes with 4. Slopes were generally calculated for the steepest parts of the curves at low [LSR]:[1] molar ratios, before any apparent "leveling off" could be seen; these molar ratios were usually less than ca. 0.2 and indicate "most positive" slope values (except for NH with 2 or 3). Overall, a high degree of isostructurality in the bound complexes of 1 with the three LSRs is implied. Relatively large slope magnitudes for aryl H-6 vs. aryl 2-methyl suggests qualitative agreement with the conformation reported for the crystal free base of dexmedetomidine (8), in which the aryl H-6 is directed back towards the imidazole moiety and the aryl methyls are directed away. In addition, the slope for the aryl H-6 is greater than for the doublet methyl protons although the H-6 proton is one bond further from bound lanthanide.

CONCLUSIONS

The 60 MHz ^1H NMR spectra of racemic medetomidine free base, 1, in $CDCl_3$ solution, have been studied with the added LSRs, $Eu(FOD)_3$, 2; $Eu(HFC)_3$, 3; and $Eu(FACAM)_3$, 4. The lanthanide-induced shifts for each hydrogen of 1 are presented, together with the normalized relative slopes for the plots of chemical shift versus [LSR]:[1] molar ratio, based on least squares line fitting. Notable differences with the three LSRs are seen with respect to LIS behavior for the NH resonance, with 2 causing substantial anomalous (upfield) shifts and 3 causing smaller anomalous shifts, with low [LSR]:[1] molar ratios. With [LSR]:[1] ratios above ca. 0.1-0.2, both 2 and 3 cause considerable downfield shifts for the NH signal. In contrast, 4 elicits exclusive downfield shifts of the NH resonance over the entire range of LSR levels studied. Substantial lanthanide-induced line broadening of the imidazole H-2 and H-5 occurs, starting even with low LSR levels. The NH absorption more abruptly broadens, at moderate or higher LSR levels. Possible chemical exchange broadening associated with NH exchange and resulting altered LSR binding sites on the imidazole tautomers may be involved. No useful enantiomeric shift differences were seen with the chiral LSRs, 3 or 4.

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