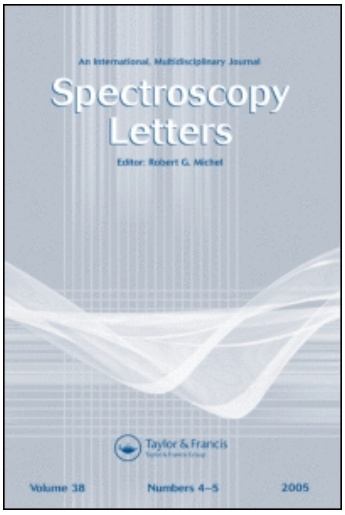

Spectroscopy Letters

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

1H NMR Spectral Simplification with Achiral and Chiral Lanthanide Shift Reagents. Clenbuterol. Method for Direct Optical Purity Determination

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To cite this Article Martin, Rolf and Rothchild, Robert(1989) '1H NMR Spectral Simplification with Achiral and Chiral Lanthanide Shift Reagents. Clenbuterol. Method for Direct Optical Purity Determination', Spectroscopy Letters, 22: 5, 511 — 532
To link to this Article: DOI: 10.1080/00387018908053901
URL: http://dx.doi.org/10.1080/00387018908053901

SPECTROSCOPY LETTERS, 22(5), 511-532 (1989)

^{1}H NMR SPECTRAL SIMPLIFICATION WITH ACHIRAL AND CHIRAL LANTHANIDE SHIFT REAGENTS. CLENBUTEROL. METHOD FOR DIRECT OPTICAL PURITY DETERMINATION

Key Words: Clenbuterol, Lanthanide, NMR, Shift Reagents, Optical Purity, Enantiomers, Chiral, Conformation, Europium

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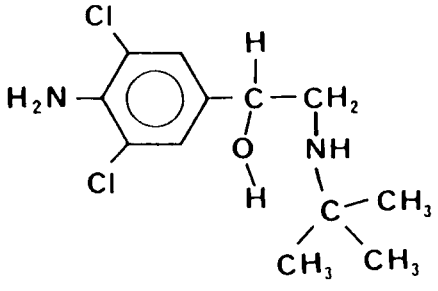
ABSTRACT

The 60 MHz ^{1}H NMR spectra of clenbuterol, 1, have been studied in $CDCl_3$ solution with the achiral shift reagent, tris(6,6,7,7,8,8,8-hepta-fluoro-2,2-dimethyl-3,5-octanedionato)europium(III), 2, and the

chiral reagent tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium(III), 3. Use of 3 resulted in observable enantiomeric shift differences, $\Delta\Delta\delta$, for the t-butyl, NH_2, benzylic CH and the aryl proton signals. Values of $\Delta\Delta\delta$ as high as 135.1 Hz (2.25 ppm) and 86.5 Hz (1.44 ppm) were seen for the methine and aryl protons, respectively, with a solution 0.1035 molal in 1 and a 3:1 molar ratio of 0.551. The aryl resonance is especially well suited for direct optical purity determinations of 1.

INTRODUCTION

Details of the synthesis of clenbuterol, 4-amino-3,5-dichloro-α-[[(1,1-dimethylethyl)amino]methyl] benzenemethanol, also known as NAB-365 or 4-amino-α-[(tert-butylamino)methyl]-3,5-dichlorobenzyl alcohol, 1, were published in 1972 along with data for an extensive array of analogs (1), following ear-



lier patents (2). Numerous studies of clenbuterol hydrochloride have shown it to be a novel broncholytic agent specific for adrenergic β_2-receptors. It has high potency, long duration of action and good efficacy after oral administration (3). Nine articles dealing with pharmacology, animal studies, reproduction toxicology, metabolic patterns, and other aspects of 1 have appeared together (4). The potent and effective action of 1 as a bronchodilator was reported in clinical trials for patients with chronic obstructive lung disease (5) and either asthma or chronic bronchitis (6).

We were interested in analytical NMR studies of 1 with lanthanide shift reagents (LSR) as part of ongoing work in our laboratories aimed at spectral simplification and direct optical purity determinations. Clenbuterol is a substituted arylethanolamine and is therefore similar to several other pharmaceutical agents, such as ephedrine, pseudoephedrine, albuterol and derivatives which have been studied with LSR; comparisons are drawn in the Discussion. The benzylic hydroxyl of 1 was expected to play an important role in LSR binding because of the possibility of bidentate chelation with the lanthanide, and could therefore offer interesting comparisons with selected

arylisopropylamines such as 3,4-methylenedioxyamphetamine ("MDA") (7) or 2,5-dimethoxy-4-ethylamphetamine ("DOEt") (8) which lack this hydroxyl. The LSR binding of the anilino nitrogen was also of interest because of earlier studies on aminoglutethimide (9). The presence of the chiral center at the benzylic position of 1 meant that use of chiral LSRs could offer a method for direct analysis of the enantiomers.

EXPERIMENTAL

Samples of racemic 1-hydrochloride (lot TK927, N-AB365CL) were provided by Boehringer Ingelheim, Ridgefield, CT 06877, through Dr. Karl Thomae GmbH, Biberach an der Riss, W. Germany, and had (uncorr.) mp 169.5-172.5 (with decomp.), lit. 174-175.5 (1). Chloroform-d (99.8 atom % D), obtained from Aldrich Chemical Corporation, Milwaukee, WI 53201, USA, or from Norell, Inc., Landisville, NJ 08236, USA, was dried and stored 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, for spectral runs with 1, an accurately weighed sample of drug (22-48 mg) was added to 520-870 mg $CDCl_3$ [containing about 0.2% tetra-

methylsilane (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 recorded immediately. All spectra were recorded using a Varian EM-360A 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 ±0.05 ppm. For runs with racemic 1 and chiral shift reagent, the reported δ values for resonances displaying antipodal differences are the average values for both enantiomers. In spectra where the TMS signal was obscured by shift reagent peaks, $CHCl_3$, present as an impurity in the solvent, was used as internal standard.

Preparation of 1-free base: In a separatory funnel was placed 1-HCl (383.6 mg, 1.224 mmol), 8ml H_2O and 2.5 ml (excess) 5.4% aqueous NaOH. The resulting free base of 1 was extracted five times with a total of 50 ml CH_2Cl_2. The combined organic extracts were washed with brine and dried over KOH pellets. Solvent was removed with a rotary evaporator (aspirator pressure, bath temperature 35-40°) to constant weight, yielding the free base of 1 as a light yellow oil (268.3 mg, 79.1% recovery)

which was stored under N_2 and which crystallized upon standing for a few days. The resulting light yellow-tan solid had mp 116.5-123°, lit. 109-115° (1) and was used for NMR studies without further purification. All free base samples of 1 were routinely stored under N_2 in a desiccator with P_2O_5.

RESULTS AND DISCUSSION

Initial runs were performed on clenbuterol solutions in $CDCl_3$ using the achiral shift reagent, tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium(III), 2, known as $Eu(FOD)_3$. The solubility of 1 as the free base was somewhat limited in $CDCl_3$. Observed chemical shifts for 1 as a 0.0882 molal solution were as follows, δ (ppm); 7.20 (ArH, 2H, s); 4.45(HCO, 1H, m); 4.39 (NH_2, 2H, br s); 2.63 (CH_2, 2H, m); 2.40 (OH, NH, 2H, br s); 1.08 (t-Bu, 9H, s). Rapid chemical exchange (on the NMR timescale) between the sidechain OH and NH resulted in a single broad averaged signal. In contrast, the aromatic amino protons provided a separate signal. The benzylic methine displayed a downfield shift reflecting effects of the aryl and hydroxyl groups. The diastereotopic methylene protons,

CH_aH_b, appeared as a complex multiplet partly obscured by the OH and NH absorption.

Incremental additions of 2 provided significant lanthanide-induced shifts, $\Delta\delta$, for all signals. The $\Delta\delta$ value is defined as the chemical shift of a particular nucleus in the presence of shift reagent minus the chemical shift of the nucleus with no shift reagent present. As expected, the magnitude of $\Delta\delta$ for the aromatic amino hydrogens was quite small, reflecting little or no binding of 2 at this site. Aryl amines normally have reduced basicity relative to alkyl amines because of mesomeric withdrawal of electron density from nitrogen by the aromatic ring.

Investigations of LSR binding to a series of anilines (10,11) indicate LSR sensitivity to both electronic and steric factors. In the case of 1, severe steric effects result from the two chlorine atoms flanking the aryl NH_2. Their influence, in part, would cause the NH_2 to twist out of plane, potentially increasing its basicity, but their inductive electron withdrawal and steric repulsions render the NH_2 a poor site for binding. The very large $\Delta\delta$ seen for the combined OH and NH signal does not of itself clarify the mode of complexation to 2, which could involve binding to either heteroatom or to

both, by a rapid-exchange monodentate mechanism or by a bidentate chelation of lanthanide through a five-membered ring. Since $\Delta\delta$ for the methine is substantially greater than for either proton of the methylene, preferential binding of 2 at OH is suggested. A very severe steric interaction of the t-butyl on nitrogen would be particularly important for complexation of the bulky LSR, possibly overwhelming the intrinsically greater basicity (for protons) of the amine versus the alcohol. The possibility of intramolecular hydrogen bonding between the OH and NH of 1 in the absence of LSR is not necessarily ruled out since steric requirements would be less demanding than for LSR complexation. In principle, extraction of vicinal coupling constants within the $CHCH_2$ moiety should provide insight regarding conformation about this carbon-carbon bond. Experimentally, this was rendered difficult by unfavorable signal-to-noise ratios because of low solubility of 1; by interferences in the $CHCH_2$ resonances from the OH, NH, NH_2 and LSR signals; and by lanthanide-induced line broadening. The possibility of lanthanide-induced conformational changes in a flexible system, such as 1, is very real, especially if intramolecular

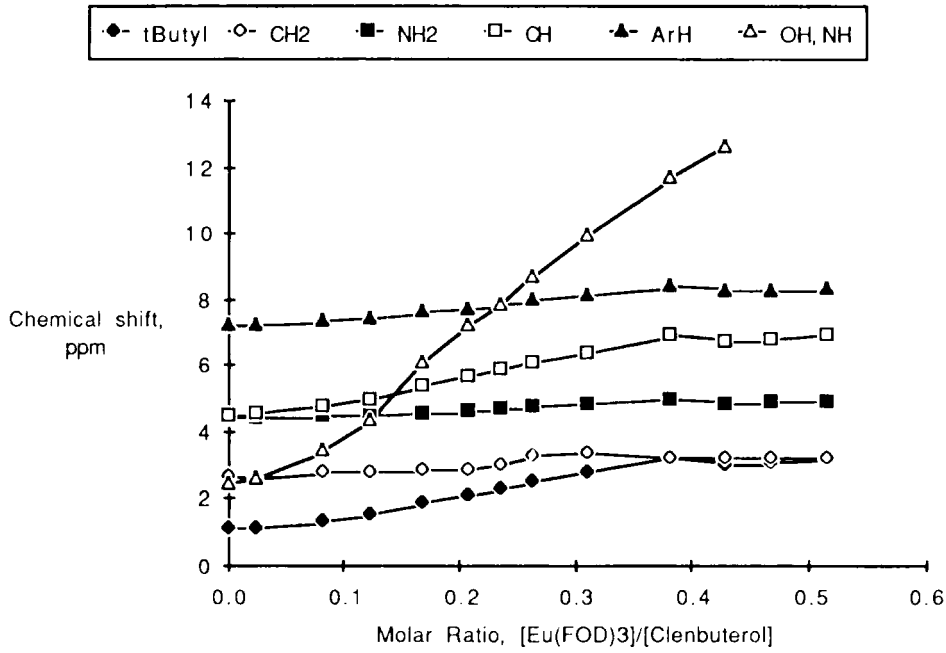


FIG. 1. Variation of chemical shifts, δ (in ppm), with molar ratio of 2:1. Note: Average chemical shifts are plotted for the diastereotopic methylene protons; these shift assignments are tentative for higher 2:1 ratios.

hydrogen bonding and steric effects are delicately balanced (12, 13). While steric repulsion could favor binding of 2 to OH and an anti orientation of the O-C-C-N moiety to minimize repulsions between LSR and t-butyl, relatively large $\Delta\delta$ magnitudes for the t-butyl resonance may argue against this. Results of runs with 2 are summarized in Figure 1.

Of greatest interest to us was the possibility of direct optical purity determinations of 1 using a chiral lanthanide shift reagent, tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium(III), 3, known as $Eu(HFC)_3$ or $Eu(HFBC)_3$. Solid increments of 3 were added to a solution of racemic 1 free base, 0.1035 molal in $CDCl_3$. Lanthanide-induced shifts using 3 closely paralleled results obtained with 2, except that the methine H-CO signal displayed greater $\Delta\delta$ magnitudes with 3 and moved downfield of the aryl protons at the highest molar ratios employed (circa 0.55). Results with 3 are presented in Figure 2. The enantiomeric shift difference, $\Delta\Delta\delta$, may be defined as (the magnitude of) the difference in chemical shifts between corresponding nuclei in enantiomers in the presence of the chiral shift reagent. In the case of 1 with 3, remarkably high $\Delta\Delta\delta$ values were observed for the benzylic methine at the chiral center and for the aryl hydrogens. For the former, $\Delta\Delta\delta$ as high as 135.1 Hz (2.25 ppm) was observed with a 3:1 molar ratio of 0.551. At the same molar ratio, $\Delta\Delta\delta$ for the aromatic ring protons was 86.5 Hz (1.44 ppm). Such high $\Delta\Delta\delta$ values have been encountered for protons directly attached to a chiral center bearing the basic

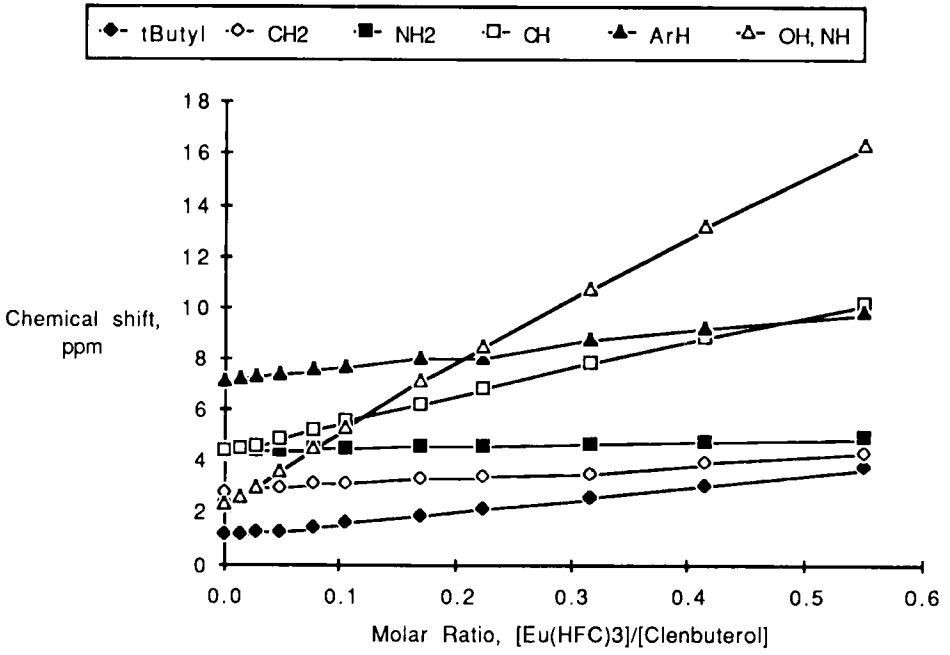


FIG. 2. Variation of chemical shifts, δ (in ppm), with molar ratio of 3:1. Where enantiomeric shift differences occur, the average chemical shift for the two antipodes is plotted. See Note for Figure 1.

atom which binds the LSR, as in α-phenethylamine (14, 15) and DOEt (8), and in structurally analogous molecules where the "marker" group is in close proximity to both the chiral center and the LSR binding site (16). The large $\Delta\Delta\delta$ value for the aryl protons of 1 with 3 strongly suggests major binding of 3 at the benzylic oxygen, four bonds removed from the aryl protons.

That signal separations observed for racemic 1 with 3 are indeed enantiomeric shift differences is supported by the following. First, corresponding separations were not observed when the achiral 2 was employed. Furthermore, if the aryl protons, $H_{2,6}$, were resolved from one another by virtue of being diastereotopic due to slow rotation (on the NMR timescale) about the aryl-CHOH bond, then the two meta protons would be nonequivalent and would be expected to show a small splitting of each other's signal. No evidence for typical meta coupling constants is seen for these peaks, which remain sharp at low shift reagent levels. Conceivably, the signal separation of the t-butyl group or of the methine could result from relatively slow rates of inversion about the secondary nitrogen (possibly resulting from LSR binding at this nitrogen) or from hindered rotation about proximal C-C or C-N bonds. However, nonequivalence resulting from such slow processes in the sidechain would arise from diastereomeric structures which are expected to be unequal in energies and populations. If these NMR signals reflected such nonequivalent populations rather than enantiomeric shift differences, then signal intensities would be unequal (i.e., would be proportional to unequal

conformer populations). In fact, observed split signals were equivalent in intensity, consistent with $\Delta\Delta\delta$ for a racemic sample. Hindered rotation of the amino NH_2 group could not result in nonequivalence of these protons unless rotation about the aryl-CHOH bond was also slow; however, the latter was ruled out by the absence of splitting between the aryl protons. We must conclude that true enantiomeric shift differences are the origin of the observed signal separations for the signals of NH_2, t-butyl, methine and aryl protons.

Despite the larger $\Delta\Delta\delta$ magnitudes seen for the benzyl proton versus the aryl protons, the latter signals have far greater analytical utility because they are more intense (corresponding to 2H rather than 1H) and because they appear as sharp singlets rather than as multiplets. Much greater signal-to-noise ratio is available with the aryl absorption, with freedom from interfering overlaps. We observed near-baseline separation of the aryl protons' signal for the two enantiomers (3.7% valley) at a 3:1 ratio as low as 0.0476 and full baseline separation (0% valley) at a molar ratio of 0.0768 ($\Delta\Delta\delta$ of 14.5 Hz). Significant $\Delta\Delta\delta$ values were also observed for the aryl NH_2 signal (15.3% valley, $\Delta\Delta\delta$ = 10.8 Hz at a

3:1 ratio of 0.416) and for the t-butyl. Optimum resolution for the t-butyl resonance was observed at a 3:1 ratio of 0.170 (9.7% valley, $\Delta\Delta\delta$ = 8.2 Hz). Interestingly, $\Delta\Delta\delta$ increased monotonically with higher levels of 3 except for the t-butyl absorption for which $\Delta\Delta\delta$ magnitude fell and rose again. This behavior could suggest a change in the sense of magnetic non-equivalence for the t-butyl in the region of a 3:1 ratio of 0.4. The aryl resonance was clearly the best signal to use for analytical optical purity determinations. Variations of $\Delta\Delta\delta$ values are summarized in Figure 3.

Spiking experiments were performed to demonstrate the use of 3 in optical purity determinations of 1. For example, a mixture of 23.3 mg (0.0841 mmol) of racemic 1, 19.9 mg (0.0718 mmol) of (+)-1 and 13.0 mg (0.01089 mmol) of the chiral LSR, 3, were combined with $CDCl_3$ (511.2 mg) to produce the results in Figure 4 and Table 1. The spectra that were obtained for these and other spiked samples clearly showed enhancement of signals for the upfield enantiomer for the absorptions of aryl, t-butyl, NH_2 and methine (CHOH) protons, indicating an upfield sense of magnetic nonequivalence for each of these signals for (+)-1 with 3 under the conditions examined.

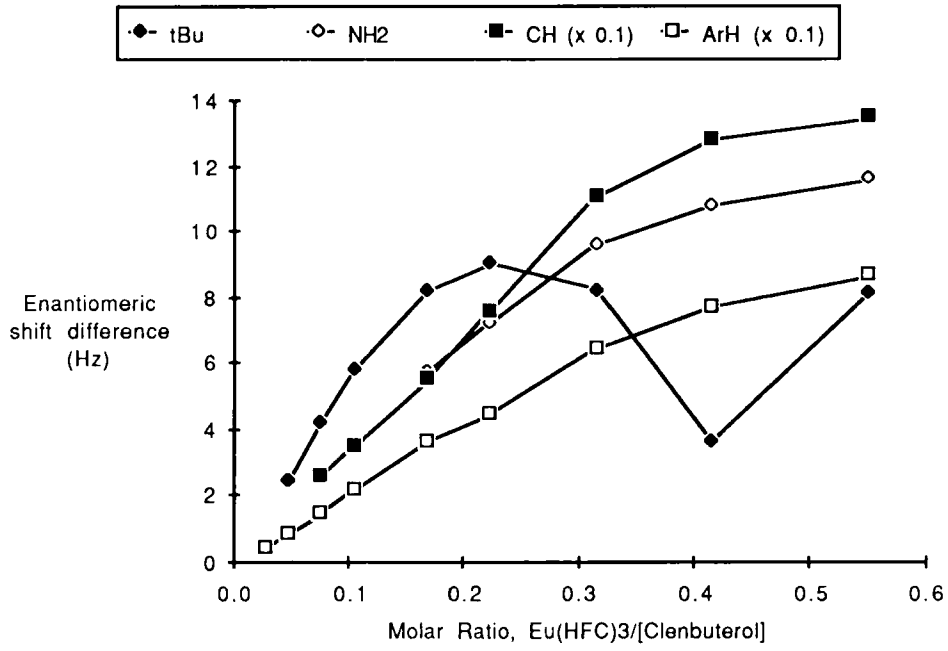


FIG. 3. **Variation of enantiomeric shift differences, $\Delta\Delta\delta$ (in Hz), with molar ratio of 3:1.**

Some closely related systems have been studied with LSR by several investigators. Phenylpropanolamine (also known as norephedrine), $C_6H_5CHOHCH(CH_3)NH_2$, was examined by Büyüktimkin (17) using 3. The benzylic methine, CHOH, exhibited analytically useful $\Delta\Delta\delta$ of up to about 30 Hz (0.5 ppm) with an LSR/substrate ratio near 0.4, allowing 7% detection limits for the minor enantiomer. No $\Delta\Delta\delta$ was reported for aryl protons; both of the methine protons were shifted

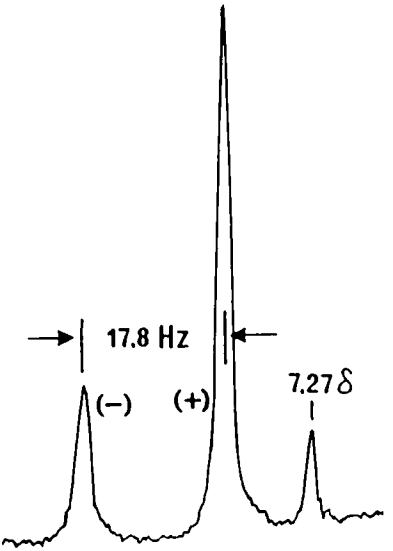


FIG. 4. The 60 MHz ^1H NMR spectrum for the aromatic hydrogen region of a sample containing racemic 1 (23.3 mg, 0.0841 mmol) "spiked" with (+)-1 (19.9 mg, 0.0718 mmol), the chiral LSR, 3 (13.0 mg, 0.01089 mmol) and $CDCl_3$ (511 mg). The nominal enantiomer ratio, (+)-1:(-)-1 is 73.1:26.9 and the molar ratio of LSR to total drug is 0.0699. Residual $CHCl_3$ in the solvent provides a marker signal at 7.27 ppm and the observed $\Delta\Delta\delta$ is 17.8 Hz. Spectrum was obtained at 2 ppm sweep width. The aryl proton absorptions are shown for (+)-1 (upfield) and (-)-1 (downfield).

downfield by LSR, with CHOH exhibiting the larger $\Delta\delta$. Hatzis (18) examined phenylpropanolamine with 2 and observed a slight upfield shift of $CHNH_2$ with the usual downfield shift for the benzylic CHOH. The angular factor in the simplified

Table 1. Optical purity determination of 1 with 3 based on step heights for replicate integral scans of the aryl proton signals of 1 for the sample described in Figure 4. Ratios are for upfield signal, (+)-1, to downfield signal, (-)-1.

Scan no.	Peak Areas (Integral Heights) (+)-1 : (-)-1
1	76.85 : 23.15
2	76.25 : 23.75
3	75.00 : 25.00
4	76.02 : 23.98
5	75.12 : 24.88
Observed mean:	75.85 : 24.15 (S.D. = 0.70; variance = 0.49)

Actual ratio: 73.1 : 26.9 (Absolute error less than 3 percent)

McConnell-Robertson equation (19), together with an LSR-induced conformational change and bidentate chelation of LSR, was tentatively suggested to account for the upfield shift.

Dillon and Nakanishi have discussed (20) absolute configurational studies in vicinal glycols and amino alcohols with tris(2,2,6,6-tetramethyl-3,5-heptanedionato)praseodymium(III), $Pr(DPM)_3$, with "(2R,3S)-3-phenylpropane-3-hydroxy-2-amine," i.e., norephedrine (phenylpropanolamine) as one substrate. The authors

proposed initial bidentate glycol bonding to the $Pr(DPM)_3$ with higher substrate concentrations favoring bismonodentate complexes, but for "bidentate amino alcohols where the amine is sterically much less hindered than the hydroxyl," (20) as in phenylpropanolamine, it was less clear whether mono- or bidentate interactions were involved. Only primary amines were included in the amino alcohols examined by these workers.

Other analogs of clenbuterol have also been examined with LSR, either as derivatives or free bases, including ephedrine, $C_6H_5CHOHCH(CH_3)NHCH_3$ (21, 22), pseudoephedrine (diastereomer of ephedrine) (21), and albuterol, 2-(t-butylamino)-1-(4-hydroxy-3-hydroxymethylphenyl)ethanol (22,23); no details concerning induced shifts were available for the last-named compound. For ephedrine, "anomalous" upfield induced shifts were reported for the NCH_3, CHOH and aryl H using 2, and for the NCH_3 using tris[3-(trifluoromethylhydroxymethylene)-(+)-camphorato]europium(III), $Eu(FACAM)_3$; only the usual upfield induced shifts were noted with $Pr(FOD)_3$ or $Pr(FACAM)_3$ (22). This last group of 1-analogs corresponds to secondary amine substrates. The observations of occasional anomalous induced shifts in the sidechains of the primary and

secondary amines noted in this and in the preceding paragraph are evidence of quite subtle effects. These effects reflect changes in the structure of the bound complex which may result from seemingly small variations in substrate or LSR. The bulky t-butyl group of 1 may very likely result in significant differences in binding LSR compared to the less hindered primary or secondary amines studied by these other workers.

Of further interest concerning conformations in related pharmaceutical substrates are the elegant conformational NMR studies on some analogous ethanolamines by Portoghese (24), who considered isomers of ephedrine, phenmetrazine and phendimetrazine.

The efficacy of 3 for optical purity determinations of 1 contrasts with a report of the use of gas chromatography on chiral stationary phases (25) for drug enantiomer separations; no success was encountered for compounds with t-butyl substituents at the amino group. The authors suggested that the bulky t-butyl group made the molecules less accessible to diastereomeric association with the stationary phase employed. This illustrates how chiral LSR techniques can complement chiral GC and HPLC.

Full elucidation of conformation and the actual binding of 1 with LSR is of considerable interest because it will extend our understanding of an important class of pharmaceutical agents. Such investigations could provide a better picture of LSR binding in flexible molecules that possess more than one LSR binding site, and may ultimately offer insight into structures of drug receptor active sites. We plan to use deuterated $\underline{d}_4$-1 to avoid OH, NH and NH_2 interferences and a higher field spectrometer to clarify some of the questions raised here.

In conclusion, we have demonstrated the potential for direct accurate optical purity determinations of 1 using the chiral LSR, 3. As little as 3% of the minor enantiomer should be detectable using the aryl signals of 1.

ACKNOWLEDGMENTS

We are grateful to Boehringer-Ingelheim, Inc. for samples of racemic clenbuterol hydrochloride and for samples of the enantiomers. Additional support has been provided, in part, by the Sandoz Research Institute, Hoffmann-La Roche Inc., the National Science Foundation Instrumentation and Laboratory Improvement Program Award #USE-8851684 and the U.S. Education

Department Minority Institutions Science Improvement Program Award no. G-008641165 (to R.R.), and by the Research Committee of West Virginia State College (to R.M.).

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Date Received: 01/17/89
Date Accepted: 02/27/89