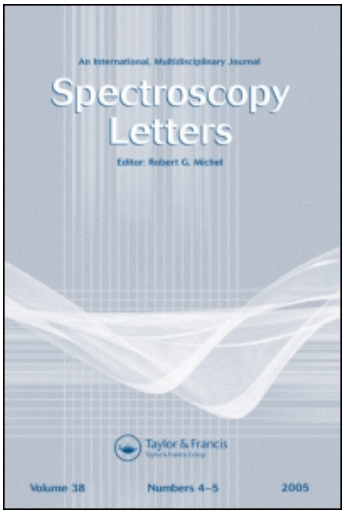

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NMR Studies Of Drugs. Chiral Solvating Agents for Direct Determination of Enantiomeric Excess of the Cardiac Antiarrhythmic, Mexiletine

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NMR STUDIES OF DRUGS. CHIRAL SOLVATING AGENTS FOR DIRECT DETERMINATION OF ENANTIOMERIC EXCESS OF THE CARDIAC ANTIARRHYTHMIC, MEXILETINE.

Key Words: Optical Purity, Enantiomer, Analysis, 1-(2,6-Dimethylphenoxy)-2-propanamine, Antiarrhythmic, Cyclodextrin, α-Methoxy-α-(trifluoromethyl)phenylacetic acid, MTPA, 2,2,2-Trifluoro-1-(9-anthryl)ethanol, TFAE.

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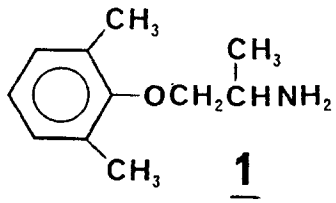
ABSTRACT

The 400 MHz ^1H NMR spectra of the cardiac antiarrhythmic, mexiletine, 1, have been studied with different chiral solvating agents (CSA) to obtain a very promising method for direct determination of enantiomeric excess (e.e.) with limited amounts of 1. The methods included the use of β-cyclodextrin (β-CD), γ-cyclodextrin (γ-CD), α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA), and 2,2,2-trifluoro-1-(9-anthryl)ethanol (TFAE). Use of TFAE in $CDCl_3$ with the free base of 1 appeared to give the best results, with enantiomeric shift differences observed for the signals of the sidechain methyl, $\underline{CH_3}CH$, and the aryl methyls.

INTRODUCTION

Mexiletine, 1-(2,6-dimethylphenoxy)-2-propanamine, 1, has antiarrhythmic activity. Both enantiomers have been obtained as (R)-(-)-1 and (S)-(+)-1 and the pharmacokinetics of the enantiomers in humans have been studied; the stereoselective disposition of 1 has been reported (1-4) as well as aspects of stereospecific binding (5,6). Various analytical techniques have been applied for enantiomeric excess (e.e.)



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determination of 1, including: (a) high performance liquid chromatography (HPLC) of diastereomers after use of a chiral derivatizing reagent (1) or with a chiral stationary phase (CSP) (2,4,6-8); (b) capillary column gas chromatography (GC) with a CSP (9). All of these analyses required prior derivatization of 1 for subsequent chromatography, either with achiral (2,4,6-9) or chiral (1) reagents. In addition, one non-chromatographic method has been described, based on ^{1}H NMR spectroscopy using chiral lanthanide shift reagents (LSR) (10). The NMR LSR technique has the advantage of being a true direct analysis, with no separate derivatization step required. However, because this earlier NMR LSR method employed a low field 60 MHz spectrometer, relatively large amounts of sample were required, ca. 40-60 mg. It was therefore of interest to extend the NMR studies for direct e.e. determination of 1 using a higher field

(400 MHz) NMR spectrometer for analyses at the level of a few milligrams or less. The techniques employed in this present work were based on the use of chiral solvating agents (CSA). In addition to elaboration of a direct NMR method using CSA for potential e.e. determinations of 1, a non-racemic sample of 1 was analyzed to determine the sense of induced magnetic nonequivalence for the two enantiomers.

NMR methods for e.e. determinations based on chiral solvating agents have been reviewed (11,12). The most attractive of these CSAs appeared to be based on the use of a trifluoromethylarylcarbinol to form short-lived diastereomeric solvates with the substrate. The carbinol CSAs, being more acidic than related amine CSAs, were considered to be more appropriate for use with a basic substrate such as 1 (13-15). Thus, studies were performed using (-)-2,2,2-trifluoro-1-(9-anthryl)ethanol, TFAE (14).

In addition, parallel studies were explored using other CSA techniques. Cyclodextrins have been found useful in allowing NMR discrimination of pharmaceutical enantiomers via formation of the inclusion complexes (16). Both β-cyclodextrin (β-

CD) and γ-cyclodextrin (γ-CD) were employed to explore different cavity sizes. Lastly, α-methoxy-α-(trifluoromethyl)phenylacetic acid, MTPA, was briefly examined as a chiral complexing agent for 1. MTPA amides and esters have been used as covalently bound diastereomeric derivatives of amines and alcohols, respectively, to allow e.e. determination by NMR or chromatography (17,18). We considered a modification using MTPA as a complexation reagent via salt formation (19).

EXPERIMENTAL

All spectra were acquired on a Varian VXR-400S FT-NMR spectrometer with a ^1H observe frequency of 399.952 MHz, using a 5mm switchable ^1H/broadband (80-160 MHz) probe, thin-walled NMR tubes and a 25° probe temperature. Typical spectrometer parameters were: 30° pulse flip angle, 5s pulse repetition time, 3s acquisition time, 32 acquisitions, 4317.8 Hz spectral width, 25984 datapoints (32 bits), and 0.05 Hz exponential line broadening. Solutions were prepared in 1.0 ± 0.1 ml $CDCl_3$ (unless otherwise noted) and used 0.03% tetramethylsilane (TMS) as internal reference (δ = 0.000). For samples requiring the free base of 1, the hydrochloride salts (1-HCl) were dissolved in a few

ml H_2O, and 2M NaOH was added to give pH ca. 12. After extraction with four portions $CHCl_3$, the combined $CHCl_3$ extracts were dried (anhyd. K_2CO_3) and the solvent evaporated. The residue was then taken up in $CDCl_3$ and dried (K_2CO_3). Evaporation of the solvent gave the free base, 1, which was used directly for NMR studies. Commercial reagents were used as supplied without further purification. Deuterium oxide (99.8 atom % D) and deuterochloroform (99.8 atom % D, containing 0.03% TMS) were from Janssen Chimica, Belgium; mexiletine samples were from Boehringer, Germany; (R)-(-)-TFAE (98%), MTPA, and δ-cyclodextrin were from Aldrich Chemical Co., Milwaukee WI, USA; β-cyclodextrin was from Sigma Chemical Co., USA. For the "spiked" run of non-racemic 1 with (R)-(-)-TFAE, a portion of (-)-1-HCl was converted to the free base (as described above) which was added to a $CDCl_3$ solution of racemic free base 1. Chemical shifts are reported in ppm; where enantiomeric shift differences were observed, the average chemical shift for the two antipodes is given.

RESULTS AND DISCUSSION

When increments of (-)-TFAE were added to a solution of racemic mexiletine free base in $CDCl_3$

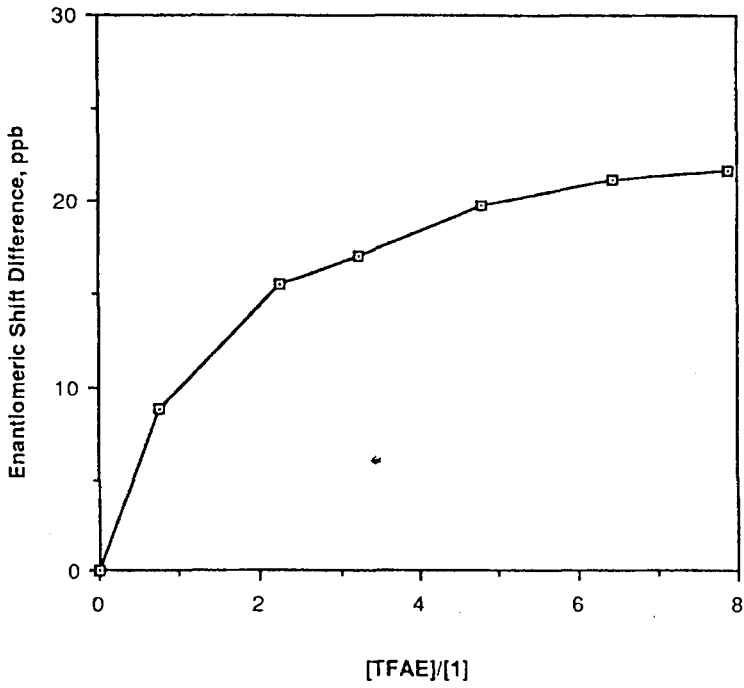

Fig. 1. Variation in enantiomeric shift difference, $\Delta\delta$ (in ppb), with molar ratio [TFAE]/[1] for a solution of 1 ca. 0.0305 molal in $CDCl_3$ at 25°. Data is shown for the $\underline{CH_3}CH$ signal.

at 25°, enantiomeric shift differences ($\Delta\delta$) were observed for both the $\underline{CH_3}CH$ methyl doublet signals and for the methyl singlet signals of the 2,6-dimethylphenyl moiety. The $\Delta\delta$ magnitudes were appreciably greater for the former signal. The variation of $\Delta\delta$ with molar ratio of (-)-TFAE/1 for the $\underline{CH_3}CH$ resonance is shown in Figure 1 and

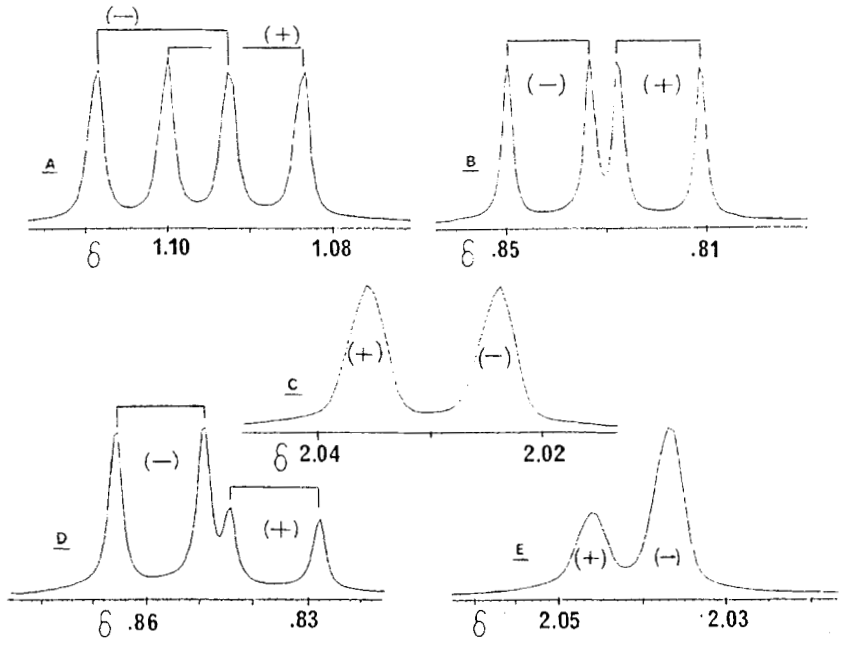

Fig. 2. Expansions of the 400 MHz ^{1}H NMR of 1 with added (-)-TFAE. For each trace, the following is specified: observed nucleus; average chemical shift (δ, ppm); enantiomeric shift difference ($\Delta\delta$, Hz); molar ratio of (-)-TFAE to total 1. (a) $\underline{CH_3}CH$; 1.096 ppm; 3.56 Hz; 0.744; (b) $\underline{CH_3}CH$; 0.830 ppm; 8.70 Hz; 7.88; (c) $(\underline{CH_3})_2C_6H_3$; 2.030 ppm; 4.8 Hz; 7.88; (d) $\underline{CH_3}CH$; 0.847 ppm; 8.44 Hz; (e) $(\underline{CH_3})_2C_6H_3$; 2.042 ppm; 3.6 Hz. Note: traces (d) and (e) were obtained from solution of Fig. 2b, above, by spiking with (-)-1.

representative spectral traces in Figure 2. The development of $\Delta\delta$ is accompanied by slight upfield shifts for both the $\underline{CH_3}CH$ and aryl methyl signals. A non-racemic sample of 1 free base was prepared by

"spiking" racemic material with (-)-1, and this mixture was subjected to treatment with (-)-TFAE. Under these conditions, the lower field doublet of the $\underline{CH_3}CH$ signals was enhanced, i.e., the doublet shifted upfield more slowly must be due to (-)-1. Thus, for the sidechain $\underline{CH_3}CH$ with (-)-TFAE added, (-)-1 has a downfield sense of magnetic nonequivalence (see Fig. 2).

In contrast, the higher field aryl methyl signal was enhanced for the spiked sample, so that an upfield sense of magnetic nonequivalence results for (-)-1 with added (-)-TFAE. The fact that opposite senses of magnetic nonequivalence are seen for the $\underline{CH_3}CH$ versus the aryl CH_3 signals means that the enantiomeric shift differences may reflect not simply a preferential binding of one of the enantiomers of 1 to the TFAE, but also geometric differences of the locations of the marker nuclei in the diastereomeric solvates. Finally, we note that the unequal peak intensities for the aryl methyl signal in non-racemic 1 with (-)-TFAE confirms that the observed peak separations result from true enantiomeric shift differences (rather than from hypothetically nonequivalent methyl groups on the aromatic ring due to slow rotation of the ring on the NMR timescale).

Even relatively small amounts of (-)-TFAE can be effective. With racemic 1 (0.0305 molal in $CDCl_3$) and a molar ratio [TFAE]/[1] of 0.744, average $\Delta\delta$ of 3.56 Hz (9.2 ppb) was seen for the $\underline{CH_3}CH$ signals. Although each enantiomer's doublet was overlapped, the average valley heights between peaks of each antipode (i.e., the two low field lines or the two high field lines) was just over 10%.

For racemic 1 in $CDCl_3$ at a molar ratio of [TFAE]/[1] of ca. 7.88 (the highest examined), a $\Delta\delta$ magnitude of about 12 ppb was observed for the aromatic methyl signal versus 21.7 ppb for the sidechain $\underline{CH_3}CH$. Under our conditions, the valley height between the aryl methyl signals was about 11% of the average peak heights from the two enantiomers, as shown in Fig. 2. Since the $\underline{CH_3}CH$ signal is a 3H intensity doublet (in the absence of CSA) and the $ArCH_3$ a 6H singlet, the lines of the aromatic methyl signals are each four times as intense as those for the sidechain methyl. (Actually, the full peak height advantage of the aromatic methyl signal is not realized since its linewidth is greater than for the $\underline{CH_3}CH$ peaks, presumably caused by long-range coupling to aryl

protons). Particularly in cases where only very small amounts of 1 are available, e.e. determinations using the aromatic methyl signals may be preferred despite their smaller $\Delta\delta$ magnitude. Alternative approaches to the direct e.e. determination using TFAE and the $\underline{CH_3}CH$ signals are described below.

Enantiomeric excess determinations for some pharmaceuticals by NMR of cyclodextrin inclusion complexes in D_2O appeared to be a promising technique (16). We examined racemic 1 as its HCl salt in D_2O using β-CD as CSA. The limited success may partly reflect the limited water solubility of β-CD (20). The largest $\Delta\delta$ magnitudes were again found for the sidechain methyl of 1, $\underline{CH_3}CH$. For example, with 1.09 mg racemic 1-HCl in 1.0 ml D_2O, observed average $\Delta\delta$ values were only 2.68 Hz (6.7 ppb) with 28.4 mg β-CD added; using 56.8 mg β-CD, the observed $\Delta\delta$ was actually less: 2.19 Hz (5.5 ppb). (The actual amount of added β-CD is nominal since both of these solutions required filtration. Solutions with lower levels of the CSA were homogeneous but $\Delta\delta$ magnitudes were smaller). Thus, the $\Delta\delta$ magnitudes were much smaller than the vicinal coupling constant and the doublets for each

enantiomer's $\underline{CH_3}CH$ signal were severely overlapped. The valley heights between adjacent lines from the two antipodes were quite high, about 43% and 48%, respectively, for the samples noted above with 28.4 and 56.8 mg of added β-CD. No discernible $\Delta\delta$ was seen for the aryl methyls at any level of this cyclodextrin CSA. Very small downfield shifts were induced for both of the methyl signals with added β-CD.

We considered the possibility that the 2,6-dimethylphenyl moiety of 1 was too large to be fully accommodated within the hydrophobic interior of the β-CD cavity. Examination of Dreiding models suggested that ca. 6.6Å would be needed for the dimethylphenyl group based on the distance between the methyl groups (21). The cavity diameter for β-CD has been estimated at 6.0-6.5Å (20) with 7.8 Å maximum diameter. If optimal enantiomeric shift difference is dependent upon the substrate's chiral center and analytical "reporter" nuclei both being close to the chiral hydroxyl group environment at the mouth of the CD cavity, this might require a greater penetration into the CD cavity by the hydrophobic dimethylphenyl moiety of 1 than physical size allows. In contrast, γ-CD has a more

spacious cavity, with estimated diameter of 7.5-8.3 Å and 9.5 Å maximum diameter (20). This would certainly allow full penetration by the hydrophobic portion of 1. In addition, γ-CD has more than twelve times the water solubility of β-CD, 23.2 versus 1.85 g/100 ml at 25° (20), and it was thought that this might prove advantageous by allowing higher molar ratios of CSA:1. However, when γ-CD was tried, $\Delta\delta$ values were far inferior to those seen with β-CD. It may be that the fit of 1 in the larger cavity was simply too floppy, and that 1 in the smaller cavity of β-CD provided a more desirable tighter fit, even though the dimethylphenyl moiety may not have been fully within the cavity; this must be considered speculative.

Use of MTPA as a CSA for NMR e.e. determination of amines has been described (19). We attempted to examine racemic 1 free base in $CDCl_3$ with added (+)-MTPA. In our hands, presumed salt formation led to precipitation from the solution, preventing spectral acquisition. This approach was not pursued further.

Of the different CSAs tried, TFAE appeared most useful. Using this reagent, we examined the

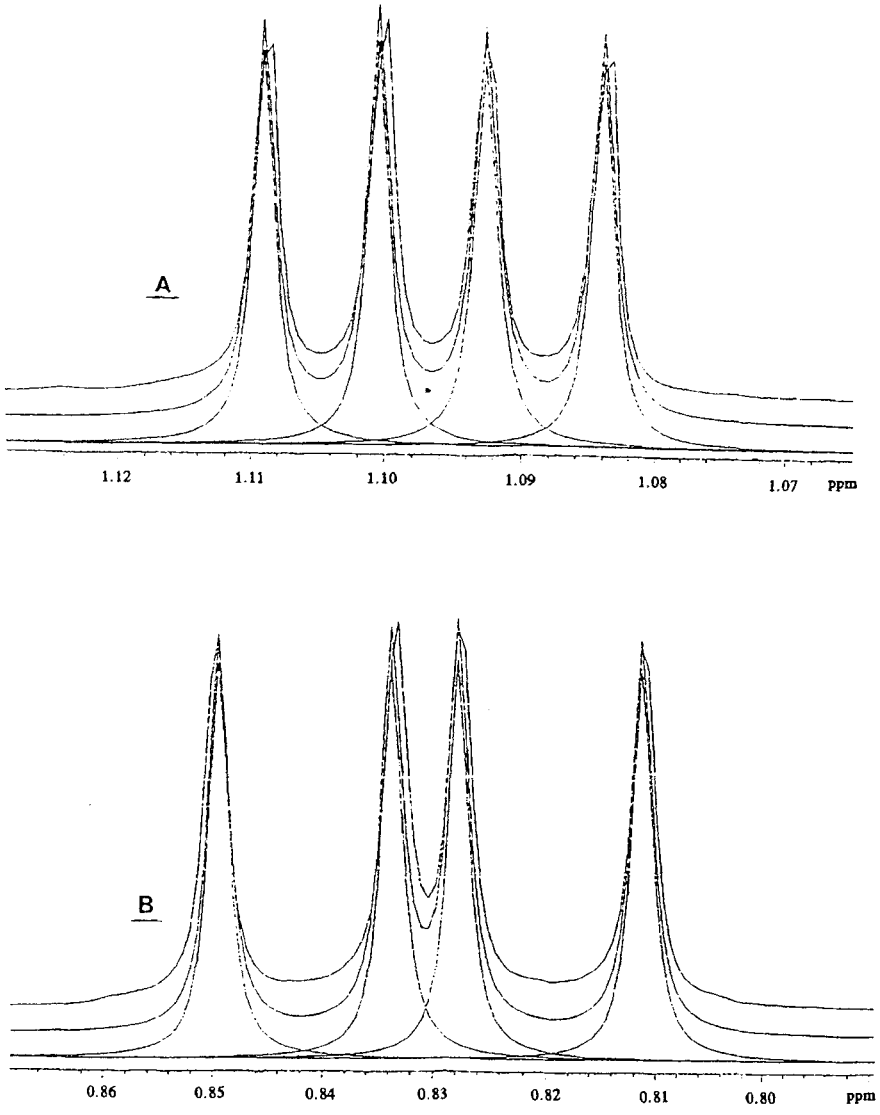

Fig. 3. Spectral deconvolutions of traces from Fig. 2a and Fig. 2b, above. For each deconvolution, (a) and (b), respectively, the uppermost trace is the actual spectrum, the center trace is the full fit, and the bottom trace shows the individual component plots. See caption for Fig. 2 for sample conditions.

use of the $\underline{CH_3}CH$ doublet signals (which exhibited the largest $\Delta\delta$ values) with the ancillary technique of spectral deconvolution by means of the standard Varian software, applying Lorentzian line-shapes. Deconvoluted spectra are shown in Figure 3. This method appeared to offer considerable promise for e.e. quantitation of the doublet signals (which are not baseline resolved). The deconvolutions appear to be excellent fits to the experimental spectra and suggest the possibility of e.e. determinations with substantially less TFAE than would be necessary for complete separation of the doublet signals. Homonuclear decoupling via selective irradiation of the methine $CH_3\underline{CH}$ to collapse the methyl doublets to singlets was also considered. But experimentally, complete decoupling was not achievable due to the extreme widths of the multiplets caused by extensive couplings and differential induced chemical shifts.

CONCLUSIONS

Different CSAs have been examined for use in the direct e.e. determination of mexiletine. The best results (largest $\Delta\delta$ magnitudes and smallest valley heights) were achieved with 1 free base in $CDCl_3$ using (-)-TFAE, based on the $\underline{CH_3}CH$ signal.

The (-) enantiomer of 1 exhibited a downfield sense of magnetic nonequivalence. We estimate that, for practical applications, a single milligram each of mexiletine sample and (-)-TFAE are sufficient for e.e. analysis with a 400 MHz (or higher frequency) spectrometer. Good results were also seen using the aryl methyl signals. Spectral deconvolution promised even greater potential. Use of β-CD in D_2O with 1-HCl was inferior, with smaller $\Delta\delta$ magnitudes and higher valley heights; γ-CD was still less effective. Use of MTPA with 1 free base in $CDCl_3$ caused precipitation and could not be applied.

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