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Optical purity determination and ^{1}H NMR spectral simplification with lanthanide shift reagents — V. Mephenytoin, 5-ethyl-3-methyl-5-phenyl-2,4-imidazolidinedione

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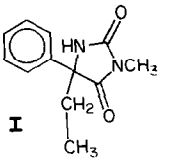
Abstract: The 60-MHz ^{1}H NMR spectra of racemic mephenytoin, **I**, have been studied with the achiral shift reagent, tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato) europium (III), **II**, and the chiral reagent, tris[3-(trifluoromethylhydroxymethylene)-*d*-camphorato] europium (III), **III**. Moderate values of the enantiomeric shift differences, Δ, were clearly observed for the NCH_3, NH, aryl and CCH_3 resonances in $CDCl_3$ solution at 28°C with added **III**. The NCH_3 and NH absorptions could be useful for direct assays of optical purity. Thus, for a 0.34 molal solution of **I** in $CDCl_3$ with a molar ratio **III:I** of 0.138, the value of Δ for the NCH_3 resonance was 3.1 Hz (0.052 ppm) with 32% valley resolution.

Keywords: *^{1}H NMR; mephenytoin; chiral lanthanide shift reagents; optical purity determination.*

Introduction

The class of compounds known as hydantoins, 2,4-imidazolidinediones, has been extensively investigated because of its important pharmacological properties, especially anticonvulsant activity [1]. The chemistry of hydantoins has been reviewed [2]. A number of optically active hydantoins have been discussed or synthesized [3, 4]. Various studies have been carried out on the pharmacological properties of the enantiomers of specific hydantoins and related compounds. For mephenytoin, **I**, the R-isomer was more active as an anaesthetic in mice than was the S-isomer and was demethylated by rats



I

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faster than the S-isomer [5]. R-(−)- and S-(+)-mephenytoins labelled with ^{14}C at the 3-methyl position have been prepared and studied [6]. The S-isomer of mephenytoin disappeared faster than the R-isomer from the plasma of dogs. The stereoselective metabolism and disposition of the enantiomers of **I** have been studied in man [7]. Enantiomers of **I** were synthesized with known absolute configurations [4]. In general, the stereochemistry of drugs is of considerable importance in relation to their pharmacological properties; this is certainly true of hydantoins and related compounds and of mephenytoin, in particular.

In this laboratory, interest has been shown in methods for the direct determination of the optical purity of drugs such as glutethimide [8] and thiamylal [9; S. Eberhart and R. Rothchild, unpublished communication No. 102, 34th Pittsburgh Conference, Atlantic City, NJ, USA (1983)]. Positive results with these compounds, and with some others, that have common structural features with mephenytoin have led to an attempt to extend the technique of chiral lanthanide shift reagents for ^1H NMR spectral simplification and determination of optical purity to **I**. This method has been reviewed [10–15] and applied to a number of drugs [16–20]. The results presented here for mephenytoin suggest that NMR spectral simplification and assay for optical purity can be accomplished.

Experimental

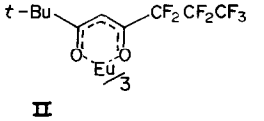
A sample of racemic mephenytoin (Mesantoin®, batch No. 931341) was kindly provided by Sandoz Pharmaceuticals (East Hanover, NJ, USA). Deuterated chloroform (99.8% purity), obtained from Aldrich Chemical Corp. (Milwaukee, WI, USA) or from Norell Inc. (Landisville, NJ, USA), was dried and stored over 3A molecular sieves. Shift reagents were obtained from Aldrich and were stored in a desiccator over phosphorus pentoxide. Materials were used as supplied except as noted.

An accurately weighed portion of drug (about 50 mg) was added to about 650 mg of $CDCl_3$ [containing about 0.2% tetramethylsilane (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 immediately determined.

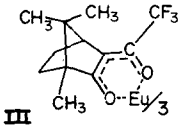
All spectra were determined on a Varian EM-360A 60-MHz ^1H NMR spectrometer at a probe temperature of 28°C. Chemical shifts (δ) are reported in parts per million relative to TMS as internal standard and are believed to be accurate to ±0.05 ppm. In spectra where TMS was obscured by shift reagent peaks, $CHCl_3$, present as an impurity in the solvent, was used as the internal standard.

Discussion and Results

The achiral lanthanide shift reagent tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato) europium (III), abbreviated as $Eu(FOD)_3$, **II**, and the chiral reagent tris[3-(trifluoromethylhydroxymethylene)-*d*-camphorato] europium (III), abbreviated as $Eu(TFC)_3$, **III** [also known as $Eu(FACAM)_3$] have been used in these studies. Achiral shift reagents have been shown to be particularly useful for enhanced dispersion of



II



III

spectral features to permit assignments and, with reagent **III**, direct determination of optical purity can be achieved. Here, parallel studies have been carried out, first with the achiral reagent **II** to facilitate spectral elucidation and then with the chiral reagent **III** to demonstrate the potential value of the technique for assays of optical purity.

In the unshifted spectrum of 0.338 molal solution of **I** in $CDCl_3$, the CCH_3 appears as a triplet at 0.9 ppm (^3J = 7.2 Hz), the diastereotopic protons of the CH_2 appear nearly as a quartet at 2.2 ppm, the NCH_3 is a singlet at 3.02 ppm, the aryl protons are a complex multiplet from about 7.2 to 7.8 ppm, and the NH appears as a broad singlet at 7.83 ppm. As increments of **II** are added, the most significant changes involve the resolution of the protons of the methylene and the separation of the *ortho* aryl protons from the remaining protons of the phenyl ring. Lanthanide-induced peak broadening is relatively slight, even for the NH proton, permitting the use of relatively high molar ratios of **II:I** (up to 1.30) in the present work. The lanthanide-induced shifts, $\Delta\delta$, were considerable. $\Delta\delta$ is defined as the chemical shift (in ppm) observed in the presence of added shift reagent minus the chemical shift (in ppm) of the unshifted substrate; in determinations with **III**, $\Delta\delta$ represents the average for the two enantiomers. For example, at a **II:I** ratio of 0.712, the diastereotopic methylene protons showed multiplets at 4.30 ppm and 5.03 ppm that are almost fully separated from each other. The NCH_3 is at 8.23 ppm, the NH at 15.83 ppm, the *ortho* aryl protons are at 10.13 ppm, whereas the *meta* and *para* protons are observed at 7.77 ppm. Apparent coupling constants for the *ortho* protons of about 2 and 8 Hz are consistent with the expected aromatic ring couplings [21]. These results are summarized in Fig. 1.

The substantial values for $\Delta\delta$ and the minor line broadening observed for **I** with **II** suggested that results with the chiral reagent **III** would be favourable in obtaining useful values of the enantiomeric shift difference, Δ. This is defined as the difference in chemical shift between the two enantiomers; the values reported here represent the magnitude of this difference. The results obtained earlier with the structurally similar compound, ethotoin [22], 3-ethyl-5-phenylhydantoin, **IV**, further suggested the possibility of readily achieving optical purity assays of **I**, which was expected to have an

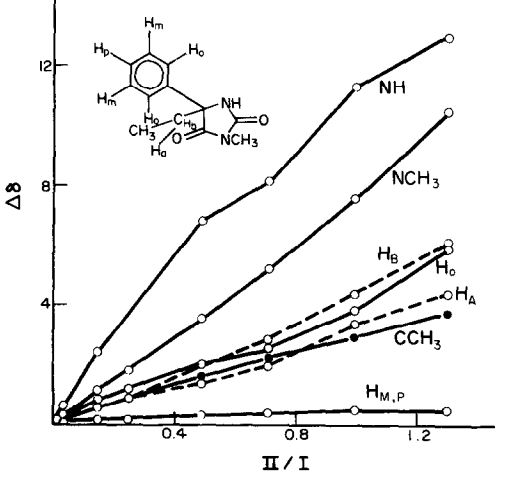
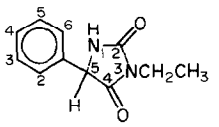


Figure 1
Variation of lanthanide-induced shift, $\Delta\delta$, with molar ratio of $Eu(FOD)_3$:mephenytoin (**II:I**).



IV

electron-rich hydantoin ring system comparable to that of **IV**. However, less steric hindrance to europium complexation would be expected for **I** because of the smaller size of the 3-methyl group of this compound. Based on electronic considerations, the C_2 carbonyl is presumed to be the favoured complexation site in each compound, the urea-type of carbonyl enjoying mesomeric electron release from two nitrogens rather than from one (as in the C_4 amide-type carbonyl group). However, as a result of europium complexation at the C_4 carbonyl position the lanthanide would be closer to the chiral centre; this would be potentially advantageous for inducing a suitable difference in the chemical shift between the enantiomers, Δ. If a number of europium-complexed species were in rapid equilibrium, there could be contributions that involved both C_2 and C_4 carbonyl coordination. C_4 carbonyl binding of lanthanide should be increasingly disfavoured with increasing size of substituents at C_5.

As increments of **III** were added to a 0.342 molal solution of **I**, distinct Δ values were observed at a **III:I** ratio of 0.072; Δ values for the NH and NCH_3 were 4.4 and 1.8 Hz, respectively. Because of greater peak sharpness for NCH_3 better resolution was observed for the latter resonance, despite the smaller Δ value, which corresponded to 50% compared with 78% valley resolution. At a **III:I** ratio of 0.138, the Δ for NH was 8.0 Hz with 44% valley resolution, whereas Δ for NCH_3 was 3.1 Hz with 32% valley resolution. A **III:I** molar ratio of 0.237 provided an observable Δ value for the CCH_3 group (1.6 Hz). For NH, the Δ value was 11.6 Hz with 30% valley and for NCH_3 Δ was 3.4 Hz with 43% valley resolution, reflecting increased peak broadening. An increase in the **III:I** ratio to 0.348 led to a decrease in Δ for NCH_3 to 1.6 Hz, while the Δ value for NH increased to 12.5 Hz with 32% valley resolution. Although not useful analytically, Δ for H_{ortho} was 7–8 Hz; this was clearly apparent by comparison with determinations using **II** and was similar to the authors' previous results for glutethimide. Further additions of **III** virtually eliminated Δ for the NCH_3 at a **III:I** ratio near 0.48; however, a low value for Δ was observed at a still higher ratio (0.658) when the Δ for NH began to decline. Lanthanide-induced broadening became severe at a **III:I** ratio of 0.937, the highest examined in this work.

These results therefore show an initial increase in the Δ value for the NCH_3 group to a maximum; this is followed by a decrease (possibly to zero) and then an increase in Δ. In the absence of pure enantiomers for spiking experiments, only the relative value of Δ can be measured and it is not possible to determine which enantiomer is responsible for the particular upfield (or downfield) absorption. It is believed that the chemical shifts of the NCH_3 for the R- and S-enantiomers of **I** must be crossing over, with the observed Δ falling to a value near zero at the cross-over point. At low **III:I** ratios, one enantiomer is further upfield, but at higher **III:I** ratios the other enantiomer must produce the upfield absorption. At the cross-over point, each enantiomer has the same chemical shift, of course (cf. Figs 2 and 3). It is suggested that this interesting observation almost certainly results from a slight change in the relative binding constants to the two isomers, due to differential saturation of the binding site, as indicated by the curvature on the individual plots in Fig. 2.

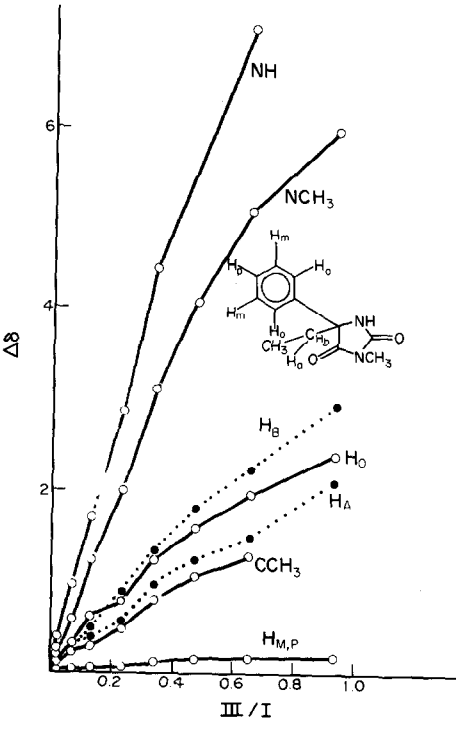


Figure 2
Variation of lanthanide-induced shift, Δδ, with molar ratio of Eu(TFC)$_3$:mephenytoin (**III:I**), where Δδ represents the average for the two enantiomers.

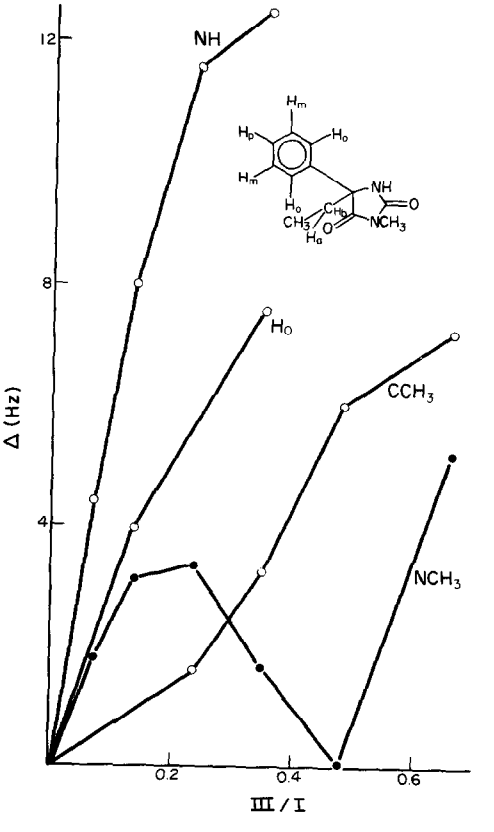


Figure 3
Variation of enantiomeric shift difference, Δ, with molar ratio of Eu(TFC)$_3$:mephenytoin (**III:I**).

For analytical purposes, the results indicate a modest ability to perform assays of optical purity using the NCH_3 resonance with a **III:I** molar ratio near 0.14, or using the NH resonance with higher **III:I** ratios of about 0.24–0.35. Experimentally, the NH resonance seems easier to use because of a broader acceptable range of **III:I** ratios. It should be possible to detect as little as 10% of the minor enantiomer, even with a 60-MHz spectrometer. Clearly, the use of a higher-field instrument would be advantageous.

Perhaps more important than the ability to determine relative amounts of the enantiomers of **I** is the significant difference between these results and those for ethotoin, **IV**. Although lanthanide-induced shifts for corresponding protons in mephenytoin and ethotoin are strikingly similar at comparable molar ratios of either **II** or **III**, the enantiomeric shift differences appear to be considerably less for **I**. This would suggest that the geometry of lanthanide complexation of **I** and **IV** is similar and that the C_5 substituents are mainly responsible for different values of Δ. It is thus proposed that the phenyl and ethyl groups at the C_5 of **I** induce less non-equivalence between the enantiomers of **I** with **III** than does the phenyl group and the C_5-proton of **IV**.

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