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Report

Optical Purity Determination of *threo*-Methylphenidate Hydrochloride Using a Chiral Europium Nuclear Magnetic Resonance (NMR) Shift Reagent

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A ^{1}H-NMR spectroscopic method for the determination of the optical purity of *threo*-methylphenidate hydrochloride is presented. Complexation of the free-base form of the substrate with a chiral Eu(III) shift reagent resulted in two distinct enantiomeric ester methyl proton signals of utility for quantitative work. The accuracy of the method was validated by analyzing six synthetic mixtures of various proportions of (+)- and (−)-*threo*-methylphenidate. In addition to yielding assay results that were in close agreement with the known weights of each enantiomer in the mixture, and a mean recovery of the (−)-*threo*-enantiomer of better than 99.0%, the proposed method was capable of accurately measuring as little as 2% of one enantiomer in the presence of the other.

KEY WORDS: *threo*-methylphenidate; enantiomers; optical purity; ^{1}H-NMR; Eu(III) shift reagent.

INTRODUCTION

Methylphenidate hydrochloride (methyl α-phenyl-2-piperidineacetate hydrochloride) is a mild central nervous system stimulant and antidepressant with pharmacological properties that are essentially the same as those of the structurally related amphetamines (1). However, methylphenidate differs from the amphetamines in that it is less potent and has a shorter duration of action (2) and in that it brightens mood and improves performance without causing excessive stimulation (3) or appreciable cardiovascular and respiratory irregularities (4).

The presence of two chiral centers in the structure of methylphenidate determines the existence of pairs of *erythro*- and *threo*-diastereoisomers, with the biological activity residing mainly with the *threo*-isomer (5,6) and with the (2*R*,2′*R*)-(+)-*threo*-enantiomer being more potent than the (2*S*,2′*S*)-(−)-*threo*-antipode (5,7,8). In the form of the (±)-*threo*-mixture, methylphenidate is clinically used in the treatment of depressive states, narcolepsy, and the hyperkinetic syndrome (1).

The resolution of the pair of *threo*-enantiomers has been accomplished by fractional crystallization (8) and by open-column chromatography on an optically active ion exchanger (9), but these methods are laborious and, more importantly, can lead to significant sample losses (9). More quantitative enantioselective analytical methods have included HPLC in the normal phase (10) and gas chromatography with electron capture detection (11,12), but these approaches necessitate the use of the expensive pure enantiomers as reference standards.

Taking advantage of the ability of paramagnetic lanthanide shift reagents of inducing frequency differences between the spectral lines of enantiomeric substrate species in achiral solvents (13,14), we have developed a method for directly determining the enantiomeric composition (optical purity) of *threo*-methylphenidate by the combined use of proton NMR spectroscopy (^{1}H-NMR) with an optically active Eu(III) chelate. In addition to providing quantitative information on enantiomeric compositions, the proposed method requires a minimum of reagents and procedural steps, and it circumvents the need for enantiomeric reference standards.

MATERIALS AND METHODS

All ^{1}H-NMR spectra were recorded on a Model EM-390, 90-MHz spectrometer (Varian Associates, Sunnyvale, CA), operating at a probe temperature of 35 ± 1°C. Chemical shifts are reported as parts per million relative to tetramethylsilane (TMS; Aldrich Chemical Co., Milwaukee, WI). TMS was made free of tetrahydrofuran by consecutive washes with sulfuric acid and saturated potassium bicarbonate, distilled, and stored over type 4A molecular sieves. The solvents carbon tetrachloride (CCl_4; Aldrich) and NMR-grade deuterated chloroform ($CDCl_3$; + 99.5 atom% D; Aldrich) were distilled prior to use and stored over type 4A molecular sieves. The shift reagents tris [3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium(III) [Eu(hfc)$_3$; Aldrich] and tris [3-(heptafluoropropyl-

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Table I. Shift Data (ppm) for the Ester Methyl Protons of (+)- and (−)-*threo*-Methylphenidate After Complexation with Various Molar Equivalents of $Eu(hfc)_3$[a]

	(−)-Enantiomer		(+)-Enantiomer		
Molar ratio	δ	Δδ	δ	Δδ	ΔΔδ
0.000	3.62	0.00	3.62	0.00	0.00
0.032	3.69	0.07	3.69	0.07	0.00
0.064	3.77	0.15	3.75	0.13	0.02
0.096	3.87	0.25	3.82	0.20	0.05
0.128	3.93	0.31	3.88	0.26	0.05
0.160	4.03	0.41	3.97	0.35	0.06
0.192	4.09	0.47	4.01	0.39	0.08
0.224	4.18	0.56	4.10	0.48	0.08
0.256	4.25	0.63	4.14	0.52	0.11
0.288	4.33	0.71	4.21	0.59	0.12
0.310	4.42	0.80	4.28	0.66	0.14
0.342	4.49	0.87	4.34	0.72	0.15
0.374	4.53	0.91	4.37	0.75	0.16

[a] Concentration of (+)- plus (−)-*threo*-methylphenidate of 0.12 *M* in CCl_4.

hydroxymethylene)-(+)-camphorato]praseodymium(III) [$Pr(hfc)_3$; Aldrich] were stored over P_2O_5 in an evacuated desiccator. To minimize contamination of the shift reagents with either ambient air or moisture, their weighing and transferring were conducted within a glove box and under an atmosphere of dry nitrogen. Moreover, all NMR tubes and syringes were dried in an oven prior to use, at least 75°C for 12 hr.

Sample Preparation. The hydrochloride salt of *threo*-methylphenidate was converted to the free-base form as follows: a quantity of drug, accurately weighed, was dissolved in 2 ml of water contained in a separatory funnel, and the solution was rendered alkaline with 0.5 ml of 3 *M* sodium hydroxide and then extracted into 3 ml of chloroform. The organic phase was decanted, evaporated to dryness under a stream of dry nitrogen, and dried *in vacuo* at 50°C. The residue was dissolved in the NMR solvent containing 1% (by volume) of TMS, and the solution was used for spectral determinations. When required, the solution was stored in a glass vial crimper-sealed with a Teflon-coated rubber septum and an aluminum seal. Aliquots of solution for analysis were withdrawn through the septum by means of a liquid-tight microliter syringe.

Lanthanide-Induced Shift Studies. Unless otherwise indicated, runs were performed using a constant concentration of substrate and variable concentrations of lanthanide shift reagent. The changes in shift reagent-to-substrate molar ratios given in Tables I and II were obtained by adding the solid shift reagent to an NMR tube prior to an appropriate aliquot of substrate stock solution (concentration of 0.1 to 0.15 *M* in either CCl_4 or $CDCl_3$). After capping the NMR tube, its contents were thoroughly mixed, allowed to stand for 10 min, and then placed in the NMR spectrometer for recording the spectrum. This procedure was repeated in an identical manner subsequent to the addition of a new aliquot of substrate stock solution, until a sufficient number of spec-

Table II. Shift Data (ppm) for the Ester Methyl Protons of (+)- and (−)-*threo*-Methylphenidate After Complexation with Various Molar Equivalents of $Pr(hfc)_3$[a]

	(−)-Enantiomer		(+)-Enantiomer		
Molar ratio	δ	Δδ	δ	Δδ	ΔΔδ
0.000	3.62	0.00	3.62	0.00	0.00
0.005	3.60	−0.02	3.60	−0.02	0.00
0.011	3.55	−0.07	3.55	−0.07	0.00
0.022	3.46	−0.16	3.48	−0.14	0.02
0.033	3.38	−0.24	3.41	−0.21	0.03
0.044	3.33	−0.29	3.37	−0.25	0.04
0.055	3.26	−0.36	3.31	−0.31	0.05
0.066	3.20	−0.42	3.26	−0.36	0.06
0.077	3.10	−0.52	3.17	−0.45	0.07
0.088	2.93	−0.69	3.03	−0.59	0.10
0.099	2.73	−0.89	2.87	−0.75	0.14
0.110	Broad	Broad	Broad	Broad	—

[a] Concentration of (+)- plus (−)-*threo*-methylphenidate of 0.12 *M* in CCl_4.

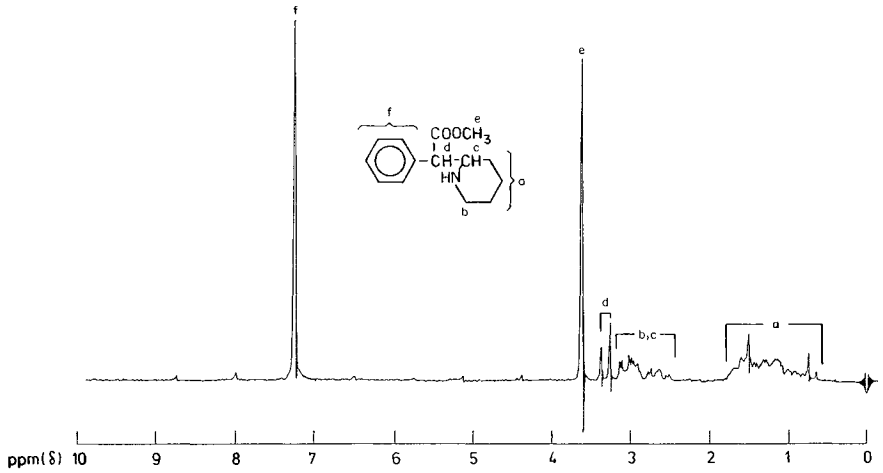

Fig. 1. ^{1}H-NMR spectrum of a mixture of (+)- and (−)-*threo*-methylphenidate in CCl_4.

tra for properly defining the effect of the molar ratio of shift reagent to substrate on the enantiomeric spectral lines had been obtained.

Determination of Optical Purity. Weigh accurately ca. 27 mg of Eu(hfc)$_3$ into an NMR tube, add a solution of 16–17 mg of *threo*-methylphenidate free base (prepared from 18–19 mg of the hydrochloride salt as described under Sample Preparation) in 0.5 ml of CCl_4 containing 1% of TMS (by volume), stopper the tube, and effect solution by inverting the tube several times. Allow the solution to stand for 10 min, and record the ^{1}H-NMR spectrum. Measure the relative intensities (peak heights or peak areas) of the resonance signals at 4.53 and 4.37 ppm, and calculate the percentage of each enantiomer in the sample from

$$\% \text{ (+)-enantiomer} = \frac{100 \times A_{(+)}}{A_{(+)} + A_{(-)}}$$

$$\% \text{ (−)-enantiomer} = \frac{100 \times A_{(-)}}{A_{(+)} + A_{(-)}}$$

where $A_{(+)}$ is the peak area (or peak height) of the signal for the (+)-enantiomer at 4.37 ppm, and $A_{(-)}$ is the peak area (or peak height) of the signal for the (−)-enantiomer at 4.53 ppm.

RESULTS AND DISCUSSION

Although every lanthanide(III) ion has been evaluated for their ability to induce chemical shift differences between nuclei of suitable polar substrate species, Eu(III) and Pr(III) complexes are regarded as the most useful (15). Of the two, Eu(III) appears as the more commonly used because it causes less line broadening than its Pr(III) counterpart (15,16). While Eu(III) chelates normally shift most signals to higher frequencies, i.e., downfield, those containing Pr(III) will induce shifts in the opposite direction (17). In this regard, Pr(III) shift reagents may be more advantageous when the addition of Eu(III) results in serious signal overlapping (15).

Examination of the ^{1}H-NMR spectrum of a mixture of the (+)- and (−)-enantiomers of *threo*-methylphenidate in CCl_4 (Fig. 1) led to the following spectral assignments: sin-

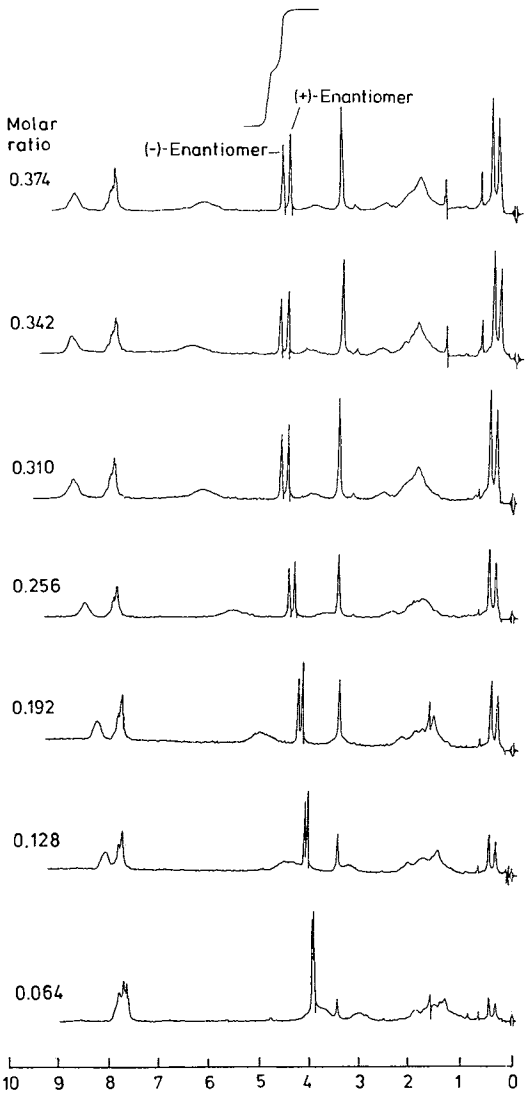

Fig. 2. ^{1}H-NMR spectra of a mixture of (+)- and (−)-*threo*-methylphenidate, combined concentration of 0.12 *M* in CCl_4, at various Eu(hfc)$_3$-to-substrate molar ratios. Signals integrated correspond to those of the ester methyl protons.

Table III. Analysis of Synthetic Mixtures of (+)- and (−)-*threo*-Methylphenidate Hydrochloride by ^{1}H-NMR Spectroscopy with Eu(hfc)$_3$[a]

	Amount added			Amount found	
Sample	(+)-Form (mg)	(−)-Form (mg)	(−)-Form (% of mixture)	(−)-Form (mg)	(−)-Form (% rec.)[b]
1	55.7	56.3	50.27	56.58	100.5
2	66.8	45.2	40.36	45.15	99.9
3	76.2	35.8	31.96	35.66	99.6
4	88.3	23.7	21.16	23.49	99.1
5	101.5	10.5	9.38	10.37	98.8
6	110.1	1.9	1.70	1.88	98.8

[a] Concentration of (+)- plus (−)-*threo*-methylphenidate of 0.12 *M* (free-base form) in CCl_4, and a Eu(hfc)$_3$-to-substrate molar ratio of 0.374. Resonances of the ester methyl protons were used in the analysis.
[b] Calculated as (amount found × 100)/amount added.

glet at 7.25 ppm, phenyl protons; sharp singlet at 3.62 ppm, ester methyl protons; doublet centered at 3.30 ppm, benzylic protons; overlapping multiplets between 3.20 and 2.50 ppm, piperidinic N-CH_2 and N-CH protons; and broad multiplet between 2.00 and 0.60 ppm, other piperidinic protons. The close proximity of the ester methyl group to the carbonyl functionality, the basic coordinating site, and a chiral center facilitated the resolution of its enantiomeric proton signals (18). Upon complexation with the Eu(III) chelate, the singlet at 3.62 ppm, representing the unresolved signals for the enantiomeric ester methyl protons in the uncomplexed spectrum, was not only shifted to a higher frequency but also split into two singlets, one at 4.53 ppm, because of the (−)-enantiomer, and the other at 4.37 ppm, because of the (+)-enantiomer (Fig. 2). Unambiguous assignment of the enantiomeric resonances was facilitated by observing the enhancement in peak intensity when one of the enantiomers was added to the test sample.

Since a lanthanide shift reagent can perturb the substrate resonances from their normal positions by an amount which depends not only on the relative concentration of shift reagent present but also on the solvent used (14), and since different shift reagents exert different effects on line width (16), the changes in induced pseudocontact shift differences ($\Delta\Delta\delta$) for the resonance lines of interest were systematically investigated as a function of the solvent, shift reagent, and shift reagent-to-substrate molar ratio used, until a maximum $\Delta\Delta\delta$ was attained (18). Larger $\Delta\Delta\delta$ were noted in CCl_4 than in $CDCl_3$ (data not shown). At equivalent low molar ratios in CCl_4, Pr(hfc)$_3$ yielded larger $\Delta\Delta\delta$ than Eu(hfc)$_3$ (Tables I and II); however, the proposed method includes Eu(hfc)$_3$ rather than Pr(hfc)$_3$ because the largest $\Delta\Delta\delta$ without evidence of both line broadening and line overlapping were obtainable only with the former shift reagent. Optimum conditions for the quantification of optical purities corresponded to a Eu(hfc)$_3$-to-substrate molar ratio of 0.374 and a substrate total concentration of 0.12 *M* in CCl_4 (Fig. 2, uppermost spectrum).

Six synthetic mixtures of the (+)- and (−)-enantiomers of *threo*-methylphenidate hydrochloride, made up in the proportions shown in Table III, were converted to the free-base forms and analyzed by the proposed method. The experimental results were found in good agreement with the known weights of each enantiomer in the synthetic mixtures. The mean ± SD recovery of the (−)-enantiomer was 99.5 ± 0.7% of the added amount, with as little as 2% of this isomer being measurable accurately.

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