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Determination of the optical purity and absolute configuration of *threo*-methylphenidate by proton nuclear magnetic resonance spectroscopy with chiral solvating agent

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Abstract: The direct determination of both the optical purity and absolute configuration of *threo*-methylphenidate has been accomplished in a simple, specific, and accurate manner by ^{1}H-NMR spectroscopy. The enantiomeric resonances of *threo*-methylphenidate were effectively resolved in $CDCl_3$ solution by the addition of the chiral solvating agents (*R*)-(−)- or (S)-(+)-2,2,2,-trifluoro-1-(9-anthryl)ethanol. Optical purities were determined on the basis of the intensities of the enantiomeric ester methyl proton resonances; the assignment of enantiomeric configurations was based on the relative field positions of these resonances and the examination of molecular models. The analysis of synthetic enantiomeric mixtures of *threo*-methylphenidate by the proposed NMR method resulted in assay values that agreed closely with the known quantities of each enantiomer in the mixtures tested. The mean ±SD recovery value for the (2*S*,2′*S*)-(−)-*threo*-enantiomer, amounting to 99.9 ± 0.6% of added (n = 10), correlated well with that previously found by ^{1}H-NMR spectroscopy with a chiral Eu(III) shift reagent. However, the present approach is simpler, shows less reliance on reagents and solvents of a high purity, and does not require strict anaerobic working conditions.

Keywords: threo-*Methylphenidate; ^{1}H-NMR spectroscopy; chiral solvating agent; enantiomers; diastereomeric solvates; optical purity; absolute configurations.*

Introduction

Methylphenidate hydrochloride is a piperidine derivative structurally and pharmacologically related to the amphetamines. Because of its mild central nervous system stimulant activity, it finds use in the pharmacotherapy of depressive states, narcolepsy, and the attention-deficit disorder of children with or without hyperactivity [1,2].

The presence of exocyclic and endocyclic stereogenic centres in this phenidate compound determines its existence as pairs of diastereomeric *erythro*- and *threo*-forms. A comparative study of the central and peripheral stimulant effects of these compounds has indicated that the *threo*-isomers are more active than the *erythro*-isomers, and that the (2*R*,2′*R*)-(+)-*threo*-form is more active than the (2*S*,2′*S*)-(−)-*threo* antipode [3–5]. Variations in the magnitude of pharmacological responses among these stereoisomers are probably due to their different affinities for cell receptor sites imposed by their particular molecular configurations and, hence, to receptor stereoselectivity [6].

For therapeutic purposes, *threo*-methylphenidate hydrochloride is commercially available as the racemic mixture. Resolution of the enantiomers has been accomplished by fractional crystallization [7] and by open-column chromatography on an optically active ion-exchanger [8], but these methods are quite laborious as well as susceptible to significant sample losses [9]. More quantitative enantioselective separations have been possible through the use of HPLC in the normal [10] or reversed [9] phase, or of gas chromatography with electron capture detection [11], but these approaches require samples of the expensive pure enantiomers for use as external reference standards. Furthermore, the use of HPLC may cause sample racemization during the separation of an enantiomer on an optically active sorbent [12].

This laboratory has recently proposed a

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simple, specific, and quantitative ^{1}H-NMR spectroscopic method with a chiral Eu(III) shift reagent for the determination of the enantiomeric composition (optical purity) of *threo*-methylphenidate [13]. Salient favourable features of this method were economy of reagents and procedural steps, non-reliance on reference standards, freedom from potential racemization, a time scale that is shorter than that of HPLC methods, and the possibility of simultaneously obtaining positive proof on the identity of the sample.

The purpose of this report is to describe an alternative ^{1}H-NMR spectroscopic method for the direct determination of the optical purity of *threo*-methylphenidate. The required resolution of the enantiomeric resonance lines is readily accomplished through interaction of the enantiomeric mixture with a chiral solvating agent. In addition to permitting further simplification of the analytical procedure, this new approach is also suitable for establishing the absolute configuration of the enantiomers.

Experimental

Apparatus

All ^{1}H-NMR spectra were obtained using a Varian EM-390, spectrometer (Varian Associates, Sunnyvale, CA, USA) operating at a probe temperature of 35 ± 1°C, and were referenced to tetramethylsilane (TMS) taken as 0.00 ppm on the δ scale.

Chemicals

TMS (>99.9%), deuterochloroform ($CDCl_3$, 99.8 atom % D) and (*R*)-(−)- and (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol (TFAE; >98%) were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). TMS was made free of tetrahydrofuran by consecutive washes with sulphuric acid and saturated potassium bicarbonate, distilled, and stored over type 4A molecular sieves.

Sample preparation

Synthetic mixtures of (2*R*,2′*R*)-(+)- and (2*S*,2′*S*)-(−)-*threo*-methylphenidate were prepared by accurately weighing the quantities of enantiomeric hydrochloride salts listed in Table 1. The free base forms were obtained by placing the sample of enantiomeric mixture in a separatory funnel, dissolving in 2 ml of water, alkalinizing with two drops of 3 M NaOH, and extracting into 3 ml of $CHCl_3$. The $CHCl_3$ extract was evaporated to dryness under a stream of dry nitrogen, and the residue was dried at 50°C *in vacuo*.

Solvating agent-induced chemical shift studies

The optimum concentration of chiral solvating agent to use was determined by obtaining the spectra that resulted from the gradual addition of increasing quantities of (*S*)-(+)- or (*R*)-(−)-TFAE to the solution of a mixture of *threo*-methylphenidate enantiomers in $CDCl_3$ containing 1% TMS.

Determination of the optical purity

An accurately weighed quantity of the dry residue of *threo*-methylphenidate free bases (*ca* 8.5 mg) was dissolved in 0.5 ml of $CDCl_3$ containing 1% TMS with gentle swirling. The solution was transferred to a NMR tube containing *ca* 50 mg of (*S*)-(+)-TFAE, mixed by inversion, and used to record the ^{1}H-NMR spectrum. After measuring the relative intensities of the enantiomeric ester methyl proton signals at 3.43 ppm [(2*R*,2′*R*)-(+)-enantiomer] and 3.33 ppm [(2*S*,2′*S*)-(−)-enantiomer], the percentage of each enantiomer was calculated from

$$\%\ (2R,2'R)\text{-}(+)\text{-enantiomer} = \frac{100 \times A_{(+)}}{A_{(+)} + A_{(-)}},$$

$$\%\ (2S,2'S)\text{-}(-)\text{-enantiomer} = \frac{100 \times A_{(-)}}{A_{(+)} + A_{(-)}},$$

where $A_{(+)}$ = peak area (or height) of the

Table 1
Shift data for —$COOCH_3$ protons of solvates of (2*R*,2′*R*)-(+)- and (2*S*,2′*S*)-*threo*-methylphenidate

	δ (ppm)	
Solvating agent	(2*R*,2′*R*)-(+)-enantiomer	(2*S*,2′*S*)-(−)-enantiomer
(*S*)-(+)-TFAE	3.43	3.33
(*R*)-(−)-TFAE	3.33	3.43

resonance signal for the ester methyl protons of the (2*R*,2′*R*)-(+)-enantiomer, and $A_{(-)}$ = peak area (or height) of the resonance signal for the ester methyl protons of the (2*S*,2′*S*)-(−)-enantiomer.

Results and Discussion

TFAE was ideally suited as a chiral solvating agent both for the determination of the optical purity and for the assignment of the absolute configurations of *threo*-methylphenidate diastereomeric solvates by ^{1}H-NMR spectroscopy. On the one hand, by possessing a group of high diamagnetic anisotropy in the vicinity of its stereogenic centre, namely the anthryl moiety, TFAE will be able to translate different average spatial environments around the enantiomeric solute nuclei into spectral lines with different chemical shifts. Such an effect is expected to be substantial in view of the two possible spatial orientations the anthryl moiety can adopt relative to the functional groups under its influence. On the other hand TFAE exhibits functionalities that are complementary to those of *threo*-methylphenidate. Due to its close proximity to a trifluoromethyl group, the alcoholic hydroxyl group of TFAE is sufficiently acidic to interact with a strong basic site in the enantiomeric solute. Additional interactions are also expected to take place at the methine hydrogen since the inductive effect of the electron withdrawing groups attached to the stereogenic centre of TFAE, i.e. trifluoromethyl, hydroxyl and anthryl, will direct an electric dipole roughly along the methine C—H bond axis to render the methine hydrogen slightly acidic and, thus, amenable to additional interactions with weak basic sites in the solute [14]. Among the various possible TFAE–solute interactions, those between the hydroxyl and imino groups are considered to be stronger than those between the methine hydrogen and carbomethoxy carbonyl. Consequently, two such points of interaction will engender greater populations of specific, non-equivalent, short-lived, chelate-like, diastereomeric solvate conformers.

Figure 1 shows the 90 MHz ^{1}H-NMR spectrum of a mixture of (2*R*,2′*R*)-(+)- and (2*S*,2′*S*)-(−)-*threo*-methylphenidate in $CDCl_3$. The singlet at 3.62 ppm represents the unresolved resonance signals for the enantiomeric methyl ester protons. Figure 2 shows the spectrum of a mixture of the diastereomeric solvates of *threo*-methylphenidate enantiomers formed upon their interaction with (*S*)-(+)-TFAE, in which the signals for the enantiomeric ester methyl protons are clearly resolved into singlets at 3.43 ppm [(2*R*,2′*R*)-(+)-enantiomer] and 3.33 ppm [(2*S*,2′*S*)-(−)-enantiomer]. Figure 3 illustrates the effect of varying the TFAE to solute molar ratio on the resolution of the resonances for the enantiomeric ester methyl protons. It is apparent that the enantiomeric shift difference (ΔΔδ) for

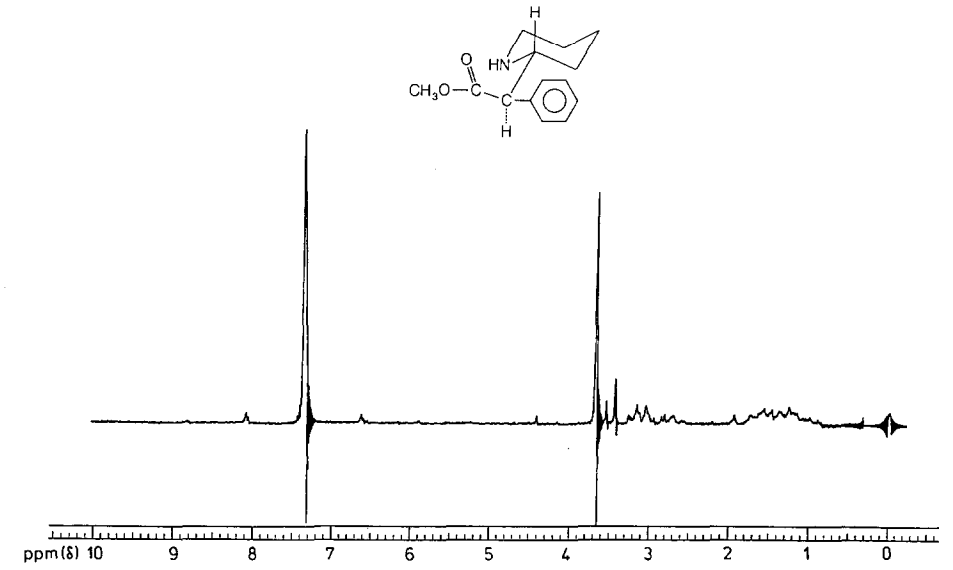

Figure 1
^{1}H-NMR spectrum of a mixture of (2*R*, 2′*R*)-(+)- and (2*S*,2′*S*)-(+)-*threo*-methylphenidate in $CDCl_3$.

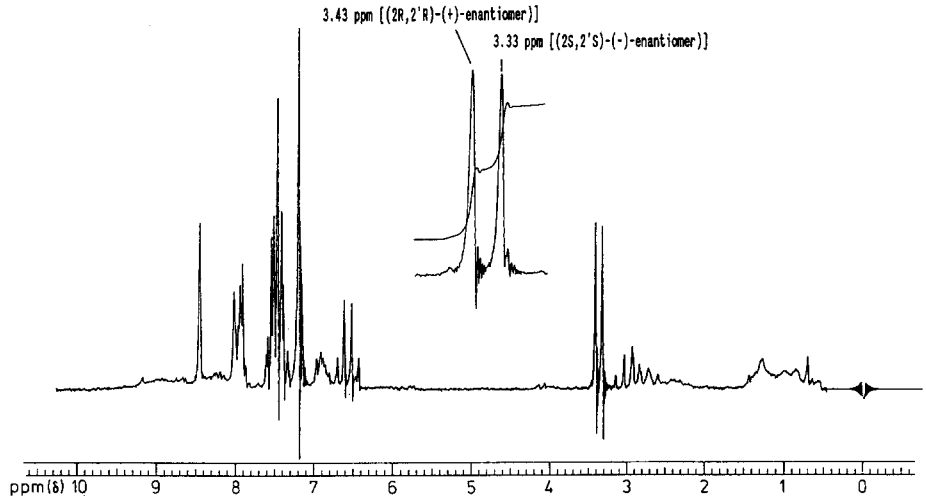

Figure 2
^{1}H-NMR spectrum of a mixture of the diastereomeric solvates of (2*R*,2′*R*)-(+)- and (2*S*,2′*S*)-(−)-*threo*-methylphenidate (combined concentration of 0.075 M) with (*S*)-(+)-TFAE (0.375 M) in $CDCl_3$.

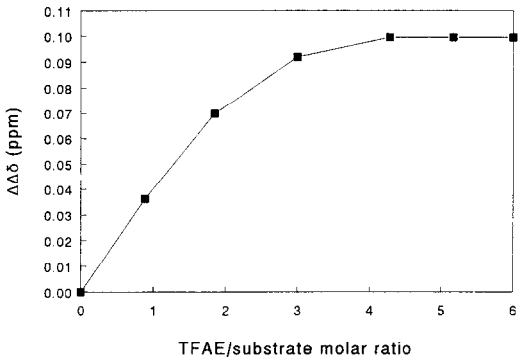

Figure 3
Variation in chemical shift differences (ΔΔδ) for ester methyl protons of enantiomers of *threo*-methylphenidate in $CDCl_3$ (combined concentration of 0.075 M) with increasing TFAE–substrate molar ratios.

these resonance signals increased in parallel fashion to the increase in molar ratio, with a ceiling effect starting at a molar ratio of about 4.0. The absence of line broadening at all molar ratios examined indicates that competing self-associations among solute or chiral solvating molecules are, at best, negligible, and that the solvent appears not to interfere with solute–chiral solvating agent interactions. In this instance, solute–solute interactions were kept to a minimum by the combined use of an excess of solvating agent with a concentration of solute that was just enough to produce an adequate signal strength.

At a molar ratio of 4.83, which corresponds to a *threo*-methylphenidate concentration of 0.075 M, spectral nonequivalences among enantiomeric resonance signals are considered to reflect structural differences inherent to each diastereomeric solvate rather than differences in the degree of association between enantiomeric solutes and the chiral solvating agent. This assumption is corroborated by observing no diminution in spectral non-equivalences at very high concentrations of TFAE, i.e. >0.362 M.

The assignment of the absolute configurations of the enantiomers of *threo*-methylphenidate was facilitated by the construction and examination of suitable ball-and-stick molecular models of the diastereomeric solvates formed with TFAE. In this manner, it was verified that the absolute configurations were also assignable on the basis of differences in chiral solvating agent–solute interactions. As can be seen in Fig. 4(a,b), it is the spatial orientation of the ester methyl group of each of the solvates that will determine if this group will be shielded or deshielded by the anthryl moiety of the chiral solvating agent. For example, the timed-average chemical shift of the ester methyl group will occur at a higher field if this group is located above or below the ring current, in other words, *cis*- to the anthryl substituent [as in (2*R*,2′*R*)-(+)-methylphenidate, Fig. 4(a)]. In contrast, the resonance signal for the ester methyl group will appear at a lower field when this group is oriented with the plane of the anthryl moiety of TFAE, that is, it is *trans*- to the anthryl substituent [as in (2*S*,2′*S*)-(−)-methylphenidate, Fig. 4(b)]. While the populations of *threo*-methylpheni-

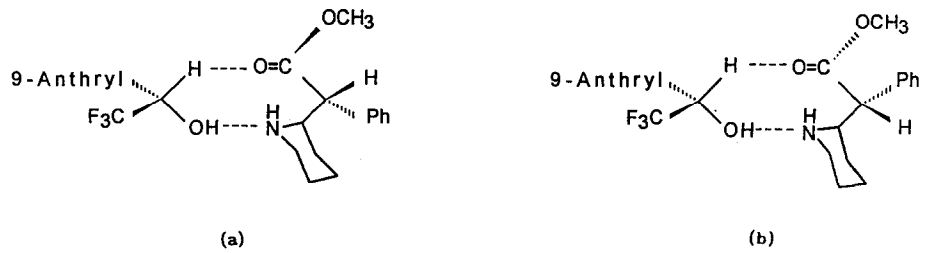

Figure 4
Most abundant conformers of solvated diastereomers of (*S*)-(+)-TFAE and enantiomers of *threo*-methylphenidate: (a) with the (2*R*,2′*R*)-(+)-enantiomer; and (b) with the (2*S*,2′*S*)-(+)-enantiomer.

Table 2
Results of assay of synthetic mixtures of (2*R*,2′*R*)-(+)- and (2*S*,2′*S*)-(−)-*threo*-methylphenidate by ^{1}H-NMR spectroscopy with chiral solvating agent*

	Amount of isomer added			Amount of (−)-isomer found	
Sample no.	(+)-isomer (mg)	(−)-isomer (mg)	(−)-isomer (% of mixture)	% of mixture	% recovered†
1	51.9	51.5	50.29	50.02	99.5
2	55.2	45.8	45.35	45.25	99.8
3	57.5	42.6	42.56	42.44	99.7
4	59.8	37.5	38.54	38.69	100.4
5	62.6	34.5	35.53	35.55	100.1
6	72.8	28.5	28.13	27.92	99.3
7	79.0	20.7	20.78	20.76	99.9
8	84.5	15.2	15.25	15.36	100.7
9	90.6	9.6	9.58	9.50	99.2
10	117.9	2.1	1.75	1.76	100.7
Mean					99.9
SD					0.6
RSD					0.6

*The total concentration of drug was *ca* 0.075 M in $CDCl_3$; the concentration of TFAE was *ca* 0.0375 M.
†Amounts recovered were calculated from (amount found × 100)/amount added, where amount found, mg (2*S*,2′*S*)-(−)-enantiomer, was calculated from: $A_{(2S,2'S)\text{-}(-)} \times$ mg taken/$[A_{(2S,2'S)\text{-}(-)} + A_{(2R,2'R)\text{-}(+)}]$.

date solvate conformers such as those depicted in Fig. 4(a,b) need not be the same, their contributions to the average magnetic environments of the resolved signals for the enantiomeric ester methyl protons of the solute are such as to result in the shielding of the ester methyl protons of the (2*S*,2′*S*)-(+)-configuration and the deshielding of those of the (2*R*,2′*R*)-(−)-configuration. These conclusions were confirmed by separately studying the interactions of each of the enantiomers of *threo*-methylphenidate with a specific enantiomer of TFAE. As presented in Table 1, in both instances the chemical shifts were of the same magnitude, but their assignments became interchanged when going from one TFAE enantiomer to the other. For the set of 10 synthetic enantiomeric mixtures listed in Table 2, made to contain various amounts of (2*R*,2′*R*)-(−)-*threo*-methylphenidate, the recoveries of this enantiomer by the proposed ^{1}H-NMR spectroscopic method with chiral solvating agent ranged from 99.2 to 100.7% of added. In general a close agreement existed between these assay results and the known quantities of enantiomer present in the mixtures examined.

The determination of the optical purity of *threo*-methylphenidate hydrochloride by NMR spectroscopy has been previously accomplished through complexation of this substrate with a chiral Eu(III) shift chelate [13]. Results by this method (mean ± SD recovery, 99.5 ± 0.7% of added; range, 98.8–100.5% of added; $n = 6$) compared very favourably with those gathered using a chiral solvating agent. However, in spite of the many advantages this approach offered over other analytical techniques, namely simplicity, economy of reagents, accuracy, and specificity, it requires the use of absolutely anhydrous reagents, solvents of a high purity, and a completely anaerobic

working environment. In contrast, the procedure presented here represents a further simplification of the previous approach, it circumvents most of the stringent requirements imposed by the earlier method and, additionally, it gives no evidence of line broadening. Furthermore, it represents an alternative approach to the determination of the absolute configuration of the enantiomers of *threo*-methylphenidate by methods entailing sequential chemical conversions to and comparisons with compounds of known absolute stereochemistry [15–17].

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