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Determination of enantiomeric purity of nicotine in pharmaceutical preparations by ^{13}C-NMR in the presence of a chiral lanthanide shift reagent

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Abstract: A method for the determination of enantiomeric composition of nicotine samples, based on ^{13}C-NMR spectroscopy in the presence of the chiral lanthanide shift reagent, tris[3-(trifluoromethylhydroxymethylene)-(+)-camphorato]ytterbium [Yb(tfc)$_3$], was developed. Observation at 100.6 MHz of the C2′ resonance of nicotine in the presence of 0.15–0.20 mol of the ytterbium complex, either in ordinary ^{13}C{^{1}H}-NMR spectra or in carbon spectra enhanced by polarization transfer (refocused INEPT), allowed precise determination of the ratios of (*S*)- to (*R*)-nicotine. At least 1% of (*R*)-nicotine could be determined in samples of (*S*)-nicotine, milligram amounts being required for the analysis. Use of the ^{13}C-NMR spectra is more advantageous than use of ^{1}H-NMR spectra. Thus, Yb(tfc)$_3$ induced separation of the proton resonances of the enantiomers of nicotine, and the shifted resonances of nicotine enantiomers could be assigned by use of ^{1}H–^{13}C heteronuclear chemical shift correlation, but the proton resonances were broad, their chemical shifts were sensitive to small variations of the ratio between Yb(tfc)$_3$ and nicotine, and signals of the enantiomer present in small amounts were easily obscured by impurities. Therefore, although ^{13}C-NMR is more time consuming, this method is more suitable for routine analysis. The method was applied for the determination of enantiomeric purity of (*S*)-nicotine in pharmaceutical formulations, including chewing gums, skin absorption patches, inhalators, and nasal sprays.

Keywords: *^{1}H-NMR; ^{13}C-NMR; INEPT; lanthanide-induced shifts; chiral shift reagents; enantiomeric purity.*

Introduction

Nicotine (Fig. 1), an alkaloid of *Nicotiana* species, is an agonist for a subclass of acetylcholine receptors known as nicotinic receptors. Apart from effects related to tobacco smoking, there has been an interest in nicotine in relation to Parkinson's [1] and Alzheimer's [2, 3] diseases. The natural nicotine present in the tobacco plant is the (*S*)-(−)-isomer, but there is an interest in the pharmacological properties of the enantiomeric (*R*)-(+)-nicotine [4, 5]. Recently, pharmaceutical companies launched numerous (*S*)-(−)-nicotine preparates aimed to aid smokers to cope with cigarette abstinence symptoms. These include chewing gums [6, 7], skin absorption patches [8–11] and tapes [12], roll-on devices [13], mucous membrane absorption devices [14], and nasal sprays [15–17]. All these applications fuel interest in methods for determination of enantiomeric purity of nicotine.

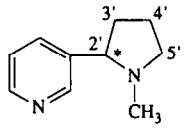

Figure 1
Structure of nicotine with atom numbering used in the text. Asterisk indicates the chiral centre.

The benzylic chiral centre in nicotine can be racemized by action of bases [18, 19]. In principle, there are several possibilities for determination of enantiomeric compositions of nicotine samples. Polarimetry, which normally requires pure material, has recently been applied in conjunction with a HPLC column employing a laser polarimeter [20]. Although this method includes a separation step and is applicable for small samples, it is not suitable for the analysis of samples with a large excess of one enantiomer.

Efforts to achieve practically useful separations of nicotine enantiomers by enantioselective chromatographic techniques have been only partly successful. Thus although nicotine enantiomers could be separated on a packed microcolumn using a mobile phase

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containing β-cyclodextrin, the method requires a 1 m long column operated at 130 atm, and the elution time exceeds 3 h [21]. No separation of nicotine enantiomers could be achieved on β-cyclodextrin bonded stationary phases [22, 23]. Similarly, capillary gas chromatography on a chiral support [24] is not easily applicable.

In this work, it is demonstrated that ^{13}C-NMR spectroscopy in the presence of a chiral lanthanide complex is a convenient method for determination of enantiomeric ratios in nicotine samples.

Experimental

General

(−)-Nicotine (+)-ditartrate, (+)-nicotine (+)-di-*p*-toluoyltartrate, and tris[3-(trifluoromethylhydroxymethylene)-(+)-camphorato]-ytterbium [Yb(tfc)$_3$] were obtained from Sigma. All salts and solvents used were analytical grade chemicals. NMR spectra were recorded on a Bruker AMX 400 WB spectrometer at 400.13 and 100.6 MHz for ^{1}H and ^{13}C, respectively.

Methods

NMR spectra were acquired in chloroform-*d* using 90° pulses and optimized acquisition parameters; sweep widths and number of data points were adjusted to obtain digital resolution in the frequency domain better than 0.3 Hz per data point. ^{13}C-NMR spectra were obtained either as normal, composite pulse (WALTZ16) decoupled spectra, or, for increased sensitivity, as refocused INEPT spectra with decoupling during the acquisition. In order to achieve maximal resolution of the ^{13}C resonances, the data were resolution enhanced by zero-filling and Gaussian multiplication prior to Fourier transformation. Longitudinal (T_1) relaxation times were measured by the inversion recovery method. Mixtures of (−)- and (+)-nicotine with known enantiomeric ratios were obtained by dissolving known amounts of the enantiomerically pure nicotine salts in 0.5 M aqueous sodium carbonate, extraction of the solutions with pentane, and evaporation of dried (magnesium sulphate) extracts in high vacuum. After determination by weighing of amounts of free nicotine base thus obtained, the samples were dissolved in 0.7 ml of chloroform-*d*, and spectra were recorded after addition of accurately weighed amounts of Yb(tfc)$_3$. The enantiomeric purity of nicotine standards was confirmed by the ^{13}C-NMR spectra as described in the text.

Analysis of (−)-nicotine formulations

Nicotine patches were cut into small pieces and extracted by soaking for 3 h in pentane. The pentane extracts were extracted four times with 0.1 M hydrochloric acid, the aqueous solution made alkaline with 0.5 M sodium carbonate, and extracted four times with pentane. The combined extracts were dried (magnesium sulphate), evaporated, and the amount of the residue determined. The residue was dissolved in 0.7 ml of chloroform-*d*, and ^{13}C-NMR spectra determined after addition of an equal milligram amount of Yb(tfc)$_3$. Typically, 6–8 k transients were collected. Occasionally, small amounts of (+)-nicotine standard (typically 1–2% of the expected amount of (−)-nicotine) were added during the extraction as controls. Nicotine inhalators were processed similarly. Nicotine nasal spray solutions were made alkaline prior to extraction with pentane as above. Chewing gums were extracted with dichloromethane overnight, the solution reextracted with 0.1 M hydrochloric acid, and the procedure continued as described above.

Results and Discussion

Although application of ^{1}H-NMR pseudocontact shifts from a chiral lanthanide reagent [Eu(tfc)$_3$] to nicotinic compounds has been mentioned in the literature [25], the method was considered not to be generally applicable [21]. In the present work, the ^{1}H-NMR spectra of mixtures of nicotine enantiomers and Yb(tfc)$_3$ were assigned by ^{1}H–^{13}C heteronuclear shift correlation experiments. Although Yb(tfc)$_3$ induces differential chemical shifts of the enantiomers, the proton resonances are strongly broadened and could be used for analysis of samples containing high ratios of the enantiomers only with difficulty (Fig. 2). In particular, the ^{1}H-NMR spectra were sensitive to the precise ratio between nicotine and Yb(tfc)$_3$, and drift of ^{1}H resonances was observed on aging of the solutions for several hours. Moreover, because of a narrow spectral width in the ^{1}H-NMR spectra, impurities were more likely to obscure quantification of the resonances. On the other hand, ^{13}C resonances experienced large in-

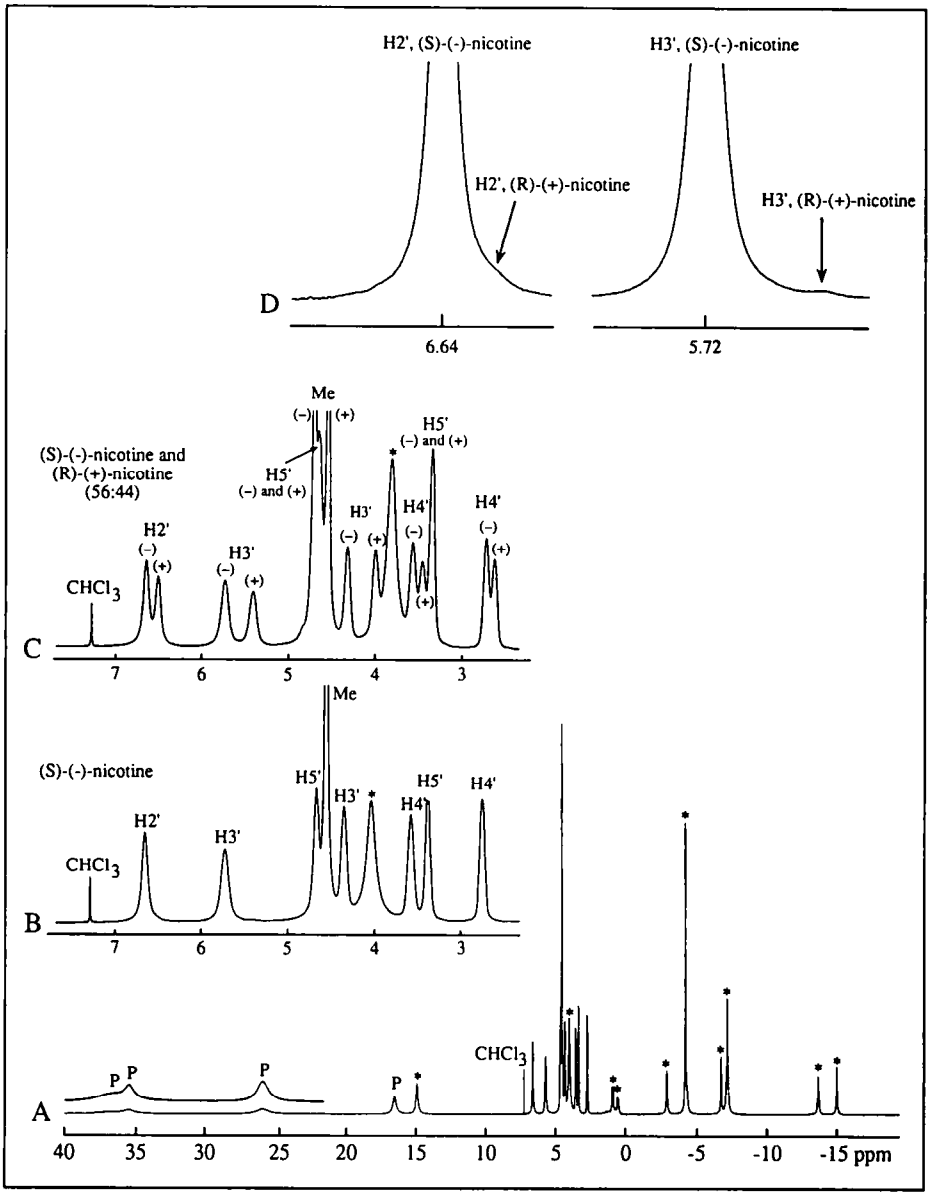

Figure 2
400 MHz ^{1}H-NMR spectra of (−)- and (+)-nicotine in the presence of Yb(tfc)$_3$ in chloroform-*d*. (A) Full spectrum of (−)-nicotine containing 1.05% of (+)-nicotine, with molar ratio between nicotine and Yb(tfc)$_3$ 1:0.17; the asterisks indicate signals originating from the shift reagent and impurities, Ps indicate resonances of the pyridyl moiety. (B) Fragment of spectrum A showing assignment of the aliphatic protons of nicotine. (C) Similar spectrum of a mixture of 56% of (−)-nicotine and 44% of (+)-nicotine. (D) Expansion of the resonances of H2′ and H3′ from spectrum B; signals from 1.05% of (+)-nicotine are apparent.

duced shifts in the presence of Yb(tfc)$_3$ (Fig. 3) without much signal broadening. The aliphatic carbon resonances could also be readily assigned, in spite of small changes of the chemical shifts according to the exact ratio between nicotine and Yb(tfc)$_3$, and exhibited good reproducibility for a given sample (no drift of resonances). Sufficient differentiation of the chemical shifts of carbons of (−)- and (+)-nicotine was observed already at a low ratio between nicotine and Yb(tfc)$_3$ (Fig. 4), and the difference was not sensitive to the exact amount of Yb(tfc)$_3$ present. Relaxation times (T_1) measurements yielded the following values (±8%) for the aliphatic carbons of nicotine in the presence of 0.18 mol of Yb(tfc)$_3$: C2′ 812 ms, C3′ 868 ms, C4′ 925 ms, C5′ 753 ms, CH_3 886 ms; no significant differences were observed between the ^{13}C resonances of (−)- and (+)-nicotine. The values were used for optimization of the interpulse delays in the acquisition of the

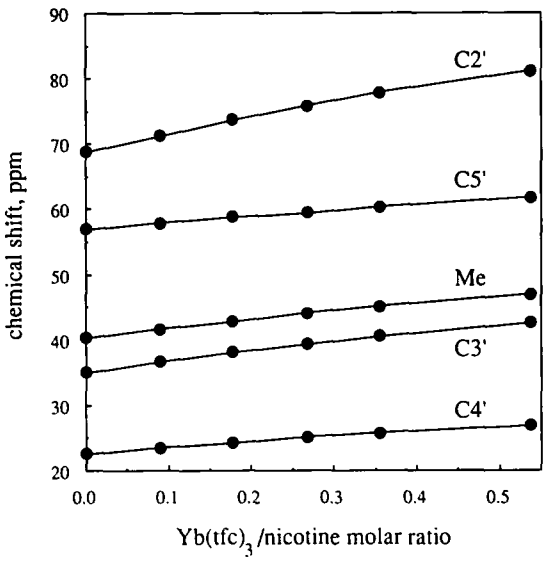

Figure 3
Dependence of chemical shifts of the aliphatic carbons of (−)-nicotine on the ratio between nicotine and $Yb(tfc)_3$.

carbon spectra. Under similar conditions but in the absence of the shift reagent the T_1 values of aliphatic carbons of nicotine were as follows: C2′ 4.3 s, C3′ 2.7 s, C4′ 2.8 s, C5′ 2.3 s, CH_3 1.9 s (oxygen not excluded). Routine determinations of enantiomeric ratios are best performed from the C2′ resonance, for which good differentiation between (−)- and (+)-nicotine was observed (Fig. 4), and a minimum of interferences from impurities was experienced. Since the signals of the enantiomers have the same line widths, quantification of the enantiomeric ratios can conveniently be done from signal heights. For increased sensitivity, the ^{13}C spectra were also recorded as refocused INEPT spectra, with polarization transfer delay optimized for $^1J_{C2',H2'} = 131.5$ Hz (determined from a gated carbon spectrum). The INEPT spectra were recorded with interpulse delays of 0.9 s (about 10 times the value of T_1 for H2′, determined to be 89 ± 7 ms).

Using this method, enantiomeric composition of nicotine samples could be determined conveniently and reliably. The method is appropriate for analysis of samples with at least 100-fold excess of one of the enantiomers (Fig. 5), being thus comparable to what can be achieved by the state-of-the-art HPLC methods using chiral supports. The method requires only crude purification of nicotine (acid and base extractions) and is largely insensitive to the presence of impurities. It requires 1–3 mg of nicotine for overnight acquisition of ^{13}C-NMR data if the amount of one enantiomer is very low; larger amounts found in pharmaceutical preparates, or samples with comparable amounts of both enantiomers, can be analysed during 1–2 h. Using this method, chewing gums, nasal sprays, patches and inhalators were analysed.

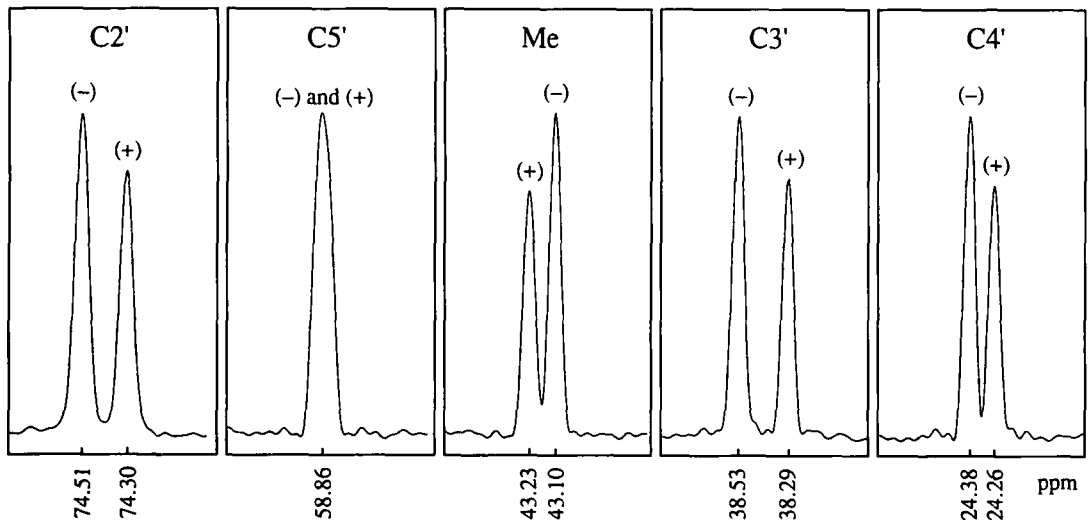

Figure 4
100.6 MHz $^{13}C\{^1H\}$-NMR spectra showing signals of aliphatic carbons of (−)- and (+)-nicotine (ratio 56:44) in the presence of $Yb(tfc)_3$. Molar ratio between nicotine and the ytterbium complex is 1:0.17, the resonances are scaled to the same intensity.

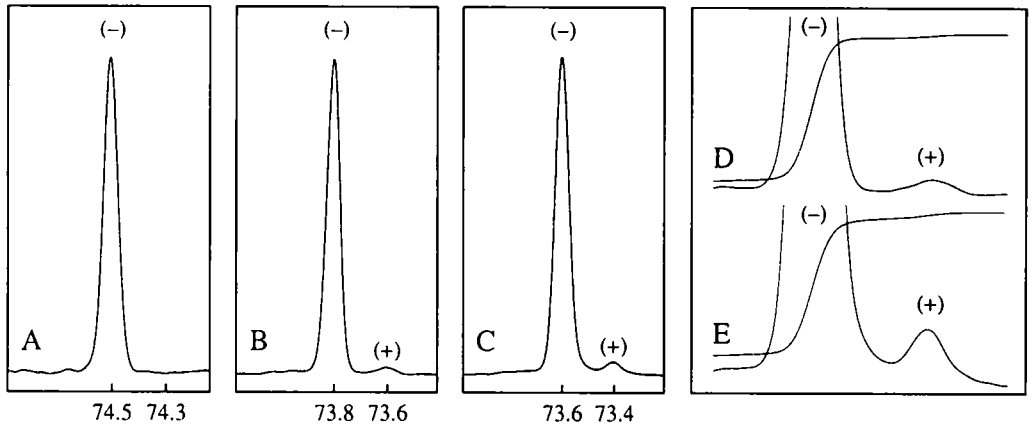

Figure 5
100.6 MHz $^{13}C\{^1H\}$-NMR spectra showing signals of C2' of nicotine enantiomers. (A) Enantiomerically pure (−)-nicotine. (B) (−)-Nicotine containing 1.10% of (+)-nicotine. (C) (−)-Nicotine containing 2.80% of (+)-nicotine. (D) and (E) are expansions of (B) and (C), respectively.

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